Although this volume focuses mainly on developments on this continent, radiobiological research became a "global market" at a very early date; ideas, and indeed scientists, crossed the Atlantic at great speed and with great frequency. As a consequence, no attempt will be made here to impose geographical boundaries on the evolution of new ideas.

There are four principal themes in the evolution of radiobiological research related to radiation therapy:

1. The oxygen effect. This was first discovered in the laboratory and eventually influenced much of clinical research in the form of high pressure oxygen tanks, neutrons, radiosensitizers, and bio-reductive drugs.

2. Fractionation. Laboratory experiments over the years shaped the protocols used in clinical practice.


4. The development of experimental biological endpoints. In the early years, plant systems were widely used, later replaced by mammalian cells cultured in vitro, clever assays for radiation damage in normal tissues of experimental animals, and a variety of transplantable tumors. Recombinant techniques in molecular biology have gradually been introduced into radiation research beginning in the 1980s. The development of these research themes will be emphasized at the end of the chapter.

**The X-Ray Era: 1910–1950**

**Leading the Way**

One of the first documented uses of X-ray therapy was the treatment of a hairy nevus by the Viennese dermatologist Leopold Freund in 1896. Priority for the first X-ray therapy has been claimed by others, including Grubbé, Despeignes, Williams, and Vogt, though these were largely one-of-a-kind efforts rather than systematic attempts to apply a new modality.¹,²

The first planned experiment in radiobiology was performed by Pierre Curie, who applied a tube of radium to his own forearm and described in detail the various phases of the resulting moist radion-epidermitis and his recovery from it (Fig. 5.1).

Radiobiological research in the early years of the twentieth century
emphasized the importance of the proliferative potential in determining the radiosensitivity of cells. Kienbock in 1901 commented that organs in which active cell proliferation occurred were especially sensitive to radiation. In 1903 Albers-Schönberg showed that radiation could induce azospermaea after irradiation of the testes, while three years later, Bergonié and Tribondeau used the same biological system to develop their "law," which proposed a proportionality between radiosensitivity and reproductive activity of cells. In these earliest years the German school dominated radiation therapy and favored the view that single dose "caustic" treatments were superior, while fractionated treatment was judged to be "weak irradiation." As Wintz, one of the proponents of caustic irradiation, argued:

The cells of the human body are endowed with variable radiosensitivity and capacity for recovery from radiation damage. It is also reasonable to assume that recovery from radiation injury depends on cellular metabolism, and further that a rapidly growing tumor cell is better able to effect recovery from injury than a connective-tissue cell with its comparatively slow metabolism. Therefore, the difference in response will favor the tumor if the cancercidal dose is not applied in the first treatment.

This is the first but not the last example to be found in our history where radiobiological ideas were used retrospectively as a rationale for clinical protocols decided arbitrarily.

The controversy between "concentrated" and fractionated treatments continued into the 1920s, each based on proposed radiobiological principles. The supporters of concentrated treatments argued that recovery from injury was more efficient in rapidly growing tumor cells so that one big dose was best, while advocates for fractionation evoked the chance of irradiating cells in a more radiosensitive phase if the dose was divided. The controversy was unresolved when Coutard (Fig. 5.2) began treating head and neck tumors with fractionated low dose-rate (LDR) beam therapy in 1919, attempting to mimic the radium technique of Regaud.

Biological support for the superiority of fractionated treatment came from the experiments of Regaud and Ferraux, published in 1927 (Fig. 5.3). It was found that rabbits could not be sterilized by exposing their testes to a single dose of radiation without extensive skin damage to the scrotum, whereas if the radiation was spread out in a series of daily fractions, sterilization was possible without producing unacceptable skin damage. 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 have been flawed, but the conclusion proved valid: fractionation of the radiation dose produces, in most cases, better results.
tumor control for a given level of normal tissue toxicity than a single large dose.

In the years that followed, the conversion to fractionated treatments became essentially complete. The success of Coutard's technique was recognized when he presented it at the second International Congress of Radiology in Stockholm in 1928, reporting better tumor control with less normal tissue damage than for single dose treatments for head and neck cancer.8

Meanwhile, treating cancer of the uterine cervix by means of radium tubes inserted into the uterus yielded success. In other sites radium needles placed interstitially were successful for some highly-localized cancers. These methods of treatment, called brachytherapy, made use of the good radiobiological properties of continuous LDR irradiation which have only been explained more recently.

 Isoeffect Curves and Overall Treatment Time

During the 1920s and 1930s a number of researchers tabulated what were termed "recovery factors" for fractionated protocols derived from daily treatments. Recovery factors were defined to be the ratio of biologically effective doses in a fractionated protocol (over a specific number of days) to the biologically effective dose given in a single sitting. Most notable were the efforts of Reisner; Duffy, Arnnesen, and Edward; and MacComb and Quimby.9,10,11 In retrospect, the most influential work was that of Strandqvist in 1944, who based the analysis of his data on a biological principle that we now know to be incorrect, namely "...radiation effect depends only on the total dose and overall time...it matters very little if the fractional doses are of variable size."12 He found that the total dose required to produce a given level of skin damage (either erythema or necrosis) was related to the overall treatment time by a simple power law:

\[ \text{Total Dose} = \text{Constant} \times T^{0.33} \]

As the X-ray era drew to a close, a most significant contribution was the publication of the nominal standard dose (NSD) concept of Ellis and his colleagues at Oxford in the late 1960s.13,14

This came about from a chance circumstance. Frank Ellis was appointed as external examiner for the doctoral thesis of Lionel Cohen at the University of the Witwatersrand in South Africa. This thesis contained extensive clinical data on acute skin reactions and the cure of squamous cell carcinoma of the skin (Fig. 5.4). The burden of the thesis was that the isoeffect curve for squamous cell carcinoma was different than for normal skin. The isoeffect curve followed a power law for both normal skin reactions and tumor cure, but the slope was less (0.22) for tumor than for skin (0.33).

Ellis reasoned that normal skin was under homeostatic control, whereas the tumor was not, and concluded that the difference in slope (0.11) could be taken as the time factor for normal tissue recovery. The formula therefore became:

\[ \text{Total Dose} = \text{NSD} \times N^{0.24} \times T^{0.11} \]

where \( N \) = number of fractions, and \( T \) = overall time in days. For all its problems, the NSD concept had a significant impact on clinical practice. In retrospect, the biggest contribution it made was to separate the effects of fraction size and overall time and to emphasize...
that fraction size or number was by far the most important variable. The NSD concept received considerable support from the animal experiments of Fowler and colleagues, who showed that for skin reactions in pigs, the number of fractions was of greater importance in determining isoeffect doses than time, at least up to twenty-eight days. In retrospect, we now know that this overall time was too short to show much repopulation in either pig or human skin.

The Oxygen Effect in Radiotherapy

The first radiation experiments in which oxygen was mentioned as a factor were performed by Wald Schwarz in 1910. He applied a radium applicator to the skin of his own forearm and observed that the subsequent reaction was reduced if the applicator was pressed hard against skin during the exposure. He concluded that metabolism was involved in the radioresistance of the skin cells but excluded oxygen as a factor because he found that if the blood flow to the arm was temporarily interrupted by the application of a tourniquet this had no effect on the radiation response. The oxygen effect could have been discovered in 1910, but it wasn’t. However, this work was the stimulus for subsequent experiments by Holthusen and Petry.

Holthusen, in 1921, studied the effect of radiation on ascarsis eggs and synchronized the division of cells by depriving them of oxygen (Fig. 5.5). He observed that cellular radiosensitivity was diminished under hypoxia, but attributed this resistance to the absence of cell division, not to the absence of oxygen. He did the right experiment but drew the wrong conclusion. Soon after, Eugene Petry compared the radiosensitivity of various grains by measuring the length of irradiated roots. Hypoxia resulted in radioresistance, from which Petry concluded that oxygen was directly or indirectly related to the damage produced by X rays. He was, therefore, the discoverer of the oxygen effect, but since both he and Holthusen published in German, their work appears to have gone unnoticed in the English-speaking world at the time.

Crabtree and Cramer in 1933 irradiated thin slices of animal tumors and
scored the percent of positive transplants and subsequent tumor growth rate. Their primary concern was to show that respiration played a key role in the radiation response, their working hypothesis being that radiation damage was mediated through the respiratory mechanism. They clearly showed that radioresistance could be conferred by hypoxia, and they were aware of the radioresistance of tumors in badly vascularized tissues and the implications of this in radiotherapy. Thus, the oxygen effect was rediscovered in Britain ten years after its discovery in Germany.

