RADIATION RESEARCH 129, 235–249 (1992)

FAILLA MEMORIAL LECTURE

From Beans to Genes—Back to the Future[1]

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HALL, E. J. From Beans to Genes—Back to the Future. *Radiat. Res.* 129, 235–249 (1992).

Developments in radiation biology have inevitably paralleled the evolution of systems and end points in the wider field of biology. I review the development of three areas that have interested me during the 35 years that I have been involved with radiation research. (1) The dose-rate effect. My first graduate student, Joel Bedford, and I demonstrated the dependence of mammalian cell killing on the dose rate at which γ rays are delivered. These studies led to the recent development of pulsed low-dose-rate brachytherapy and the design of a new machine for clinical use. (2) Hypoxic radiosensitizers and bioreductive drugs. The hypothesis that the presence of foci of hypoxic cells in human tumors could limit their curability by γ rays led to the development of nitromidazales that preferentially sensitize hypoxic cells. Our contributions included the observation that misonidazole was also cytotoxic to hypoxic cells, that sensitization was increased by prolonged preincubation, and that the mechanism involved depletion of thiols. More recently we have collaborated with Ged Adams and Bob Sutherland in the development of a new generation of hypoxic cell cytotoxins. In particular, we have been concerned with the assessment of oncogenicity in relation to drug structure. (3) Oncogenic transformation *in vitro*. The use of *in vitro* assays for oncogenic transformation has represented a major interest for this laboratory largely due to collaborations first with Carmia Borek, and more recently with Richard Miller and Tom Hei. We published the first dose–response curves for γ rays, and more recently for a range of neutron energies and for charged particles of defined LET. Radiation-induced oncogenic transformation in C3H 10T1/2 cells has been shown to be due to a dominant acting transforming gene, which has been isolated and is in the process of being characterized and sequenced by Greg Freyer.

INTRODUCTION

I am very pleased to receive the Failla award. It is an honor I fully and deeply appreciate. I am proud to see my name added to such an impressive list of past recipients. I regard the Radiation Research Society as my "home" society. I've had more to do with this society than any other—held most offices, sat on most committees, including the geriatric committee of past presidents. So I am especially pleased and grateful to those who thought me worthy. The Failla award is special to me, too, because every working day I sit in what was once Failla's office, and even use his old battered desk. I wouldn't claim to follow his footsteps—he walked with too majestic a stride for that—but the laboratory that he founded is still inevitably influenced by his work.

The early lecturers all knew Failla personally; this is a tradition that is increasingly difficult to maintain as the years go by, and so a whole generation has grown up in the field who never met Failla. Consequently, I thought it appropriate to say a few words about this man who was largely responsible for founding both the Society and the Journal (Fig. 1a).

Gioacchino Failla was born in Sicily, in a small town a few miles from Palermo. He came to New York at age 15, following his widowed mother who had emigrated a few years earlier to establish a home—part of the flood of immigrants from southern Europe seeking a better life in the New World at the turn of the century. They lived on the lower East Side, and Failla partly supported himself through high school by working in a factory that produced artificial flowers. He finished with a Master's degree from Columbia in physics. With one of those chance circumstances that shape the future of one's life, one of Failla's professors was a friend of Dr. Janeway, one of the early well-known surgeons at Memorial Hospital. Failla was hired to build and operate a radon plant in 1915 and became probably the first medical physicist in the United States. We celebrated the 75th anniversary of the laboratory in 1990.

In 1920, Failla spent a year in Paris to finish his thesis for the degree of Doctor of Science, under the direction of Madame Curie. While in Paris, he witnessed the early radiobiological experiments involving the irradiation of the testes of rams. It was found that animals could not be sterilized with-

[1] This is the text of the 28th Failla Memorial Lecture presented at the 9th International Congress of Radiation Research, Toronto, Ontario, Canada, 1991.

0033-7587/92 $3.00

FIG. 1a. Gioacchino Failla as the Janeway Lecturer of the American Radium Society in 1939. Photograph from the archives of The Center for Radiological Research Columbia University.

out producing necrosis in the skin of the scrotum if the radiation were delivered in a single acute exposure, but if it were spread out over several weeks in a series of daily fractions, the animals could be sterilized without unacceptable damage to normal tissue. Reasoning that the testes were a model for a rapidly growing tumor, while the skin of the scrotum represented a dose-limiting normal tissue, fractionated radiotherapy as we know it today was born. These experiments made a strong impression on Failla, and when he returned to New York he made the observation in his D.Sc. thesis that, while the quantification of dose in medical physics was an important problem that urgently called for a solution, in the long run the major advances would come from biology. Consequently, from that time on, the staff of the Radiological Research Laboratory always included several biologists.

Following the second World War, during which Failla was a consultant to the Manhattan District Project, he moved the Radiological Research Laboratory across town from Memorial Hospital to Columbia University, where it remains to this day.

Failla was a man of extraordinary energy and many talents, an engineer/physicist by training, but a biologist by interest, very much the American equivalent of Hal Gray (Fig. 1b). He was chairman of the committee that founded the Radiation Research Society, and also an important force behind the creation of the journal *Radiation Research*; Titus Evans, the journal's first Editor-in-Chief, had been a student of Failla at Columbia.

In recent years, the Failla lecture has become a more personal account of the career and experiences of the lecturer; I will continue this tradition and offer my experiences as a cautionary tale for young people just entering the field! My own career is, to a large extent, a tale of two cities—of Oxford and of New York—since I've had only two jobs in my life. In many ways it is hard to credit that Oxford and New York are on the same planet—they are so different and represent the extremes of life in Western industrialized societies. New York is a vibrant bustling city, a melting pot of all nations; Oxford, home of sleepy spires and lost causes, takes pride in preserving archaic reminders of its medieval past. The two cities have one thing in common, that both have seen better days. Yet I settled in both—was productive in both—and enjoyed the very different attributes of both.

The development of radiation biology has inevitably paralleled the evolution of systems and end points in the wider field of biology. When I started in Oxford in 1955:

—The double helix structure of DNA had only recently been discovered.
—We did not know that DNA was the principal target for radiation damage.
—Mammalian cells could not be grown as single cells in culture.
—Survival curves for mammalian cells and the effective D_0 were not known.

People worked with animals, bacteria, yeast, and—I favored a system much used by physicists who had turned to biology—the bean root. And yet with such simple systems, we knew:

—the oxygen effect, and the OER for different radiations;
—the dose-rate effect;

FIG. 1b. Gino Failla and Hal Gray at the first International Congress of Radiation Research in 1958 (Center for Radiological Research Archives).

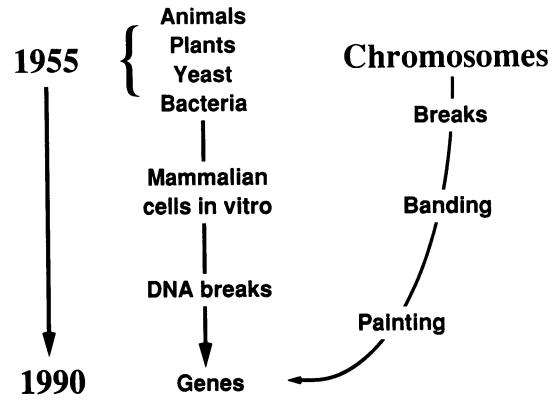

FIG. 2. Illustrating the evolution in experimental systems and end points used in radiation biology over the past three decades, as whole animals, plants, and bacteria have given way to cells in culture, DNA damages, and genes.

—the effect of fractionation;
—the greater RBE of neutrons and α particles;
—protectors and sensitizers;
—the mutagenic and carcinogenic potential of X rays.

In short, we knew most of the basic principles of radiation biology—except sensitivity changes with cell age and reoxygenation—before a mammalian cell could be grown in culture. This is a sobering thought. The evolution of experimental systems used in radiation biology is illustrated in Fig. 2.

A problem I had in preparing this Failla lecture is that I've been a Jack-of-all-trades, and I have not concentrated on any one thing: I want to give you a sample of several of my interests over the years. I've chosen three specific areas:

—the dose-rate effect,
—hypoxic cell radiosensitizers and bioreductive drugs,
—oncogenic transformation *in vitro*.