Meanwhile, in 1935 Mottram, using seedlings of *via faba*, demonstrated the modifying influence of oxygen and discussed its importance in radiotherapy. Eleven years earlier he had shown that the skin reaction produced by X rays on a rat's tail was reduced if blood circulation was stopped by a ligature applied at the base of the tail, but attributed the resistance to reduced blood flow, not to reduced oxygen. Again, the right experiment but wrong conclusion. Years later, in light of the report of Crabtree and Cramer, he reinterpreted his data in terms of oxygen. It was Mottram who introduced Gray and Read to the use of the bean root as an experimental system (Fig. 5.6). There followed the classic series of papers published in the *British Journal of Radiology* between 1942 and 1952 in which Read, Gray, and colleagues investigated X rays, neutrons, alpha particles, mixtures of high- and low-linear energy transfer (LET) radiations, and the effect of oxygen. They published the first numerical estimate of the oxygen enhancement ratio. In this way the stage was set for the subsequent era, in which the presumed limitation of radiotherapy was the radioresistance of hypoxic cells.

One lesson we can learn from the early experiments with the oxygen effect is the danger of preconceived ideas. The dogma of the day, epitomized in Bergonié and Tribondeau's so-called law, was that radiosensitivity depended on cell division. The discovery of the oxygen effect was delayed time and time again because investigators interpreted their data to support the fashionable ideas of the day and thereby missed a new discovery. How often do we do the same thing today?

**Neutrons and Heavy Ions**

The medical application of heavy particles was to blossom and reach its peak in the 1970s and 1980s at a time when physics research *per se* had lost some of its glamour; research directed toward more humanitarian goals became more fashionable. Nevertheless, it must be remembered that particles for radiation therapy are a spin-off from the incredible achievements of the halcyon era of nuclear physics. By 1919 Rutherford had demonstrated artificial transmutation of atomic nuclei. Alpha particles were used as projectiles to bombard nitrogen nuclei; the absorption of an alpha particle and ejection of a proton caused a nitrogen nucleus to become an oxygen nucleus. The alpha particles came from the only source then available, which were naturally occurring radioactive elements. As president of the Royal Society in 1927, Rutherford (Fig. 5.7) expressed a wish for a supply of "atoms and electrons which have an energy far transcending that of the alpha and beta particles from radioactive bodies."

The race was on. From the outset, two of the big rivals were the Cavendish Laboratory in Cambridge and the Lawrence Berkeley Laboratory in California. At Cambridge, Cockroft and
Walton used a voltage multiplier and won the first race when they demonstrated transmutation using an artificially accelerated particle. Their generator and most similar devices of the day relied on high potentials, which are difficult to contain. The cyclotron, invented by Ernest Lawrence in 1931, was based on the principle of using the same electrical potential over and over again—an idea in fact demonstrated by Widerøe in 1928. Lawrence's contribution was the circular form (Fig. 5.8).

The first successful cyclotron, built by Lawrence and his graduate student, could fit in the palm of a hand and accelerated a few hydrogen ions to 80,000 electron volts (Figs. 5.9 and 5.10). In the following year, 1932, James Chadwick demonstrated the existence of the neutron at the Cavendish Laboratory (Fig. 5.11).

In spite of the difficulties of the depression years, Lawrence succeeded in financing the design and construction of a series of cyclotrons of increasing size and energy. The 37-inch cyclotron was used as a source of neutrons to treat cancer patients from September 1938 to June 1939; figure 5.12 shows the first patient treated by John Lawrence and Robert Stone. The 60-inch cyclotron became available in December 1939, accelerating 16 megaelectron-volt (MeV) deuterons that were used to generate neutrons. Several hundred cancer patients were treated before the effort was terminated by the entry of the United States into World War II. The trial, not based on any particular radiobiological principle, was conducted at a time when little was known about the biological properties of neutrons and led to the conclusion that neutrons were not suitable for radiotherapy because of the disproportionately serious late effects produced in normal tissues.

Meanwhile, on the other side of the Atlantic, radiobiological experiments by Gray and his colleagues were setting the scene for a post-World War II resurgence of interest in the neutron as a particle for radiotherapy, based now on a definite radiobiological principle—the oxygen effect. Gray was a product of the Cavendish Laboratory at Cambridge, the arch rival of Berkeley in the early years. The graduate student class at
Fig. 5.10 Ernest Lawrence (right) and his second cyclotron. A series of cyclotrons of increasing size were built in the 1930s. They made possible the production of artificial radioactive isotopes, used in radiotherapy and nuclear medicine and now in many branches of medical research. (Courtesy of the Lawrence Berkeley Laboratory)

Fig. 5.11 Sir James Chadwick, who discovered the neutron in 1932.

Cambridge in 1934 included an astonishing number of individuals destined to play a major role in medical physics and radiation biology (Fig. 5.18). Of them all, no one was to have more impact than Gray. After developing his theory of the cavity ionization chamber (which has led to the unit of radiation dose being named the gray [Gy]), he became a hospital physicist with Mottram at Mount Vernon Hospital in 1932. From his vision and perseverance grew the Medical Research Council cyclotron at Hammersmith Hospital, the first cyclotron dedicated to medical use and the first to be used for a random-
ized clinical trial of fast neutrons. But that is another story reserved for the following era.

**THE SUPERVOLTAGE ERA: 1950–1970**

*In Vitro Survival Curves*

By the 1950s the lead in radiation research had crossed the Atlantic, largely as a consequence of the Manhattan District Project. The successful development of the atomic bomb, which abruptly ended World War II, led to the establishment of the Atomic Energy Commission (AEC), which made research funds available in the United States in previously unimagined quantities for the development of the peaceful uses of atomic energy ("Atoms for Peace") and the investigation of the biological effects of radiation (Figs. 5.14a and b).

A major breakthrough came from outside the field of radiation oncology when Puck and Marcus at the University of Colorado (T. T. Puck was professor and chairman of the Department of Genetics) developed techniques to grow single human cells in culture, leading to the first radiation survival curve. Many researchers previously had grown mammalian cells in mass culture, but the growth media of the day were not sufficiently sophisticated to allow cells to be grown at low density under conditions where the ability of individual cells to grow into a colony could be observed—the essential prerequisite for the assessment of cell survival following radiation (Fig. 5.15). Puck and his colleagues had the same problem as everyone else, and the (possibly apocryphal) story is told that the breakthrough came when they
were visited by Szilard, who said in his broken central European accent, “Don’t let them know they are alone.” This inspired suggestion led to the idea of feeder cells. The viable cells, whose integrity was to be tested, were mixed with large numbers of radiation sterilized “feeder cells,” which could attach and grow for a while, but could never form colonies. Feeder cells allowed mammalian cells to be used for survival experiments by the same techniques that had been used for decades with bacteria.

**Split Dose Experiments—Elkind Repair**

Cell culture techniques spread like wildfire on both sides of the Atlantic. The split dose experiments of Mortimer M. Elkind had a particularly strong influence on radiation therapy (Fig. 5.16). Elkind was working with a line of cells derived from a Chinese hamster (developed initially because they have half the number of chromosomes as human cells and are therefore easier for cytogenetic experiments). These cells grow rapidly in culture with a cycle of about ten hours and are characterized by having a very large shoulder on their radiation survival curve. It was possible to demonstrate that splitting a radiation dose into two fractions separated by times ranging from one-half hour to several hours led to an increase in survival. This was interpreted in terms of the repair of sub-lethal damage. The importance of this in vitro finding to radiotherapy was evident, since clinical protocols almost always consisted of multiple small doses separated by at least twenty-four hours—long enough for sublethal damage to be repaired. In truth, the experimental observation was not new, since the effect of splitting a dose into two fractions separated by various time intervals had been investigated by John Read before World War II. But Read had used seedlings of *vicia faba*, and his results were not applied to radiotherapy. Elkind’s experiments followed on the heels of Puck’s development of the survival curve for mammalian cells, and the importance in radiotherapy was recognized immediately.

**Radiosensitivity Through the Cell Cycle and Potential Lethal Damage**

Len Tolmach spent many years at Washington University in St. Louis. As a result of a falling out with his colleagues in radiation oncology, much of that time was spent in the anatomy department, but in the short space of those years he was involved in three key discoveries in radiation biology (Fig. 5.17). First, in collaboration with William Powers, head of radiotherapy in St. Louis at the time, he demonstrated the
existence of viable hypoxic cells in mouse tumors which dominated their radio-curability. This is further described later in this chapter.

Tolmach's second discovery was that the radiosensitivity of cells varied with the phase of the cell cycle. With Terasima, a postdoctoral fellow in his laboratory (who subsequently returned to Japan and held several senior positions including that of associate director of the unit at Chiba), Tolmach synchronized HeLa cells by shaking off those in mitosis and showed that their radiosensitivity varied as they moved through the cycle. A more complete study of the "age response function," as it is often known, "including full dose-response curves for the various phases of the cycle" was later published by Warren Sinclair (Fig. 5.18). Similar patterns of the variation of sensitivity through the cell cycle were later demonstrated in organized tissues synchronized by hydroxyurea first by Hall, Brown, and Cavanagh in a plant meristem and later by Withers and colleagues in the mouse intestinal epithelium.

Tolmach's third contribution was to coin the term "potentially lethal damage," defined to be that component of radiation damage that can be influenced by the postirradiation conditions. He showed that more cells survived a given dose of X rays if they were kept at a lowered temperature or in a depleted medium for several hours after irradiation. It was left to Jack Little and George Hahn to show that potentially lethal damage could be repaired if cells were kept for a few hours in plateau phase after irradiation and that there is a significantly enhanced cell survival in animal tumors if they are left in place for several hours postirradiation before being excised and assayed for clonogenicity. This later work is more frequently quoted because the postirradiation conditions have more direct relevance to radiotherapy, but the concept of potentially lethal damage came from Tolmach. It is of interest to note that Len Tolmach grew up in New York City only a block from Robert Kallman, who coined the term reoxygenation; between them they were responsible for two of the four Rs of radiotherapy.

**In Vivo Survival Curves**

The next important development came from workers at Westminster Hospital and Hal Gray's British Empire Cancer Campaign (BECC) radiobiology laboratory in London. The collaboration of a pathologist (Harold Hewitt) with a medical physicist (C. W. Wilson) led to the development of the dilution assay technique, making possible the determination of a quantitative cell survival curve for cells of a lymphocytic leukemia. This was the first in vivo survival curve. It is true that it related only to a "fluid" tumor, i.e., to leukemia cells in the peritoneal cavity of mice, but the idea was soon extended to solid tumors if they could be dissociated into a single cell suspension. Of more importance in the long run was the arrival in this same
laboratory at this time of a young radiotherapist from Australia, H. Rodney Withers, who was assigned the Ph.D. project of obtaining a dose response curve for cells of a normal tissue irradiated in vivo (Fig. 5.19). He chose to work with mouse skin and developed the ingenious skin colony assay in 1967. He described the mouse skin cells as having "an O.K. D_0 of 125 rads"—i.e., the sensitivity of cells irradiated in situ in mouse skin was not significantly different from the sensitivity of single cells in a petri dish in the in vitro assay. This important result was first publicly reported in the same room at 32 Welbeck St. at the British Institute of Radiology where in 1957 Spear had cast doubt on the usefulness and relevance of Puck's initial work.

It was in the same room, as well, that in 1959 Gray had pointed out that the in vitro survival curves of Puck for HeLa cells, with their D_0 of 960 rads became coincident with the in vivo survival curves of Hewitt and Wilson, with their D_0 of 140 rads, when the dose was corrected by a factor of 1.45 (because Puck irradiated cells attached to glass) as described by Morkin and Feldman.

In those heady days, people thought that all cell survival curves had the same D_0 and the same extrapolation number of two because of the two strands of DNA. This simplistic view did not last long. Incidentally, it was the same Arnold Feldman who changed places with this author in an Oxford-Chester exchange visit so that I could learn the then new technique of mammalian cell culture and continue my transition into radiobiology.

After completing his Ph.D. at the University of London, Rod Withers moved to the United States and developed a whole range of systems which allowed survival curves to be obtained in a variety of normal tissues, including mouse jejunum with Mort Elkind at the National Institutes of Health, testes when he moved to M. D. Anderson, and kidney at Los Angeles. Rodney Withers was the most creative experimentalist in the field. All these assay systems devised by Withers involved the scoring of regenerating clones in situ in normal tissues. In fact the very first system involving the regrowth of the clones from a single cell in a normal tissue was devised in Canada by McCulloch and Till. They took cells from nucleated bone marrow from donor mice, inoculated them into the tail veins of recipient mice, and scored the colonies that developed in the spleen. At a much later date, Clifton, Jirtle, and Gould developed similar cloning techniques in vivo for the hormonally controlled cells of normal thyroid and breast tissue, transplanting irradiated cells of these tissues from donor animals to grow as clones in the fat pads of recipient animals.

**Mouse Tumor Hypoxic Cells: Reoxygenation and High Pressure Oxygen**

In the early 1950s several groups tested the effect of breathing oxygen, at atmospheric or increased pressure, on the response of tumors to X irradiation. Gray's work attracted particular attention among radiotherapists, due to the suggestion that oxygen might sensitize a tumor to a greater extent than the surrounding normal tissues, i.e., improve the therapeutic ratio. Just fourteen months after the appearance of Gray's paper, 1. Churchill-Davidson treated his first patient in high pressure oxygen (HPO).
Gray and his colleagues had pointed out that HPO might sensitize normal tissues to some extent, and the work of E. A. Wright and P. Howard Flanders underlined this danger. Unfortunately, no account was taken of this possibility when randomized clinical trials were performed, with the exception of the small trial by H. A. S. van den Brenk, until the second trial of Henk in 1977. The work of H. B. Hewitt, initially carried out at Westminster Hospital, was of great importance in the development of experimental radiotherapy. Using tumors of spontaneous origin in his own strain of inbred mice, he developed an end-point titration method which provided survival curves for tumor cells in vivo (Fig. 5.20). His estimates of $D_0$ (oxic) were in general agreement with the in vitro studies of Puck. Hewitt’s technique also made it possible to estimate the state of oxygenation of tumor cells. Leukemic cells infiltrating the liver were found to be entirely “oxic,” i.e., gave a $D_0$ characteristic of well-oxygenated cells. The hypoxic base-line could be established by irradiating a dead mouse. The cells of a solid sarcoma were found to be predominantly hypoxic.

Powers and Tolmach in St. Louis, using Hewitt’s technique, were able to show that a particular tumor, the Gardner lymphosarcoma, was a mixture of oxic and hypoxic cells. They also showed that HPO reduced the hypoxic cell fraction but never abolished it. In fact, every experiment with murine tumors and HPO has indicated the difficulty of fully oxygenating tumors, particularly if they are large. The publication by Powers and Tolmach prompted many investigators to estimate the proportion of hypoxic cells in many different mouse and rat tumors, and it turned out that 10 to 30 percent was a common value.

The experimental work which led to the introduction of HPO in the clinic had all been carried out with single doses. The next vital step was taken in the late 1960s at Stanford University, where Robert Kallman collaborated with a number of Europeans on sabbatical, including van Putten from the Netherlands and Norman Bleehen from London (Fig. 5.21). The critical observation made at about that same time by Herman Suit at the M. D. Anderson Hospital and Hugh Thomlinson at the Hammersmith MRC Unit was that the proportion of hypoxic cells in a tumor, which rose to 100 percent immediately after a large dose of X rays, returned to its preirradiation level within a few hours of the radiation exposure. Kallman, and at the same time Thomlinson in London, coined the term reoxygenation for this phenomenon (Fig. 5.22). The discovery of reoxygenation by Kallman and van Putten suggested to some radiotherapists that, provided treatment was fractionated, HPO might have no place in radiotherapy. However, the extensive studies of Suit on murine tumors showed that
HPO is generally more effective when combined with fractionated radiation than with single doses (Fig. 5.23). The contrasts between this result with HPO and the effect of hypoxic sensitizers are striking; hypoxic sensitizers are less effective when given in association with fractionated radiation.