THE DOSE-RATE EFFECT

In the fall of 1962 I arrived in Denver, Colorado for a 1-year visit as a Fulbright Exchange Visiting Assistant Professor. Joel Bedford was assigned to me for a research project as part of his Master's degree—and this began the long-term friendship between Joel and me—as well as our long-standing interest in dose rate. Joel was my first graduate student and, after the year in Colorado, returned with me to Oxford to complete his doctoral studies (Fig. 3). Figure 4 illustrates the dose-rate effect for cells in culture, in this case HeLa cells, showing that when the delivery of the dose is protracted over a period of time there is a sparing effect (*1–3*). With his first graduate student Jim Mitchell, Joel Bedford went on to show the inverse dose-rate effect, whereby over a narrow range of dose rates cells tend to pile up in G_2 phase and become more sensitive as the dose rate is lowered (*4*). The combined work of a number of individuals resulted in the understanding of the overall dose-rate effect, which is illustrated in Fig. 5. The dose–response curve for acute doses is characterized by a broad initial shoulder. As the dose rate is lowered, the dose–response curve becomes progressively shallower due to the repair of sublethal damage during the exposure. Over a narrow range of dose rates cells move through the cycle and pile up in G_2 phase, which is a radiosensitive phase. Consequently the dose–response curve becomes steeper again, the so-called reverse dose-rate effect. If the dose rate is further reduced, cells are released from the G_2 block, pass into mitosis, and so cell death can be balanced by cell birth.

My long-standing interest in the dose-rate effect is illustrated by a recent paper in collaboration with David Brenner in which we introduced the concept of *pulsed low-dose-rate brachytherapy* (*5*). The idea here, illustrated in Fig. 6, is to implant a tumor with a series of catheters and then to pulse a single source through them. As the source steps through each catheter, the dwell time can be adjusted according to the linear activity required, so producing perfect dose optimization. As a result of the analysis of the survival curve characteristics and repair half-times of a large number of cells of human origin, it was proposed that a 10-min pulse every hour would be indistinguishable from continuous low-dose-rate irradiation (*5*).

Pulsed brachytherapy has a number of advantages: (1) improved dose optimization, (2) a simplified afterloader,

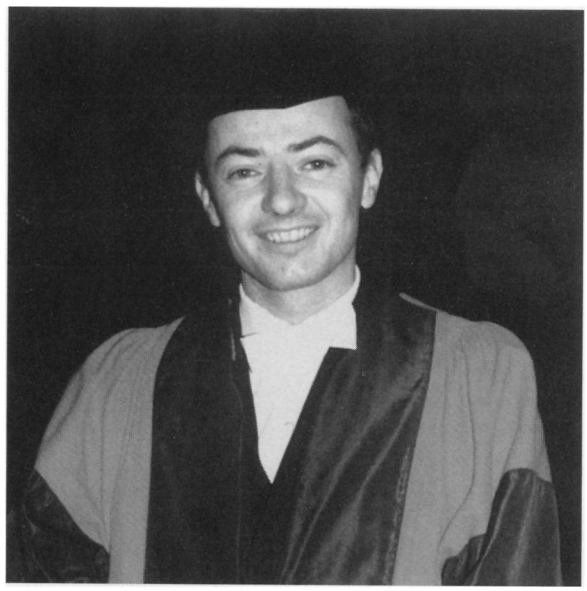

FIG. 3. Joel Bedford graduating from Oxford with the D.Phil. degree in 1966. Joel was my first graduate student for a Master's degree at The University of Colorado in 1962, and returned with me to Oxford to complete his doctoral studies.

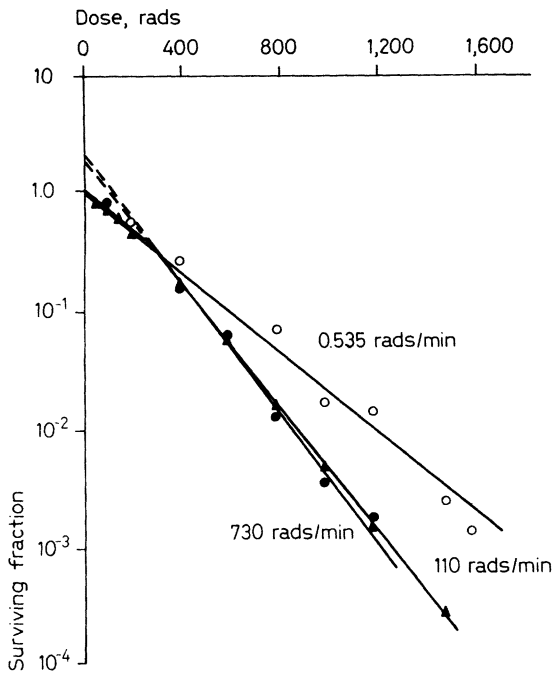

FIG. 4. The first demonstration of the dose-rate effect with mammalian cells in culture. HeLa cells were irradiated in an acute exposure at high dose rate with a teletherapy unit, or at protracted exposures by filtering or pulsing the beam. As the dose rate is reduced, the slope of the dose–response curve becomes shallower and the extrapolation number tends to unity [Data from Refs. (*1*, *2*); reproduced, by permission of the publisher, from Ref. (*3*)].

(3) one source to be replaced instead of many, (4) good radiation protection, and (5) the ability to retain the same *average* dose rate as the source decays; the pulse length is simply elongated to balance the decay of the radioactive source (Fig. 7).

In the case of mammalian cells, the repair of sublethal damage is still an operational term since we still do not know the specific molecular lesion(s) or the detailed biochemical repair processes involved. Attempts to understand this phenomenon better are being made in a number of laboratories, including our own under the direction of Howard Lieberman. Figure 8 shows recent data in which a repair gene has been identified and sequenced in the yeast *Schizosaccharomyces pombe* (*6*, *7*). The radiation-sensitive strain containing the mutant allele *rad 9-192* is about 10 times more sensitive than the wild type to γ rays. The *rad 9+* and *rad 9-192* genes have been isolated and their DNA sequences determined; there is a single nucleotide base-pair difference, a CG substituting for a TA. This would cause the coding for proline in place of leucine in the mutant *rad 9*, which could result in a dramatic change in the three-dimensional structure of the protein. This change has evidently destroyed *rad 9* protein activity and resulted in the dramatic increase in radiosensitivity exhibited by *rad 9-192* cells. The alteration of one base pair leads to this dramatic difference in radiosensitivity. It is not certain at the present time whether the gene identified is truly a repair gene or a gene that controls cell-cycle progression. Experiments are underway in many laboratories, including our own, in an attempt to identify similar genes in human cells.

HYPOXIC RADIOSENSITIZERS AND BIOREDUCTIVE DRUGS

An area that has involved a great deal of effort and interest over the years has been the development of compounds designed specifically to sensitize hypoxic cells to the effects of ionizing radiations, i.e., to replace or mimic oxygen. This is an area in which we became interested in the early 1970s, and we were able to make two modest contributions to this field; this is work in which I collaborated with Myles Astor and Laurie Towle (Fig. 9). Our first discovery was that misonidazole (Miso), introduced into clinical trials specifically to radiosensitize hypoxic cells, was in fact itself preferentially cytotoxic to cells that were deficient in oxygen (*8*).

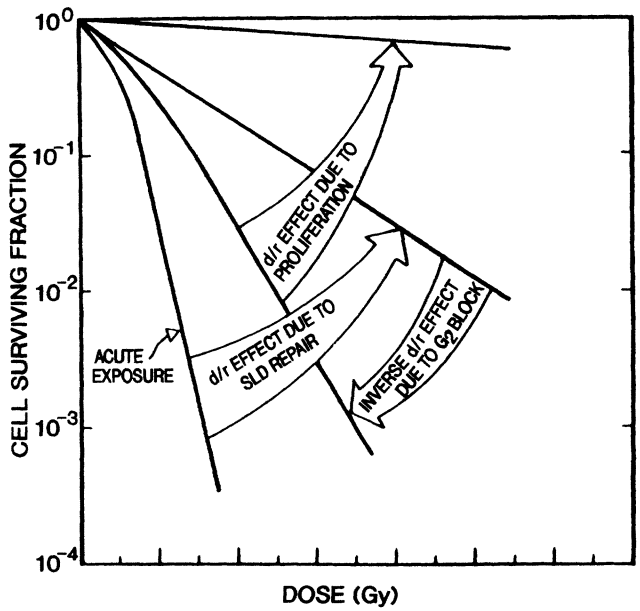

FIG. 5. The dose-rate effect due to repair of sublethal damage, redistribution in the cycle, and cell proliferation. The X-ray dose–response curve for acute exposures is characterized by a broad initial shoulder. As the dose rate is reduced, the survival curve becomes progressively shallower as more 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 as cells can progress through the cycle to pile up at a block in G_2, a radiosensitive phase, but still cannot divide. A further lowering of dose rate allows cells to escape the G_2 block and divide; cell proliferation may then occur during the protracted exposure, and survival curves become shallower as cell birth from mitosis offsets cell killing from the irradiation.(From an idea by Joel Bedford.)