Neutrons and Charged Particles: The Effect of Radiation Quality

As the European countries recovered from the devastation of World War II, they planned their response to the atomic age and the need to understand the effects of ionizing radiations. In the Netherlands the effort was concentrated in a central government funded Radiobiological Research Institute (TNO) at Rijswijk. The group of workers here had a major influence on radiobiological research of relevance to radiotherapy during the 1960s and 1970s, quite disproportionate to the size of their homeland.

In 1956 Eddie Barendsen was a post-doctorate fellow in "physics applied to archeology" at Yale University, where he witnessed Ernest Pollard irradiating enzymes with cyclotron-accelerated alpha particles to study inactivation cross sections (Fig. 5.24). Upon his return to the Netherlands he combined this technology with the new Puck culture technique for growing cells of human origin. He first analyzed the relative biological effectiveness (RBE) and oxygen enhancement ratio (OER) of natural alpha particles and later used deuterons and helions from the Hammersmith cyclotron in London. These latter studies were started after he was introduced to Jack Fowler at a meeting in Oxford in 1961 by Hal Gray. Later studies involved neutrons of various energies in Rijswijk as well as in London. While many other investigators filled in the details later, these experiments by Barendsen showed the broad picture of the variation of biological effectiveness with radiation quality and the variation of the importance of the oxygen effect with radiation quality. This set the scene for the exploration of both neutrons and accelerated charged particles for radiotherapy.

Neutrons

This era saw the research efforts that resulted in the use of neutrons in place of X rays for the treatment of cancer. The first use of neutrons at Berkeley in the late 1950s and early 1960s had not been based on any particular radiobiological rationale. The post-World War II
neutron effort at the Hammersmith Hospital was based squarely on:

(1) The experimental observation that neutrons were less dependent than X rays on molecular oxygen to produce their cytotoxic effect, i.e., OER was smaller for neutrons than for X rays.

(2) The premise that the control of human tumors by X rays was limited by the presence of viable hypoxic cells.

The cyclotron at Hammersmith Hospital was the brainchild of Hal Gray, based on experiments he and his collaborators had performed over the years to elucidate the biological properties of neutrons using a deuterium on beryllium machine that he and John Read had built in the late 1930s for $500 (Fig. 5.25). Following a dispute with the medical director, Gray left Hammersmith to found the BECC Radiobiological Research Unit at Mount Vernon Hospital, which became the Gray Laboratory after his death. The preclinical neutron studies at Hammersmith were carried out by Tikvah Alper, Shirley Hornsey, Juliana Denckamp, and Stan Field, with Jack Fowler, head of medical physics, coordinating some of the effort (Fig. 5.26). Jack Fowler had been a hospital physicist, first at

Newcastle and then at King's College London (Fig. 5.27). He accepted the position of professor of medical physics at the Royal Postgraduate Medical School at Hammersmith, with responsibility for all aspects of a large program in medical physics. During this era, though, he is remembered most for his work on neutrons, using pig skin as a biological endpoint.

Neutron experiments were carried out with a wide range of biological systems, from cells in culture to mouse tumors to mouse normal tissue systems to pig skin. The basic principles of neutron radiobiology became clear. These included the variation of RBE with dose per fraction and the great variation of the RBE between different tissues. This work led to the first clinical trials of neutrons at Hammersmith, led by Catterall. These early trials were greatly hampered by the physical characteristics of this low energy cyclotron-produced beam, poor depth doses, a fixed horizontal beam, and primitive collimating devices. The interpretation of the results of the early trials became highly controversial, but at the time neutrons appeared to yield superior local tumor control and set the stage for a massive research effort involving neutrons in the United States.

The Perceived Problem of Hypoxia

This era also saw the genesis of other strategies to overcome the per-
ceived problem that the presence of hypoxic cells limited the curability of human tumors by X rays. A common sight in many radiation therapy departments in the 1960s was the hyperbaric oxygen tank. Patients were equilibrated with pure oxygen at a pressure of 2 or 3 atmospheres prior to their treatment with X rays. The first efforts were by Churchill-Davidson at St. Thomas Hospital in London and van den Brenk in Melbourne, Australia, but many efforts were to follow.\textsuperscript{54}

These procedures involved heroic efforts. Because of the technical difficulties, treatment protocols were frequently changed to only a few dose fractions. When this was done, HBO treatments were certainly superior to the controls breathing air. The art and science of clinical trials were at a primitive state at this point, and it was a long time before it was appreciated that a fair test of the importance of HBO must include a control arm involving a conventional number of fractions. Dedicated teams on both sides of the Atlantic and in Australia carried out a variety of HBO trials which showed a modest gain for HBO—but not enough to justify the trouble and expense involved in the treatments. Two retrospective observations are in order.

First, the gains produced by HBO, while modest, have seldom if ever been achieved by any other technical modification since HBO was abandoned. Second, it is a miracle in hindsight that no patient was incinerated during the treatment of thousands of individuals scaled in high pressure oxygen tanks.

One of the reasons for the demise in the popularity of HBO treatment was the promise that chemical sensitizers would soon be available that would achieve the desired end of sensitizing hypoxic cells without the expense, technical problems, and hazard of high pressure tanks. Led largely by Ged Adams, the 1960s saw the beginning of the effort to produce hypoxic cell radiosensitizers, chemicals that would specifically and differentially sensitize hypoxic cells to killing by X rays while having no effect on normal aerated cells (Fig. 5.28).\textsuperscript{55} Some of the principles involved in these oxygen-mimicking compounds were understood, but the compounds available in bacteria were much too toxic for use in animals, much less humans.\textsuperscript{56} It was, however, the birth of a field that was to assume great importance in the next era, because it led to the concept of bioreductive drugs and hypoxic cytotoxins.

Cell, Tissue, and Tumor Kinetics

The 1950s and 1960s saw the introduction of autoradiography, made possible by the availability of radionuclides as labels, a direct spin-off from the Manhattan District Project of World War II. This resulted in
some of the most clever radiobiological research ever performed. During this era cell population kinetics had a major impact on the thinking of radiotherapists, but any direct impact on clinical practice was delayed to a later time.

The availability of radiolabeled precursors of DNA synthesis led to the important work of Howard and Pelc in delineating the phases of the cell cycle (Fig. 5.29). With a light microscope, the only event that can be seen is mitosis itself, in preparation for which cells round up and the chromosomes condense. Using labeled thymidine and autoradiography, they showed that DNA was synthesized only during a discrete period of the cycle, which they called “S”. Mitoses and DNA synthesis were separated by the “first gap in activity” (G1), while there was a second “gap in activity” (G2) before the subsequent mitosis. These terms have been retained to the present day and are widely used throughout biology. Alma Howard was a Canadian who spent most of her life in Great Britain, an example of the reverse “brain drain.” Pelc was a refugee from Hitler’s Europe who also settled in London.

Labeled precursors of DNA also made possible the analysis of the length of the cell cycle and its constituent parts by means of the “percent labeled mitoses technique,” first used by Quastler and Sherman to analyze the mitotic cycle of cells in the mouse intestinal epithelium. Autoradiography was soon applied to transplanted tumors in experimental animals. Mendelsohn showed that only a proportion of cells were in cycle at any given time, which he called the “growth fraction.” The other important concept developed was that most cells produced by mitosis are lost from the tumor, the so-called “cell loss factor,” first estimated for animal tumors by Steel and colleagues. Steel is also credited with inventing the Tpot, the potential doubling time.

Following the successful application of the techniques of population kinetics to animal tumors, groups in France and in England performed similar studies on a limited number of cancer patients (Figs. 5.30 and 5.31). This gave new insight into the pattern of growth of human tumors; the discrepancy between the cell cycle of individual cells (a few days) and the gross volume doubling time (months) was accounted for by the fact that only a proportion of tumor cells were in cycle at any given time and, in particular, by the realization that the cell loss factor in human tumors averaged about 70 percent. Comparable studies were not performed in the United States because of the ethical and legal problems inherent in giving patients radiolabeled thymidine.

As long as cell cycle analysis depended on autoradiography, results could
never be used prospectively to modify the protocol of an individual patient because of the long time (six to eight weeks) required to obtain an image. Rapid techniques to assess cell cycle parameters had to await the development of flow cytometry and appropriate probes not based on radioactive labels. The first example, involving measurement of $T_{pot}$ from a single biopsy specimen following administration of bromodeoxyuridine, is described later.