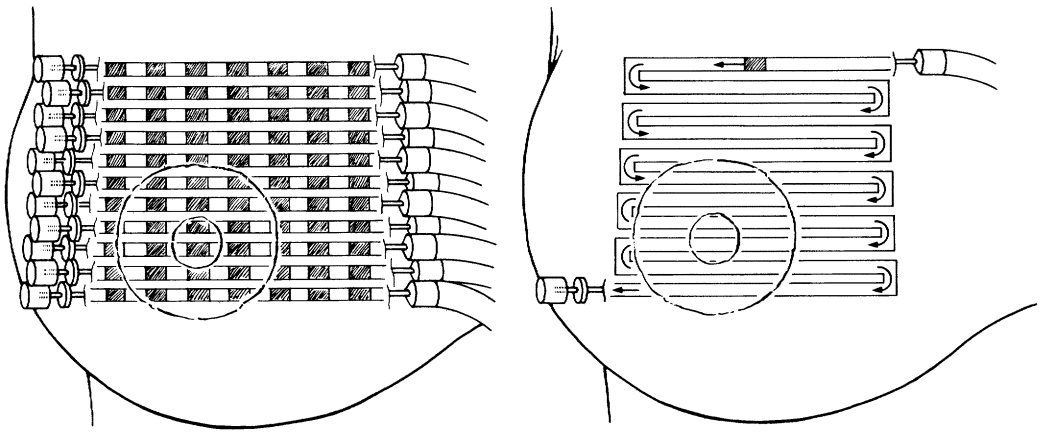

FIG. 6. In a conventional interstitial implant, catheters are implanted in the tumor and radioactive sources of iridium-192 are subsequently loaded into the catheters and left in place for about a week. An inventory of sources must be maintained and replaced every few months as the radionuclide decays (left). In pulsed low-dose-rate brachytherapy (right) catheters are implanted as usual, but now a single iridium-192 source (of about 0.5 Ci) steps through each catheter in turn under computer control, with the dwell time in each position adjusted to reflect the activity required in that position. The source would take about 10 to 20 min to step through the entire implant before returning to the protected safe—to be repeated once an hour for the same overall time of about a week.

This is shown in Fig. 10. This observation, subsequently investigated in detail by ourselves and others, did much to open up the field of bioreductive drugs. We also went on to show that the radiosensitizing effect of Miso could be greatly increased if the cells were preincubated with the drug for a period of time prior to irradiation. This came to be known as the preincubation effect (*9*). This is illustrated in Fig. 11a. (A similar phenomenon was discovered independently about the same time by Wong and Whitmore in Toronto.) Our data were published in a joint paper with John Biaglow in which it was suggested that the preincubation effect was due to the depletion of cellular glutathione; the same paper first demonstrated that hyperthermia could enhance the cytotoxicity of misonidazole as illustrated in Fig. 11b (*9*). These data were elaborated upon in a later publication (*10*).

By the time Miso had been replaced in clinical trails by etanidazole (*11*), we felt that further *in vitro* studies of radiosensitization or cytotoxicity were of little interest since the action had now moved to the clinic. So instead we turned our attention to a study of the oncogenicity of these drugs. Working with Tom Hei we made the interesting observation that etanidazole, the very compound selected for use in the clinic, was the most oncogenic nitromidazole tested (*12*). This is shown in Fig. 12a. The probable reason for this is the presence of the amide in the side chain (Fig. 12b). We went on to make a calculation of the possible number of second malignancies that could be induced in long-term survivors treated with etanidazole (*12*, *13*). The calculation was based on the following assumptions: (a) A total-body dose of 1 Gy of low-LET radiation in the human at high dose rate results in a cancer death rate of about 7%. This is the assessment of the UNSCEAR committee based on a recent reanalysis of the Japanese A-bomb survivors (*14*). (b) Relative oncogenic transformation rates between agents assessed *in vitro* reflect the same ratio for cancer induction in the human for a total-body exposure.

If these assumptions are accepted, at least for the purposes of discussion, then the relative oncogenic transformation rates quoted in this paper can be translated into cancer induction in patients treated with bioreductive drugs. For example, in the Radiation Therapy Oncology Group (RTOG) protocols to test the efficiency of etanidazole as an adjunct to radiation therapy, 17 doses of 2 g/m^2 are delivered (*15*). The average half-life of the drug in the human is about 5 h (*16*). This results in an average cumulative dose of 36 m*M*-hours spread over a period of 5 to 6 weeks.

It can be seen from Fig. 12 that a cumulative dose of 36 m*M* of etanidazole (delivered in 4 h) results in an oncogenic transformation incidence comparable to an acute γ-ray exposure of about 5 Gy. According to the UNSCEAR estimate, this would result in an incidence of fatal cancers amounting to 35%. This is clearly an overestimate based on a single treatment in 4 h rather than multiple fractions over many weeks. The dose-rate effectiveness factor is not known with any certainty for radiation, much less for bioreductive drugs; the best estimates for radiation range from 2 to 10, with a most probable value in the human between 2 and 3 (*14*).

It might be expected, therefore, that if etanidozole were used widely in the clinic it may well result in an incidence of second malignancies of 14%, which is much higher than that now seen following radiotherapy alone.

At about this time Ged Adams and his group at the Medi-

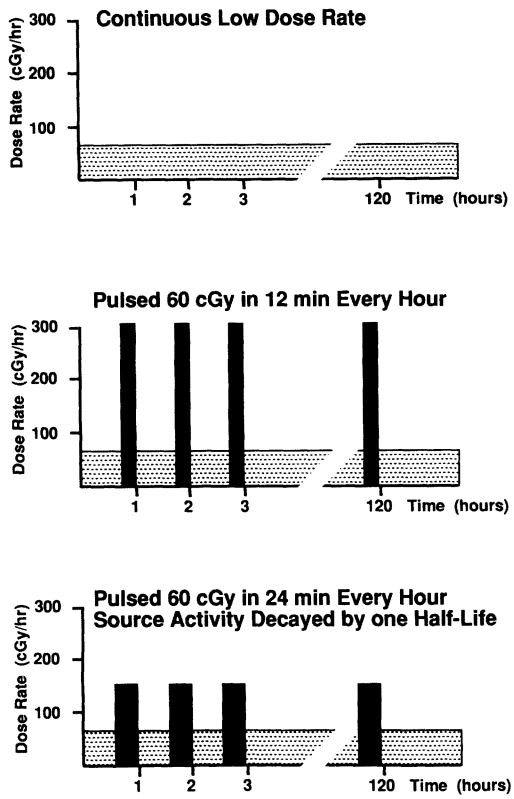

FIG. 7. Illustrating the principle of pulsed brachytherapy. Continuous low-dose-rate irradiation at (for example) 60 cGy per hour is replaced by a relatively high-dose-rate pulse of 60 cGy delivered once per hour. The pulse, during which the single iridium-192 source steps through the implant, would take about 12 min, depending on the activity of the source and the size of the implant. Over a period of months as the activity of the iridium-192 source decays, the dose per pulse, and therefore the average dose rate per hour, can be maintained by simply increasing the pulse length. After one half-life of the radionuclide, which is 70 days, the pulse length would be doubled to 24 min in each hour.

cal Research Council in the United Kingdom were developing a new series of bifunctional bioreductive drugs based on the parent compound RSU-1069 (*17*, *18*). We have been privileged to play a small part in the development and screening of these compounds, in collaboration with Ged Adams in the United Kingdom, Bob Sutherland at Stanford Research International, and Dietmar Sieman at University of Rochester. The structures of the group of bifunctional bioreductive drugs investigated are shown in Fig. 13. Our role in this was to test the derivatives of RSU-1069, since the parent compound is much too toxic for clinical use! Figure 14 shows the oncogenic transformation incidence in C3H 10T1/2 cells resulting from exposure to these various compounds as a function of drug concentration. It is evident that the oncogenic potential can be modified greatly by methylating the aziridine ring at the terminal end of the side chain of the nitroimidazole compound. The compound in which the ring is fully methylated (RB-7040)