**THE MEGAVOLTAGE ERA: 1970–PRESENT**

New Fractionation Concepts

The first mammalian cell survival curves of Puck and Marcus (1956) were obtained with HeLa cells, which are characterized by a very small initial shoulder and were fitted to a multi-target model having two components, $n$ and $D_0$, the extrapolation number and the 37-percent dose slope. In the context of modeling fractionation and dose rate effects, it soon became apparent that to account for experimental observations, a model involving a nonzero initial slope was essential. The area received a new momentum in 1976 when Douglas and Fowler adopted the linear-quadratic (LQ) model, originated in the late 1930s by Lea (Fig. 5.32) and Catcheside and derived on biophysical grounds by Kellerman and Rossi. The important point they made was that the ratio of the constants in the dose response relationship, i.e., $\alpha/\beta$, could be deduced from fractionation experiments in a tissue or organism where measurement of surviving fraction could not be determined. In fact, the principle of using isoeffect fractionation data to estimate the survival curve parameters in circumstances where cell survival cannot be measured per se had been described fifteen years earlier. However, that was in the context of multi-target survival curve models and experiments involving *via a two*.

In the years following the publication of Douglas and Fowler's work, the LQ isoeffect model grew in influence because it predicted that the fractionation sensitivity of a tissue may be classified according to the ratio $\alpha/\beta$. Thanes's work helped to spread the influence of this model (Fig. 5.33). However, the key step forward came from the recognition by Withers that early responding tissues tended to have large values of $\alpha/\beta$ (about 8 to 10 Gy), while late responding tissues were characterized by smaller $\alpha/\beta$ values (2 Gy). Barendsen made a similar suggestion in the same year. This translates into a more curvy dose response relationship for late responding tissues and a greater sensitivity to frac-
tionation. This observation explained the fact that fractionation spares late responding tissues more than early responding tissues, an observation which assumed prominence in a large number of studies of modified dose fractionation as well as neutron therapy in the late 1960s and early 1970s.

This observation of Rod Withers, from laboratory animal data, was confirmed first by data from patients obtained in Göteborg (in Strandqvist's former department) by Turesson and Notter. Other workers, especially Thames at Houston and more recently Soren Bentzen in Denmark, have developed methods of extracting alpha/beta and other radiobiological data from animal and clinical data, with widely influential results on new clinical protocols of fractionated radiotherapy.

The other important step forward in the understanding of the radiobiology of normal tissues came from the experimental work of a number of investigators, notably Juliana Denekamp at the Gray Laboratory and Bert Van de Kogel, who was a graduate student with Eddie Barendsen in the Netherlands and later worked at Los Alamos National Laboratory (Fig. 5.34). Working with mouse skin, Denekamp showed that, when irradiated with a fractionated schedule, skin did not begin to proliferate until about twelve days after the start of treatment—but that once it started, the extra dose required to counter proliferation increased rapidly as a sigmoid function of time reaching 130 cGy/day. By contrast, in the rat spinal cord, proliferation does not occur until much later, and, thereafter, the extra dose required to counter proliferation increases more slowly. In interpreting these data and extrapolating them to the case of a patient undergoing radiotherapy, Fowler made the following important points:

First, increasing overall treatment time spares early responding tissues, but has essentially no effect on late responding tissues, at least within the normal radiotherapeutic range. This contrast

Fig. 5.32 Douglas Lee was trained as a physicist at Cambridge but produced some of the earliest radiation survival curves for bacteria. In collaboration with Catcheside, he introduced the linear-quadratic formula to describe dose-response relationships for chromosomal aberrations.

(Courtesy of Mrs. Eileen Lea)

Fig. 5.33 Howard Thames is a brilliant mathematician, modeler, and biostatistician at the M.D. Anderson Hospital and collaborat-ed with Rod Withers and Lester Peters to develop models of the response of early and late responding tissues.

(Author's collection)

Fig. 5.34 Juliana Denekamp made many contributions to radiation biology in the areas of cell kinetics, tumor biology, hyperthermia, and tumor blood flow. She succeeded Professor Fowler as director of the Gray Laboratory. She is shown here sharing a lighter moment with Eric Hall at the International Conference on Hyperthermia in 1980. (Courtesy of the archives of the Center for Radiological Research, Columbia University, New York)
was a radiobiological explanation of the policy of using a large number of small fractions in six to eight weeks that has been used and advocated by the Houston group, first under Gilbert Fletcher, with a wide following in the United States. It is contrary to the United Kingdom and ex-British-Empire policy of using fewer and larger fractions but in shorter overall times of three to five weeks. It is only in the last decade that the advantages and disadvantages of both approaches, due to the application of flow cytometry to measure proliferation rates ($T_{\text{p}}$) in tumors, are being clarified.

Second, the biology implies that in resting normal tissues, compensating proliferation does not occur until two to four weeks in human mucosa or skin but then increases rapidly; this is in contradiction to the constant power law time factor in the NSD equation. In the 1980s it was recognized that there was an onset of accelerated regrowth after a lag period in tumors receiving XRT analogous to that in normal tissues, a phenomenon reported twenty years earlier in animal tumors.$^{29,78}$

These new biological concepts, namely the different sensitivity to fractionation of early and late responding tissues, the pattern of proliferative response of steady state normal tissues, and the accelerated regrowth of tumors, provided a convincing rationale for the clinical trials of hyperfractionation and accelerated treatment, which are at the cutting edge of clinical investigations at the present time.

The linear quadratic model has, to a large extent, replaced the NSD concept for the calculation of doses used in different fractionation schemes to arrive at the same biological effect. The basic idea for these calculations came from Thames, but its use was popularized largely by Jack Fowler.$^{74,75}$

Central to the development of a biological rationale for altered fractionation patterns was the group of radiobiologists that flourished around Gilbert Fletcher (Fig. 5.35) at the M. D. Anderson Hospital in Houston. Fletcher himself was a giant in the field of radiation oncology and a master of clinical research, but he also fostered a most important group of laboratory researchers and modelers. First was Herman Suit in the 1960s and 1970s, who was followed as head of experimental radiotherapy by H. Rodney Withers and later by Lester Peters and Howard Thames. The importance of their contributions cannot be overestimated.

**Particle Therapy**

The years following 1970 saw the interesting development of a major initiative in particle therapy in the United States. The earliest neutron experience of the Medical Research Council at Hammersmith Hospital had produced encouraging results despite the physical limitations of the low energy cyclotron that was available. This stimulated great interest in neutron therapy in the United States, Europe, and Japan.$^{76}$ In the United States the first efforts involved three high-energy cyclotrons that had initially been built for physics research but were by then largely obsolete for that purpose and could be adapted for medical use. They were located at College Station, Texas; the Naval Research Laboratory in Washington, D.C.; and the University of Washington in Seattle. A substantial effort was mounted in pre-clinical studies of physics and biology, but the subsequent clinical results never produced results quite as dramatic as those...
claimed by Hammersmith Hospital. It was recognized from the outset that using treatment machines in physics research installations was suboptimal, to say the least. The 1970s saw the most coordinated effort ever mounted by the radiation oncology community to obtain substantial support for a specific initiative. Many millions of dollars were allocated by the National Cancer Institute to build three dedicated hospital-based cyclotrons to produce neutrons, as well as supporting trials of negative pi mesons at Los Alamos National Laboratory and heavy ions at the Lawrence Berkeley Laboratory.

Three hospital-based neutron facilities were built at the University of Washington in Seattle (Tom Griffin), the University of California at Los Angeles (Robert Parker), and the M.D. Anderson Hospital in Houston (Lester Peters). These facilities joined the Fermilab (Lionel Cohen), a medical neutron facility that operates parasitically from the huge physics research accelerator at Batavia. Again there was a considerable preclinical effort in physics and biology to ensure uniformity of dosimetry and RBE values prior to the mounting of a number of clinical trials involving many disease sites. Machine reliability and patient accrual proved to be a problem everywhere but Seattle, and after fifteen years, most clinical trials showed no advantage for neutrons except possibly for carcinoma of the prostate, salivary gland tumors, and soft-tissue sarcomas. 77

Meanwhile, Morton Kligerman had obtained funds for a medical pion facility to be appended to the mile-long high-energy proton accelerator at Los Alamos National Laboratory, with the aim of treating cancer patients (Fig. 5.36). 78 The physics and biology research efforts were impressive, though as more data were accumulated, the advantage of pions over X rays looked less obvious. The problems of coordinating clinical trials high on a mesa in New Mexico proved to be insurmountable, and as research funds became more difficult to obtain, the project came to an end without definitive clinical conclusions.

The history of heavy ions is more complex and took longer to unfold. In the race to be the first to accelerate high-energy heavy ions, the Princeton particle accelerator was several months ahead of the Lawrence Berkeley Laboratory in producing a beam of nitrogen ions. The first radiobiological data were published by the Columbia group in 1973. The Princeton particle accelerator was closed down soon thereafter by the Nixon administration, leaving the BEVALAC at Berkeley as the only heavy ion machine in the world (Fig. 5.37). Led by Tobias, the Berkeley group, with help from colleagues across the United States, made a detailed investigation of the biological

Fig. 5.36 The Clinton P. Anderson Los Alamos Meson Physics Facility. This machine was over half a mile in length and accelerated an intense beam of protons to 800 MeV, which were then used to produce negative pi mesons for high-energy physics research and biomedical applications. It was built by the United States Atomic Energy Commission (now the Department of Energy) at a cost of $57 million. Its enormous size and spectacular location high on a mesa in New Mexico are both evident from this aerial photograph. The first pion treatment for a cancer patient was given in October 1974 by Dr. Morton Kligerman. The facility was closed some ten years later. (Courtesy of Los Alamos Scientific Laboratory, New Mexico)
of radiation therapy, beams of heavy charged particles combine the advantages of improved dose distribution, as obtained with protons, with the high-LET advantages of neutrons. It came as something of a surprise (and disappointment) that it was necessary to push to atomic numbers characteristic of argon and silicon before high-LET properties of the beam, spread out to cover a tumor of realistic dimensions, equaled those of neutrons.

Many patients were treated with a variety of charged particles. The sophisticated treatment planning techniques which were developed for the particle therapy program have proved to be of great benefit in external beam radiotherapy with X rays. There have been anecdotal reports of favorable
results in patient treatments with heavy ions, but no definite prospective controlled clinical trials were completed before the BEVALAC closed in 1993. It is ironic that there is no heavy ion machine for medical use in the United States at a time when new facilities have been built and are in use in France, Switzerland, Germany, Japan, and Russia.

It was the insights obtained from the difference in the LQ parameter alpha/beta between early and late-responding tissues that eventually clarified high-LET radiotherapy. At high LET it is the alpha component, the initial slope, that is increased with no change in beta. Therefore, most of the sparing effect, which is proportional to alpha/beta, was lost at high LET. The late-responding tissues suffered more damage relative to tumor cell kill, because they had more repair capacity to lose. High-LET treatments are no longer so popular, although the good physical dose distributions of heavy ion beams are a real advantage. These physical advantages can be obtained with proton beams, which are easier and cheaper to generate than the heavier ions of helium, carbon, or argon or than pi meson.

In the long run the least “exotic” heavy particles, protons, have had a more lasting impact on radiation therapy than neutrons, pions, and heavy ions combined. Facilities are in use in the United States, Sweden, and Russia; more than ten thousand cancer patients have been treated, the vast majority of them at the Harvard cyclotron in a team effort led by Herman Suit.81

**Hypoxic Cell Radiosensitizers and Bioelective Drugs**

The era opened with the knowledge that the potential of a compound as a hypoxic cell radiosensitizer, i.e., to mimic oxygen, depended on its electron affinity.82 Misonidazole was soon identified at the Gray Laboratory as a potent radiosensitizer of hypoxic cells in vivo and in experimental animal tumors and was offered for clinical trials. This compound proved to be much less exciting in the clinic than in the laboratory because the dose that could be administered was limited to suboptimal levels due to the development of neurotoxicity. However, a recent retrospective meta analysis of the twenty or so clinical trials involving misonidazole shows a small but clear improvement in local control when the drug is combined with radiation.83

There followed a clever piece of research which led to the synthesis of etanidazole by Stanford Research International (SRI). Bill Lee of SRI and Martin Brown of Stanford University were making and testing analogs of misonidazole when Brown took off for a year's sabbatical in Cambridge, England (Fig. 5.38). By chance, he met Paul Workman there and began doing pharmacokinetics on some of the compounds. As a consequence, they measured the pharmacokinetics of a whole series of compounds with slightly different structures that all had the same radiosensitizing potential in vitro.84 Etanidazole was chosen, which is a hydrophilic compound, does not cross the brain barrier and penetrates poorly into nerve tissue. The limiting normal tissue toxicity is still neurotoxicity but occurs at much higher drug doses than for misonidazole. At the time of writing, the clinical evaluation of this compound as a chemosensitizer and radiosensitizer is still in progress.

However, the direction of developments in this field changed as a consequence of laboratory studies. In the

Fig. 5.38 Dr. J. Martin Brown trained at Oxford and eventually became director of research in radiation oncology at Stanford University, California. He was responsible for major advances in the design and synthesis of hypoxic cell radiosensitizers. (Courtesy of Dr. J. W. Osborne and the archives of the Radiation Research Society)
early 1970s Sutherland showed that metronidazole caused damage in the central regions of spheroids, while Hall and Roizin-Towle showed misonidazole to be preferentially cytotoxic to hypoxic cells. Over the past twenty years, it has been slowly realized that it may be much more efficient to kill hypoxic cells in a tumor with a hypoxic cytotoxin than to attempt to radiosensitize them with a hypoxic cell radiosensitizer. Perhaps this should have been obvious from the start, but it was slow to be appreciated. The upshot is that effective new compounds that operate primarily as hypoxic cell cytotoxins have been synthesized by two groups on opposite sides of the Atlantic, led by God Adams and colleagues at the U. K. Medical Research Council and by Martin Brown and colleagues at Stanford. This is a clear change of direction and an exciting development that gives new life to the field. These drugs must still be combined with X rays, however. The radiation kills the aerated cells, and the drug kills the hypoxic cells; hence, the strength of each modality is exploited.

**Predictive Assays**

Predictive assays have come of age during the modern era. They come in three types: assays for intrinsic radiosensitivity, assays for hypoxia, and assays for proliferative potential.

Assays for inherent radiosensitivity owe much to the work of Malaize and colleagues in Paris and to Gordon Steel and his colleagues in London, who divided tumors into several histological categories and showed that the steepness of the initial slope of the cell survival curve correlates with clinical responsiveness. The first serious attempt to correlate clinical outcome in individual patients with the survival of cells derived from their tumors and grown in a cell adhesive matrix assay was by William Brock, Lester Peters, and colleagues at the M. D. Anderson Hospital in Texas. They focused attention on the SF2, the surviving fraction at 2 Gy, as a predictor of outcome in head and neck cancer patients receiving postoperative radiotherapy. Although a trend was observed toward higher recurrence rates in patients with more radiosensitive tumor cells in vitro, this was not statistically significant, possibly reflecting the variability of tumor burden in patients treated postoperatively. This group has also studied the relationship between normal tissue radiosensitivity and tolerance to radiotherapy and has recently demonstrated a significant correlation between late effects in normal tissues and the radiosensitivity of the patient's fibroblasts assayed in vitro. The first positive correlation of in vitro radiosensitivity and tumor control was published by West and her colleagues at Manchester. In patients with carcinoma of the cervix, the SF2 was measured by growing cells in soft agar; the probability of local control was poorer in patients for whom the SF2 was above 0.55.

The need to identify patients whose tumors contain a substantial proportion of hypoxic cells and might, therefore, stand to benefit from hypoxic cell radiosensitizers or neutrons was acknowledged from the outset. A great deal of work was performed in the 1960s to measure oxygen concentration in tumors and to correlate the results with the outcome of radiotherapy for carcinoma of the cervix. A major step forward in recent years was the development of the Eppendorf probe, which allows a rapid profile of pO2 levels in a tumor to be measured. Hockel and his colleagues in Germany successfully used this improved technology in patients with locally advanced carcinoma of the cervix and found that patients whose tumor exhibited median pO2 values less than 10 millimeters Hg had a significantly lower recurrence-free survival compared to patients with tumors that showed higher pO2 values.

At about the same time, Chapman (Fig. 5.39) and his colleagues in Philadelphia successfully linked a radionuclide, iodine-123, to a nitroimidazole, which allows hypoxic regions to be identified by a SPECT scan, since the nitroimidazole breaks down and deposits the radioactive label only in regions of...
cell kinetic studies on individual patients were used successfully to select a subgroup of patients who could benefit from a new treatment protocol.