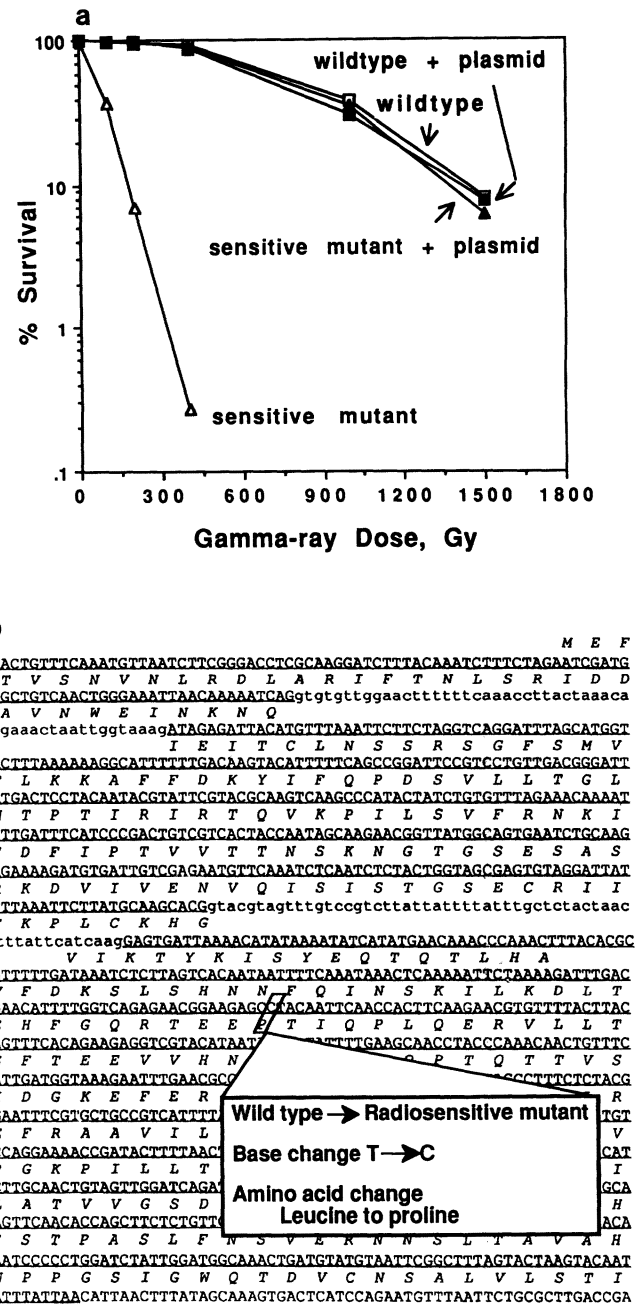

FIG. 8. (a) Gamma-ray dose–reponse curves for the yeast *Schizosaccharomyces pombe*. Data for the wild type are shown as open squares, those for the radiosensitive mutant as open triangles. The closed triangles show data for the radiosensitive strain, into which a cosmid had been transfected containing the *rad 9* wild-type gene; wild-type resistance is restored by this means. Transfecting the cosmid into wild-type cells did not affect radiosensitivity (closed squares). [Redrawn from Refs. (*6*) and (*7*)]. (b) The radioresistant wild-type *S. pombe* contains the *rad 9+* gene. The radiosensitive strain contains the mutant allele *rad 9-192*. Both genes contain a 1092 bp open reading frame. A comparison of the DNA sequence of the two genes reveals a single nucleotide base-pair difference, a CG substituting for a TA. 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. [Redrawn from Refs. (*6*) and (*7*).]

FIG. 9. Laurie Roizin-Towle and Myles Astor were my first graduate students at Columbia University, and collaborated with me in the study of hypoxic cell radiosensitizers. Also shown are Charles Geard and Basil Worgul, who were marshalls at commencement in 1980.

shows no transformation incidence above background. Of course the cytotoxic activity of the compound also varies with methylation, and it is a question of reaching a compromise between activity as a hypoxic cytotoxin and oncogenicity.

The studies of Adams, Sutherland, and Siemen have shown RB-6145 to be a particularly effective cytotoxic agent, specific for hypoxic cells, as well as an efficient chemosensitizer. For example, Fig. 15 from Adams and Stratford shows the cell killing in a KHT mouse sarcoma following a dose of 10 Gy that sterilizes most of the aerated cells.[2] Our own studies referred to above indicate that RB-6145 exhibits only a modest oncogenicity comparable to misonidazole. Based on these collaborative studies from several different institutions on both sides of the Atlantic, this compound has been chosen to move forward into phase I clinical trials as an adjunct to radiation therapy. This area of drug research has involved active collaboration and created warm friendships (Fig. 16).

ONCOGENIC TRANSFORMATION *IN VITRO*

In December of 1968 I moved from Oxford to take up a position at Columbia University in New York at the invitation of Dr. Harald Rossi, then Director of the Radiological Research Laboratory. Soon thereafter I met and hired Carmia Borek, who 3 years earlier had been the first person to demonstrate that X rays could transform fresh explants of Syrian hamster cells *in vitro* (*19*). Figure 17 is a photograph taken in 1978 of Harald Rossi, who recruited me to Columbia, and Carmia Borek. At the time I was very impressed with the ease with which a transformed colony could be identified, and so I collaborated with her in making the system into a quantitative assay. The first dose–response curve for transformation by X rays, or indeed for any agent for that matter, is shown in Fig. 18 as it appeared in *Nature* in 1973 (*20*). Over the years the development and use of transformation assays have occupied a major place at the laboratory at Columbia. The Syrian hamster embryo cells are used as well as established cells lines such as the C3H 10 T1/2 cells. A major effort is underway to use cells of human origin in transformation assays, but while human cells can be transformed, no really quantitative assay is available at the present time.

There are many examples of the pragmatic uses of oncogenic transformation assays; I will quote just one from a study of radon in collaboration with Dr. Tom Hei. In 1987 the NCRP published the pie diagram shown in Fig. 19 indicating that radon represents the largest component of the effective dose equivalent to the U.S. population (*21*). The decay scheme for radon is shown in Fig. 20. The dose to lung tissue from radon daughters is due principally to two α

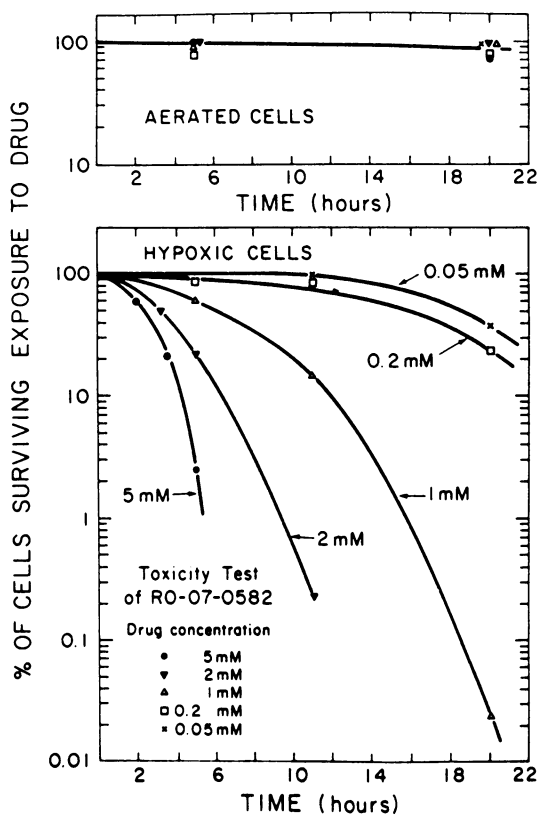

FIG. 10. Survival of V79 Chinese hamster cells stored for various periods of time at room temperature in the presence of misonidazole. There is no toxicity to aerated cells over the range of times and concentrations tested (top), whereas toxicity to hypoxic cells is a function of both time and drug concentration (bottom). This was the first demonstration of the preferential hypoxic cytotoxicity in mammalian cells (*8*).