The Gray Laboratory

In terms of research that was to have an impact on clinical radiotherapy, it is impossible to exaggerate the importance of the Gray Laboratory. Founded by Gray as the BECC Radiobiological Research Unit in 1954, it was renamed in his honor at his death. The next director was Oliver Scott, whose ideas influenced so much of the early work on the oxygen effect and whose family had played an important role in founding the lab (Fig. 5.40). When Oliver retired in 1968 due to ill health, Jack Fowler was appointed as his successor. This was a highly imaginative appointment at the time, and he was given a mandate to concentrate the considerable resources of the lab in research in “experimental radiotherapy”—the study of the effects of fractionated radiation on tumors and normal tissues in the mouse. The Gray Laboratory is so important, not only because of key contributions from its well-known staff that included Ged Adams, Jack Boag, Juliana Denckamp (the present director), Harold Hewitt, Barry Michaels, and Adrian Begg, but also from the impressive list of students and visitors who have spent time there and have been inspired to devote their research efforts to radiotherapy; these include Rod Withers, Lester Peters, Liz

low pO₂. This represents a noninvasive test that can be performed prospectively on individual patients and is an exciting development. Similar labeled compounds have been developed by Koh and colleagues in Seattle.

The cell kinetic studies conducted in the past, based on classical autoradiography techniques, were never suitable as predictive assays in individual patients because of the long time necessary to get a result. The breakthrough came with the development of flow cytometers and the use of nonradioactive dyes as markers. Adrian Begg developed a method to estimate the $T_{pot}$ of a tumor within one day by flow cytometry measurements of cells from a single biopsy taken some hours after an injection of bromodeoxyuridine. This work started while he was at the Gray Laboratory in London but reached fruition after he had moved to the Netherlands.

$T_{pot}$ was measured in a group of patients with head and neck cancer in a study by the European Organization for Research and Treatment of Cancer, comparing an accelerated protocol with conventional therapy. If all patients were considered together, there was no significant difference between the conventional and accelerated arms of the study. However, in a small group of patients with fast growing tumors, in which $T_{pot}$ was four days or less, the accelerated protocol resulted in a much improved level of local control. This is the first report in which

Fig. 5.40 Sir Oliver Scott was a close friend and collaborator of Hal Gray and became director of the laboratory when Gray died. (Courtesy of Dr. J. W. Osborne and the archives of the Radiation Research Society)
Travis, Janet Rasey, and Jim Fisher. Many of these individuals returned (or emigrated) to the United States after their stay at the Gray Laboratory, bringing their ideas and enthusiasm with them. It is significant and regrettable that there is no comparably large group in the United States, with stable long-term funding, devoted to experimental radiotherapy. The closest equivalent would be the group at the M. D. Anderson Hospital that grew up around Fletcher.

The Dose-Rate Effect and Brachytherapy

The discovery of sublethal radiation damage repair in mammalian cells by Elkind and his group in the late 1950s led to many attempts to model dose response relationships for LDR and also to experiments to determine cell survival curves for protracted LDR irradiation.98,99,100 These efforts were aimed at providing an experimental basis for brachytherapy. LDRs permit most of the repair that can take place to do so. Because tumor cells have, usually, less repair capability than late-responding tissues, LDR therefore protects the tissues in which late complications occur more than it protects tumors. LDR therefore good news, from a therapeutic ratio point of view, and we depart from that only with caution.

During the 1960s Paterson and Ellis independently published isoeffect curves for interstitial implants, relating the total dose as a function of implant time that would result in the same level of normal tissue tolerance.101 In retrospect, these isoeffect curves equate well to radiobiological models based on the linear-quadratic formalism, using values for alpha/beta appropriate for late-responding tissues. There was a long standing controversy throughout the 1970s and 1980s between those who followed the advice of Paterson and Ellis to correct total dose for overall time and the followers of Pierquin and the Paris School, who maintained that no dose correction was necessary for implant times between three and nine days.102,103 This implied the absence of a dose-rate effect between 30 and 100 cGy/hour, which was in direct conflict with radiobiological measurements. This controversy was not resolved until the 1990s when a retrospective analysis of the impressive body of data from the Paris group showed that for tumors of the mobile tongue and floor of mouth, both tumor control and the incidence of necrosis depended on dose-rate.104 For breast cancer, too, local control varied with dose-rate.105 In both cases, the variation was as predicted from radiobiological data.106

The 1980s, too, saw the proliferation of high-dose-rate (HDR) afterloaders, supplanting in many institutions the LDR intracavitary techniques that had been used to treat carcinoma of the cervix for three quarters of a century. HDR afterloaders have the advantage of improved radiation protection and allow outpatient treatments, but they also caused great controversy. The outcome of clinical use indicates HDR results that are comparable to LDR, both in terms of local control and the incidence of complications.107 Radiobiological modeling has been used extensively to suggest HDR protocols in a limited number of fractions (typically five to twelve) that are comparable to LDR.108

A further development made possible by the availability of computer-controlled remote afterloaders is pulsed brachytherapy.109 In this technique, a single radioactive source steps through the catheters of an interstitial implant or through the catheters of an intracavitary applicator, with the dwell time in each source position adjusted to optimize the required dose distribution. This technique buys a number of advantages including only one source to replace, no source preparation, improved dose optimization, better radiation protection, and a constant average dose-rate.

Hyperthermia

The supervoltage era saw the blossoming of hyperthermia as a vigorous and well-funded research area. In various forms the use of hyperthermia as a modality to treat cancer predates the discovery of X rays, but the modern development of hyperthermia based on laboratory research dates from the work of Dewey, Westra, and Eugene Robinson.
mia produced dramatic "cures" in transplantable mouse tumors, perhaps because the encapsulated nature of the tumors and the poor blood supply exaggerated the temperature differential between the tumors and the surrounding normal tissues. Exhaustive studies of normal tissue effects in rodents led to studies in larger animals, including dogs. The stage was set for the trial of hyperthermia in the treatment of human cancer. By the 1990s more than twenty thousand cancer patients had received hyperthermia and several lessons had been learned. First, hyperthermia alone has a very limited place as an anticancer modality, except for palliation. Second, as an adjunct to X rays, hyperthermia was shown to increase the response rate of many types of human cancer and thousands of patients have been treated, but final proof in the form of prospective randomized controlled clinical trials has been slow in coming. The only phase III trial showing the efficacy of hyperthermia came from the European cooperative group who showed that radiation plus heat doubled the complete response rate and the five-year local control rate of malignant melanoma, compared with radiation alone.

A major and persistent problem was the difficulty of maintaining a uniform high temperature throughout the tumor volume by means of an external heating device, whether ultrasound, microwaves, or a capacitative heating system. As early as the second International Conference on Hyperthermia, it was recognized that the best way to handle thermotolerance was to avoid it.

Modest treatments with hyperther-

(Figs. 5.41 and 5.42), who showed that cell survival curves for heat were similar to X rays, with "time at the elevated temperature" replacing absorbed dose. Research into the biological effects of hyperthermia mushroomed, and, as often becomes evident, the biological properties of hyperthermia made it highly suitable as an anticancer modality. It was discovered that the sensitivity through the cell cycle complemented that for X rays, with radioreistant S-phase cells sensitive to heat.

Hypoxic cells were not resistant to cell killing, as they are to X-ray cytotoxicity, while cells deprived of nutrients or at low pH were found to be highly sensitive. All of these properties favored the killing of cells by heat in large necrotic tumors. There were disadvantages, to be sure, including the phenomenon of thermotolerance, whereby cells became resistant to subsequent heating by a prior heat exposure (Fig. 5.43).

In most cases the synthesis of heat shock proteins (also called stress proteins) correlates with the development of thermotolerance. This finding provided the first evidence for the function of these primordial proteins and paved the way for molecular biology in hyperthermia. Thermotolerance makes multiple heat fractions impractical, and it was argued that the best way to handle thermotolerance was to avoid it.
on Hyperthermia Oncology in Fort Collins in 1980, it was pointed out that, while the biological properties of heat were very favorable for its use as a modality for the treatment of cancer, the physical principles involved in heating by any of the modalities then in use were not amenable to manipulation to produce controlled uniform volumes of elevated temperature. The slogan, “The biology is with us, but the physics is against us,” was controversial and hotly debated in 1980, but the clinical experiences of the next decade proved this prophecy to be more accurate than anyone could have known at the time!