[2] G. E. Adams and I. J. Stratford, personal communication (1991).

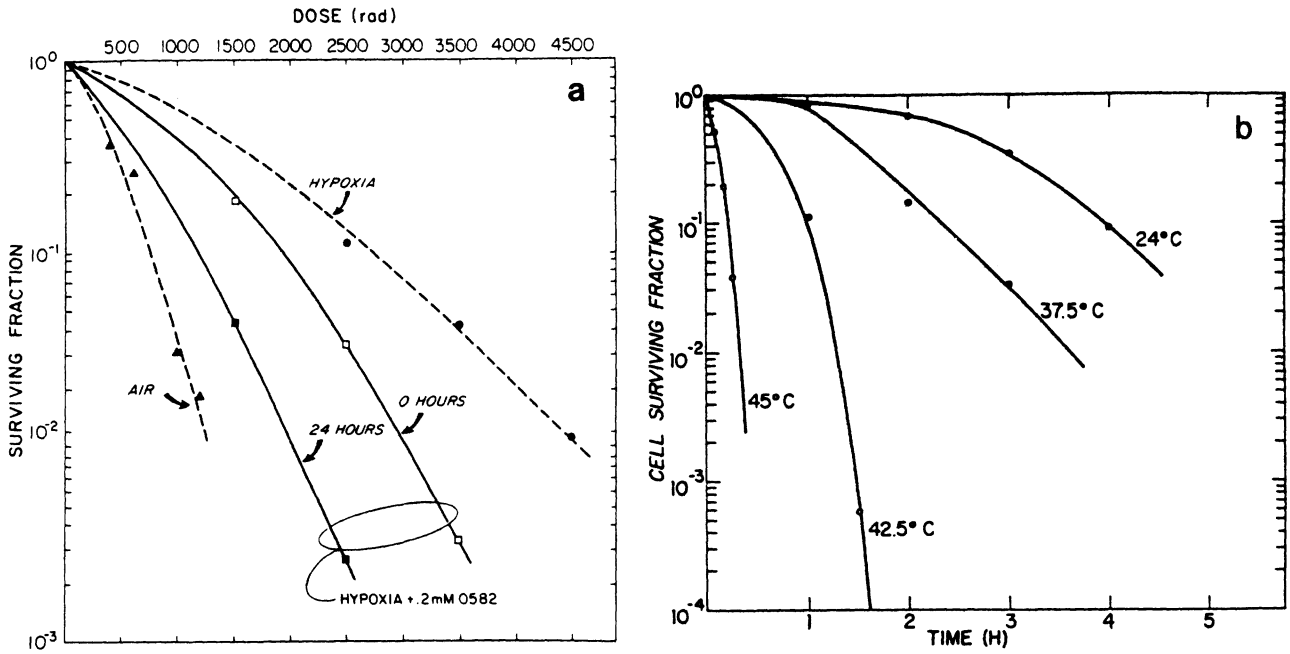

FIG. 11. (a) Illustrating the "preincubation" effect. The closed triangles and circles represent the survival of Chinese hamster cells irradiated with ^{60}Co γ rays under aerated and hypoxic conditions. The survival of hypoxic cells irradiated in the presence of misonidazole at a concentration of 0.2 m*M* is represented by open and closed squares. Open squares refer to irradiations carried out immediately after the drug was added and the cells made hypoxic. Closed squares refer to irradiations carried out after the cells were stored at room temperature for 24 h following the addition of the drug, a treatment which killed about 80% of the cells. Reproduced, by permission of the publisher, from Ref. (*9*). (b) Illustrating the temperature dependence of the hypoxic cytotoxicity of misonidazole. Survival of V79 Chinese hamster cells exposed to 5 m*M* misonidazole for various periods of time at four different temperatures (*9*).

particles of 6 and 7.7 MeV. These densely ionizing a particles turn out to be highly effective at producing oncogenic transformations.

Figure 21 shows cell survival and the induction of oncogenic transformation in C3H 10T1/2 cells irradiated with either γ rays or helium-3 ions, chosen to simulate the α particles from radon daughters (*22*). The survival curve for γ rays has a characteristic initial shoulder region before becoming steeper and approaching an exponential function of dose at higher doses. By contrast, the cell-survival curve for the high-LET helium ions closely approximates an exponential function of dose, indicating single-hit kinetics—i.e., the cell is killed by the traversal of a single particle. However, at a dose corresponding to a surviving fraction of 37%, about 14 particles traverse the nucleus of each cell killed. C3H 10T1/2 cells are quite large when stretched out and attached to a culture dish, with an average nuclear cross-sectional area of about 240 μm^2. This conclusion is quite interesting. While one particle kills the cell, on average about 13 particles traverse the cell without killing it for every one that does! This may be a clue to the high efficiency with which high-LET particles induce transformed foci.

Transformation data are most commonly plotted in terms of transformation incidence per surviving cell as a function of dose. This is convenient since this is the way in which the transformation data are collected. In the case of both X rays and helium ions transformation rises as a function of dose, eventually reaching a plateau. However, in applying these *in vitro* data to whole organisms, a more relevant quantity is oncogenic transformation incidence per initial cell at risk. This is also shown for γ rays and helium ions in Fig. 21 (*22*). The overall curve shape reflects the combined effects of oncogenic transformation induction and cell lethality. In the case of γ rays, oncogenic transformation incidence per initial cell at risk rises with dose at low doses, reaches a maximum at a few hundred centigray, and subsequently falls at higher doses as cell killing becomes dominant, eventually becoming parallel to the cell survival curve. A dose–response relationship of this general shape has been observed for a number of tumors in experimental animals induced by whole-body irradiation. In the case of the high-LET particles, transformation incidence per initial cell at risk rises very sharply indeed at low doses, reaches a narrow maximum at about 50 cGy, and subsequently falls rapidly to parallel the steep, shoulderless cell-killing curve.

The interesting point is that, at a dose of about 50 cGy, the transformation incidence per initial cell at risk for helium-3 ions is higher by a factor of three than can be

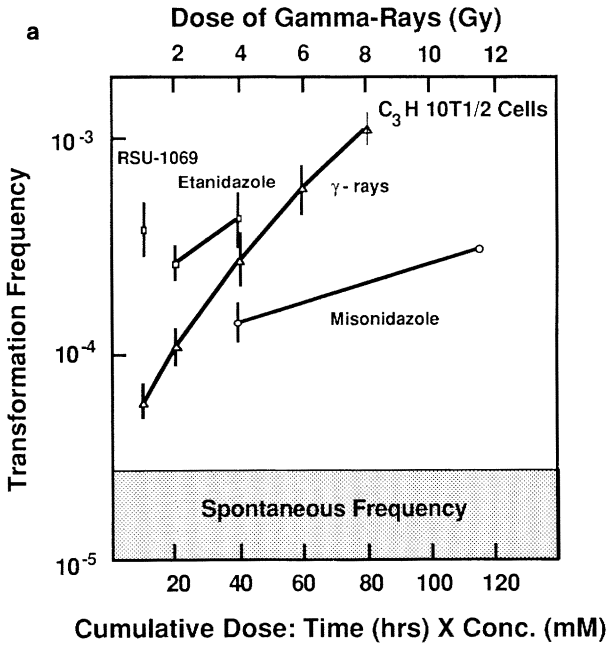

FIG. 12. Transformation incidence per surviving cell in C3H 10T1/2 cells as a function of cumulative dose for several nitroheterocyclic compounds. The exposures to the drugs were under aerated conditions. Note that while misonidazole and etanidazole are equally effective as hypoxic cell radiosensitizers, etanidazole is much more oncogenic. Also shown for comparison is the γ-ray dose–response relationship for transformation. Reproduced, by permission of the publisher, from Ref. (*12*). (b) The structures of misonidazole (Ro-07-0582) and etanidazole (SR-2508). Both are 2-nitromidazoles, but have different side chains which results in different pharmacokinetics. The amide group in the side chain of etanidazole probably accounts for its oncogenicity.

achieved by *any* dose of γ rays. This is presumably a consequence of a differing balance between the induction of transformed cells and the killing of transformed cells. High-LET particles are much more effective at cell killing than are X rays, but they are *even more* efficient at inducing transforming events.

As part of our general interest in the radon problem, Charles Geard (Fig. 9, far right) and David Brenner have made a detailed study of the morphology and morphometry of lung sections from several hundred normal individuals obtained from the Pathology Department at Columbia–Presbyterian Medical Center. The range of depths of serous and basal cells are shown in cartoon form in Fig. 22, as well as representations to scale of the ranges of the two α particles emitted by radon daughters. If α particles are emitted perpendicular to the epithelial surface from radon daughters deposited there, they have ample range to reach the basal cells. However, most particles will be emitted at an angle to the surface and then, if they reach the basal cells at all, it will be close to the end of the range of the α particles as they are slowing down. We have performed oncogenic transformation experiments using C3H 10T1/2 cells exposed to α particles in the track segment mode with LET values up to 200 keV/μm. Using these data, the quality factor, or relative effectiveness, of α particles as a function of depth has been calculated by David Brenner (*23*). The results are shown in Fig. 23, and compared with the values suggested by ICRU (*24*), which are based on chromosome aberrations and which show relatively little variation with depth until just before the end of the particle's range. These data demonstrate that the biological effectiveness of radon-daughter α particles may vary significantly as a function of depth through the bronchial epithelium, depending critically on the saturation characteristics of the biological end point of interest. For the end point of oncogenic transformation, which may be rather more relevant than chromosome aberrations, it would appear that the carcinogenic effectiveness of the α particles emitted by radon-daughter products in human lungs may be less than previously thought because of the range of the particles and the location of the cells at risk.

ACTIVATION OF ONCOGENES

The past two decades have witnessed some dramatic advances in our understanding of the mechanisms of carcino-

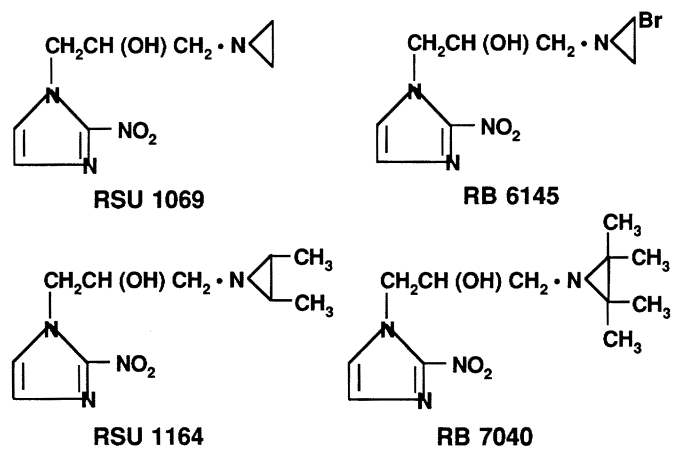

FIG. 13. RSU-1069, the lead compound, is a bifunctional drug (*34*). It is a 2-nitroimidazole with an aziridine ring at the end of the side chain. RSU-1069 is too toxic and oncogenic for clinical use. Various analogues were made in which the aziridine ring was methylated to reduce its activity. RB-6145 is a pro drug; it is much less toxic than RSU-1069, but inside the cell the aziridine ring closes and it becomes RSU-1069.

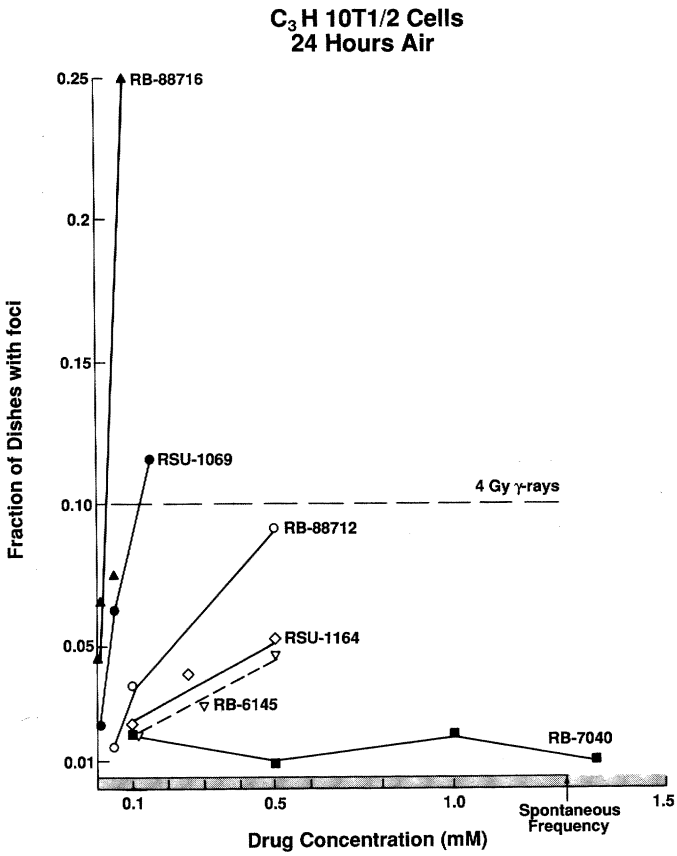

FIG. 14. Transformation incidence (fraction of dishes with foci) in C3H 10T1/2 cells as a function of drug concentration (24 h exposure) for a variety of bioreductive drugs. The parent compound RSU-1069 is a 2-nitroimidazole with an aziridine ring at the terminal end of the side chain. Successive methylation of the aziridine ring reduces the oncogenicity of the drug. Complete methylation (RB-7040) results in a transformation incidence barely detectable above background. RB-6145 is a pro drug for RSU-1069 and shows modest oncogenicity; RB-88716 is a nitrofuran which is too active to be considered for clinical use and also shows considerable oncogenicity. (Unpublished data of Dr. Tom Hei.)

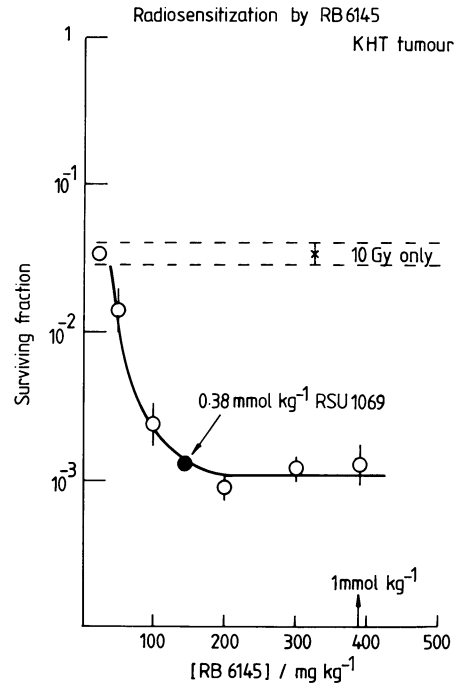

FIG. 15. Illustrating the cytotoxic action of RB-6145 on hypoxic cells. Surviving fraction is plotted as a function of drug concentration in KHT mouse sarcomas, following a 10-Gy dose of X rays, which kills essentially all of the aerated cells. (Unpublished data of G. E. Adams and I. Stratford.)

genesis. Some human cancers, especially the leukemias and lymphomas, have been shown to be associated with the activation of a dominant acting oncogene; many solid tumors appear to be associated with the loss of a suppressor gene or anti-oncogene. In a few instances, notably colon cancer, the multistep nature of carcinogenesis has been worked out in some detail, and it appears that both the activation of oncogenes and the loss of suppressor genes are involved in the progression from preneoplasia to a frankly malignant metastasizing cancer.

There are three principal mechanisms by which proto-oncogenes 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 (*25*). For example, a point mutation in N-*ras* is found in the cancer cells of most patients suffering from acute leukemia (*26*).

(2) A chromosomal rearrangement or translocation can occur, often placing a proto-oncogene next to a strong promoter sequence leading to its overexpression, or producing a new fusion gene whose product acquires a new transforming activity. Ionizing radiations are very effective at producing DNA breaks, which may rejoin as a dicentric or as a translocation. Translocations have been shown to be asso-

FIG. 16. Collaborative research in bioreductive drugs has forged many lasting friendships. Ged Adams (center) and Bob Sutherland (left) are particularly good friends of mine and collaborated in the development and assessment of RB-6145.