The most promising area of hyperthermia research in the 1990s is the combination of an interstitial implant and hyperthermia also induced by implanted electrodes. It should have come as no surprise that the combination of the best radiation dose distribution with the most uniform heat distribution would result in superior clinical results.

Molecular Techniques in Radiation Biology

Developments and advances in radiobiological research inevitably mirror advances in the broader fields of biology and general science. The past decade or so has witnessed the introduction of the techniques of molecular biology into the field of radiation research. At the time of writing, molecular biology has done little more than influence the outlook and thinking of the radiation biology researcher; there are few examples of impact and none of real importance. Nevertheless, advances in recombinant technology, and particularly in the molecular genetics of cancer, must revolutionize radiation biology as they have changed every other field of biology. For this reason a brief history is included.

Four families of genes have been identified in mammalian cells: repair genes, check point genes, oncogenes, and suppressor genes.

Repair genes involved in the repair of damage by ultraviolet light and by mitomycin-C have been cloned and sequenced. One repair gene in the mammalian cell involved in ionizing radiation repair has also been identified and sequenced, but it may not be a typical example; the radiosensitive mutant for which it corrects was only slightly radiosensitive. By contrast, a number of radiosensitive mutants have been identified in yeast and the genes involved cloned and sequenced. At first it was thought that the genes in yeast were involved solely in the repair of DNA damage by radiation. In fact it turns out that they are also molecular check point genes. Molecular check point genes in general serve the function of ensuring that the initiation of late events are dependent upon the completion of early events. In cells exposed to radiation, the function of the check point gene is to hold the cells in G2 in order to check for the integrity of their chromosomes before the complex task of mitosis is begun. Cells that are deficient in this gene do not stop in G2 after radiation but proceed immediately through mitosis, and many more die as a consequence.

In making the jump from laboratory research to the genetics of human disease, the study of individuals that are heterozygous for ataxia telangiectasia (AT) is a very important consideration. AT heterozygotes comprise only about 1 to 5 percent of the Caucasian population in the United States, but they have a cancer risk two to three times higher than the general population and may account for 9 to 18 percent of breast cancer in young patients. There is also some evidence that AT heterozy-
gotes have a much higher susceptibility to radiation-induced breast cancer than the normal population.

The discovery of the importance of oncogenes in human cancer made it possible to understand why agents as diverse as a retrovirus, ionizing radiation, or chemicals could result in tumors that were indistinguishable one from another. The retrovirus inserts a gene into the cell, while radiation or chemicals produce a mutation in a gene that is already present in the cell.

Today well over a hundred oncogenes have been identified in human cancer with more than 80 percent of the transforming genes belonging to the ras family. However, activated oncogenes are associated with only 10 to 15 percent of human cancers and tend to be associated with leukemias and lymphomas and less with solid tumors. Oncogenes have been shown to be activated by: (1) a point mutation (for example, ras), (2) a deletion (fos), (3) a reciprocal translocation (myc), or (4) gene amplification (myc). Ionizing radiations are not particularly efficient at producing point mutations, but they do cause interstitial deletions and reciprocal translocations with high efficiency. Consequently, in assessing the possible mechanisms by which radiation induces cancer, deletions or translocations would seem to be the most likely candidates.

A number of studies have been performed to investigate the influence of oncogenes on radiosensitivity. In the first such publication, Sklar used NIH3T3 cells, which are immortal, and showed that transfection of ras resulted in resistance to radiation, at least at high doses. In a more comprehensive study, McKenna and colleagues used primary rat embryo cells. It was found under these circumstances that transfection of myc or ras alone had only a modest effect on the radiosensitivity. On the other hand, if myc and ras were transfected together the cells showed a marked resistance to radiation, particularly in the low dose region at doses of approximately 2 Gy, i.e., at doses comparable to the daily dose used in radiation therapy. This paralleled the earlier work that showed that cooperation between two oncogenes was necessary to produce oncogenic transformation. Although many reports have appeared in the literature showing that transfecting oncogenes into cells in vitro can induce resistance, the data are equivocal. There is no real evidence that radioreistance in human tumors is associated with oncogene activation.

Suppressor genes are recessive acting in that both copies must be lost or inactivated for the cell to express the malignant phenotype. Suppressor genes were in fact discovered before oncogenes, at least with cells in culture. Stanbridge and colleagues in the 1970s showed that, if a hybrid was made by fusing a normal human fibroblast to a malignant HeLa cell, the normal cell suppressed the expression of malignancy by the HeLa cell. It was shown further that if, during the repeated subculture of the hybrid cells, chromosome 11 was lost, then the malignant phenotype was restored. From this result, it was inferred that chromosome 11 in the normal human fibroblast contained a gene capable of suppressing the malignant phenotype.

The importance of suppressor genes became evident from the work of Knudsen with retinoblastoma. Retinoblastoma appears in a familial form with high incidence and in a sporadic form at very low incidence. In the familial form, one mutant allele with lost function is inherited from the affected parent. A somatic event during embryogenesis then inactivates the normal allele inherited from the unaffected parent. The probability of this occurring is high and almost all such children are born with bilateral retinoblastoma. On the other hand, in sporadic retinoblastoma two somatic mutational events are necessary, the second in a descendant of a cell that suffered the first. This is much less likely to happen, which accounts for the much lower incidence of the sporadic form of retinoblastoma. Knudsen elaborated this two-hit hypothesis in the early 1970s. By the mid-1980s the location of the gene was identified on chro-
mosome 13 and eventually the Rb gene was cloned and sequenced.\textsuperscript{133,134} While the Rb gene is associated in 100 percent of cases with retinoblastoma, it is also associated with various other tumors, such as sarcomas, small cell cancer, bladder cancer, and mammary cancer in a relatively smaller proportions of cases.

An obvious mechanism for a suppressor gene to be deleted is by the action of radiation. Since a suppressor gene acts in a recessive way, the deletion would have to occur in both chromosomes of a pair, which of course would be a very low frequency event. In practice, in many cases, it is found that rather than two separate deletions the loss of the pair of suppressor genes occurs by the process of somatic homozygosity.\textsuperscript{135} This has been shown to be the mechanism in cases of retinoblastoma, small cell lung cancer, and glioblastoma. The latter is particularly interesting in as much as somatic homozygosity occurs in two different chromosomes for this high grade tumor to be produced. What happens is that one chromosome of a pair is lost, and the remaining chromosome with the deletion is replicated. At the present time there are at least six suppressor genes whose location and function are known. The two most common and most intensively studied are the Rb gene and the p53 gene. Both of these are involved with the arrest of cells in $G_1$ and in tumor differentiation. It should be noted at this point that double strand breaks in chromosomes have been consistently identified as a type of lesion responsible for most radiation induced cellular responses—including cell lethality and carcinogenesis.

A report in \textit{Science} by the Johns Hopkins group showed that all cases of hereditary nonpolyposis colorectal cancer (HNPPC) showed an alteration on chromosome 2, which may be described as a mutator gene, leading to genomic instability.\textsuperscript{156} In a high proportion of the HNPPC tumors, as well as sporadic colon cancer, there was a high incidence of mutations in \textit{K-ras}, p53, and \textit{APC} genes, though in no instance was the mutation rate 100 percent. The notion that the basic causative event is an alteration in a mutator gene leading to genomic instability may explain what was seen in the past with colon cancer—that there are a whole sequence of changes between the normal epithelium and a metastasizing malignant tumor, including mutations in oncogenes, loss of suppressor genes, and other unidentified chromosomal alterations.\textsuperscript{157} The interpretation based on the new data is that genomic instability leads to a cascade of events.

These recent findings may have a dramatic impact on the diagnosis and treatment of cancer because they imply that in many forms of cancer, the disease occurs with significantly high probability in predisposed individuals and that in the near future, these individuals can be identified at birth. This is the challenge that all cancer therapists must face as the next era opens.

**REFERENCES**

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Two classic clinical efforts to use high-pressure oxygen in X-ray treatments are: Churchill-Davision, Sanger, and Thomson, "High Pressure Oxygen and Radiotherapy," and van den Brink, "Hyperbaric Oxygen in Radiotherapy.