FIG. 17. Photograph taken in the late 1970s. Harald Rossi recruited me to Columbia in 1968. Carmia Borek was the first professional that I hired after arriving in New York. She was the first person to show that X rays could transform Syrian hamster embryo cells *in vitro*. We worked together for many years on the development and use of this *in vitro* assay for oncogenic transformation.

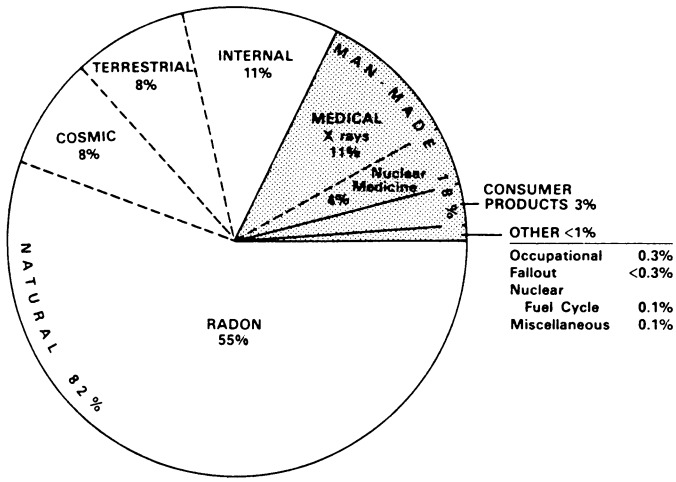

FIG. 19. The percentage contribution of various radiation sources to the total average effective dose equivalent in the U.S. population. Taken from NCRP Report 93 (1987), this recognized for the first time that the contribution from radon is larger than that from all other sources combined. Reproduced, by permission of the publisher, from Ref. (*21*).

ciated with several human cancer cells involving *myc* genes. For example, a very specific translocation between chromosomes 8 and 14 is responsible for *myc* activation in 75% of all Burkitt's lymphoma (*27*). The other 25% also involve very specific translocation but between 8 and 2 or 8 and 22. In all cases the exchange point of the translocation is precisely at the location of the c-*myc* oncogene on chromosome 8.

(3) Gene amplification, where many extra copies of a proto-oncogene exist in a cell, is associated with the activation of oncogenes in several cancers. The presence of multiple copies of a proto-oncogene leads to its overexpression. Gene amplification of N-*myc* is characteristic of many neuroblastomas (*28*).

In no instance has a specific oncogene been identified as the causal step in a radiation-induced tumor *in vivo*, or even with radiation-induced transformation *in vitro*. Pellicer showed that, in X-ray-induced lymphomas in mice, activated *ras* could be identified in 9/37 or 24% of the tumors, and that seven of these involved the same mutation. In

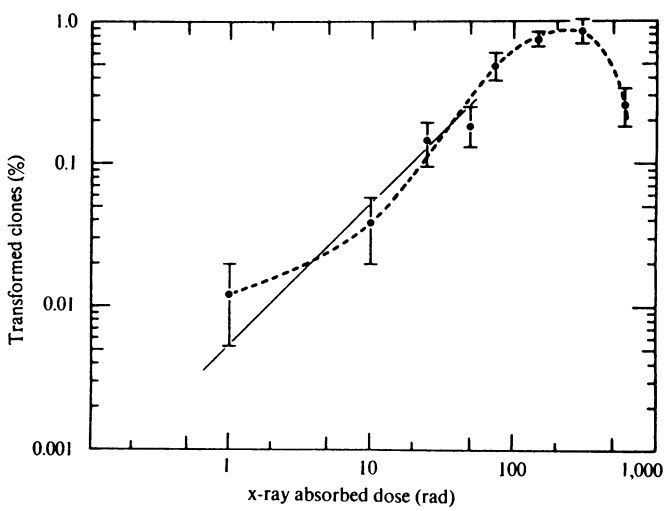

FIG. 18. Incidence of hamster embryo cell transformation following exposure *in vitro* to X irradiation. The broken line is drawn by eye to the mean data points; the full line has a slope of +1 and passes through the error bars of each data point. This was the first published dose–response curve for X-ray transformation *in vitro* as it appeared in *Nature* in 1973. Reproduced, by permission of the publisher, from Ref. (*20*).

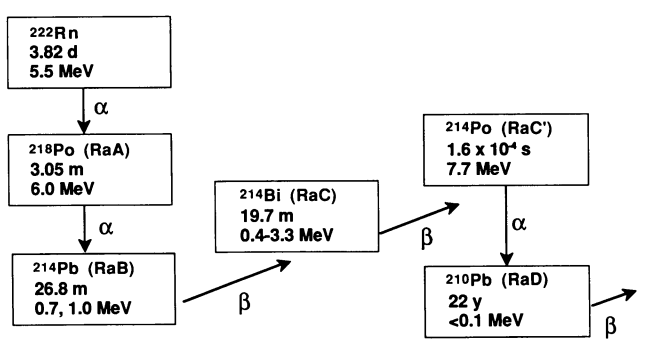

FIG. 20. A section of the decay scheme from uranium to lead that involves radon, the only element in the series that is a gas. Two of the radon daughter products decay with short half-lives emitting α particles of 6 and 7.7 MeV. These constitute the most biologically important emissions from radon daughters.

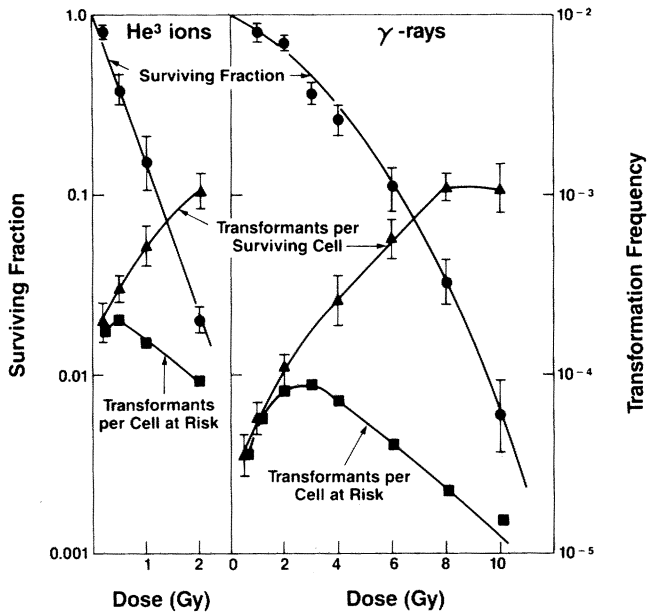

FIG. 21. Cell survival (closed circles) and transformation incidence per surviving cell (closed triangles) in C3H 10T1/2 cells exposed to γ rays or ^{3}He ions having an LET of 120 keV/m. The more relevant end point when extrapolating *in vitro* data to the irradiation of a whole organism may be transformants per initial cell at risk (closed squares). For both γ rays and ^{3}He ions, this quantity rises rapidly to a peak, and off at higher doses as cell killing dominates. The peak incidence of transformants per initial cell at risk, which occurs at a dose of about 30 cGy for ^{3}He ions, is five times higher than at any γ-ray dose. Reproduced, by permission of the publisher, from Ref. (*22*).

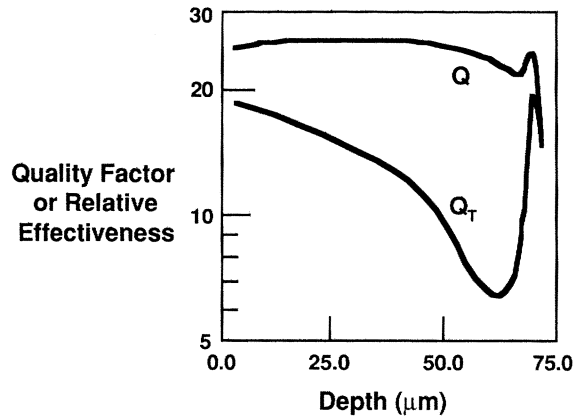

FIG. 23. Variation in effectiveness (relative to 250-kVp X rays) as a function of depth, as derived using the transformation data for C3H 10T1/2 cells (Q_T), and also as obtained using the ICRU 40 function $Q(y)$ (*24*). Averaged over the whole epithelium (10 to 72 μm), the dose mean effectivenesses are $\langle Q_r \rangle = 15$ and $\langle Q \rangle = 26$. [Redrawn from Brenner (*23*).]

further studies with neutrons, activated *ras* was observed in only 4/25 or 16% of the tumors and each instance involved a different mutation. It was concluded from these studies that while the activated *ras* oncogene may well be involved with the development of the tumor, at least in some cases, it was not the causal event (*29–31*).

During the past year, Greg Freyer in our laboratory has isolated a putative transforming gene (*32*). A library was made of the genomic DNA from a focus of C3H 10T1/2 cells transformed by γ rays. This consisted of about 300,000 pieces of DNA each 35 to 40 kb. These were inserted into cosmids, together with neomycin and ampicillin resistance markers and a *cos* site to facilitate the packaging of the cosmids for amplification. Figure 24 illustrates the technique. About a quarter of a million cosmids were screened, but only two proved to be capable of propagating the malignant phenotype. Attention has focused on one cell line (LNγ3), grown up from a type III focus, which resulted from the transfection of a cosmid into untransformed C3H 10T1/2 cells. The piece of DNA consists of about 35 kb, but studies of the mRNA indicate that the coding sequences amount to only about 2 kb.

The transforming gene does not hybridize with any of the 22 known oncogenes listed in Table I, indicating that it is a new, previously unidentified, gene. Carmia Borek in our laboratory, as well as Jack Little and his colleagues at Harvard, had previously reached a similar conclusion, namely that radiation-induced transformation may be due to a new previously unidentified dominant acting gene, but in neither case did their methodology allow for the isolation of the gene (*33*, *34*). The transforming gene isolated by Freyer is in process of being characterized and sequenced. A Southern blot analysis (Fig. 25) indicates that the DNA from the transformed cell line is changed compared with control cells in at least two instances when cut with restriction enzymes. A Northern blot analysis (Fig. 26) indicates that the message is smaller in transformed cells. Both pieces

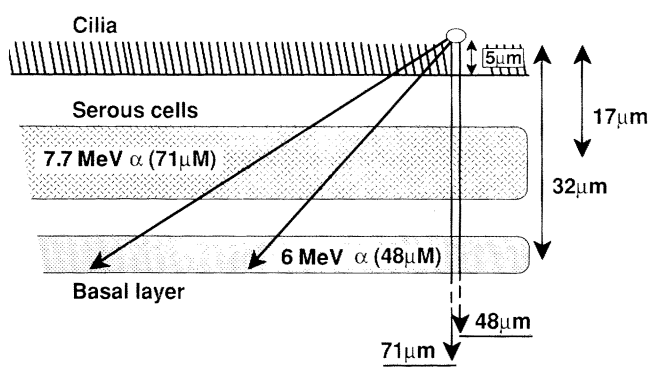

FIG. 22. Illustrating the average depths of the serous and basal cells in the human lung based on the study of several hundred "normal" lung sections from the Pathology Department at Columbia Presbyterian Medical Center. Also shown on the same scale are the ranges of the two α particles emitted by radon daughter products deposited on the lung surface. (Based on data collected by Drs. Charles Geard and David Brenner.)

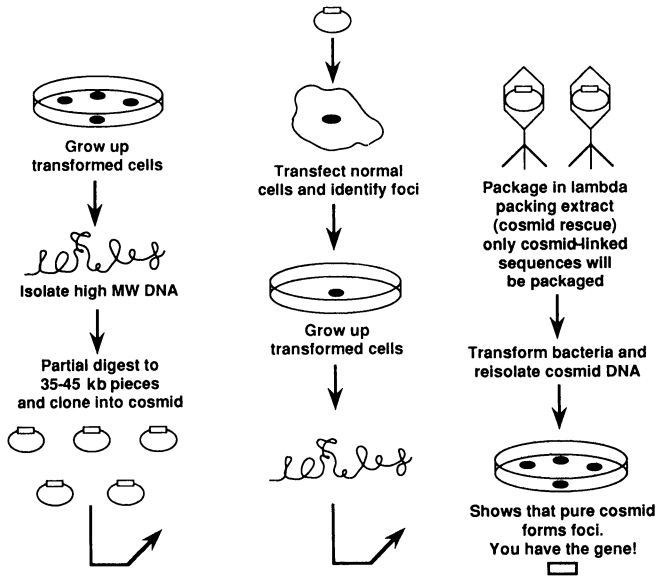

FIG. 24. Protocol used in experiments designed to identify and isolate a new dominant transforming gene involved in radiation-induced oncogenic transformation. A DNA library was constructed from a γ-ray-induced transformed focus, consisting of about 300 pieces of about 35 to 40 kb, packaged into cosmids also containing selectable markers. Two of these cosmids transmit the malignant phenotype. An attempt is being made to identify and sequence the dominant acting transforming gene in one of them, which from RNA studies appears to be about 2000 bp, located somewhere within the 35-kb DNA piece. [Unpublished figure; described in publication (*33*).]

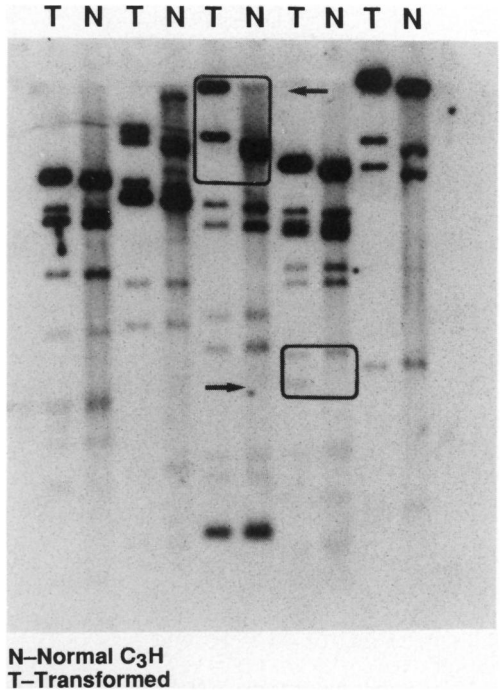

FIG. 25. Southern blot from a γ-ray-transformed focus of C3H 10T1/2 cells (T), compared with "normal," i.e., untransformed, cells (N). Six restriction enzymes were used, from left to right, *Pst*I, *Eco*RI, *Bam*HI, *Sac*I, *Pvu*II, and *Bgl*II. In two cases, indicated by the boxes, there is a difference between the DNA of normal and transformed cells which is consistent with a deletion in the DNA of the transformed cells. [Unpublished figure; described in (*33*).]

of evidence are consistent with the possibility that a deletion is responsible for the activation of the transforming gene in this γ-ray-transformed cell line.

CONCLUSION

During the time that I have been involved with radiobiology, which spans more than three decades, the tools of the trade at the cutting edge of research have changed from whole organisms, to cells, to DNA, to genes. We have gone from observing phenomena to understanding mechanisms.

TABLE I
Oncogenes Tested for Homology to pC[γ]3-1

1. N-*neu*	9. c-*myc*	17. *rel*
2. v-*ras*^{k}	10. *fos*	18. n-*myc*
3. N-*ras*^{n}	11. v-*erb*B	19. v-*fms*
4. v-*ros*	12. v-*myb*	20. v-*fes*
5. *ret* 3.3	13. c-*yes*	21. v-*abl*
6. B-*lym*	14. c-*mos*	22. v-*src*
7. *ret* 1.2	15. v-*sis*	23. v-*erb*
8. c-*raf*.1	16. v-*ras*^{h}	

In many ways the field has come of age. This thought prompted the title "From Beans to Genes."

At the same time we have, in a sense, come full circle. Much of the excitement at the moment revolves around the exploitation of the techniques of molecular biology in the field of radiation biology. But when we have sequenced the repair genes in human cells, and identified the oncogenes activated, or suppressor genes deleted in radiation-induced cancer, what then? We will still be faced with the same pragmatic aims of radiation biology that were prominent on the agenda in 1953, namely: (1) How do we arrive at realistic risk estimates for radiation-induced cancer and genetic effects—i.e., how safe is safe enough? (2) How do we further improve treatment strategies for the radiotherapy of human cancer?

These are still the principal goals of radiation research, the pragmatic aims that justify the search for mechanisms. They are a challenge to the new generation of radiation researchers just as much as they were a challenge when I was a graduate student at Oxford. The more things change, the more they stay the same, which is why this lecture carries the subtitle "Back to the Future."

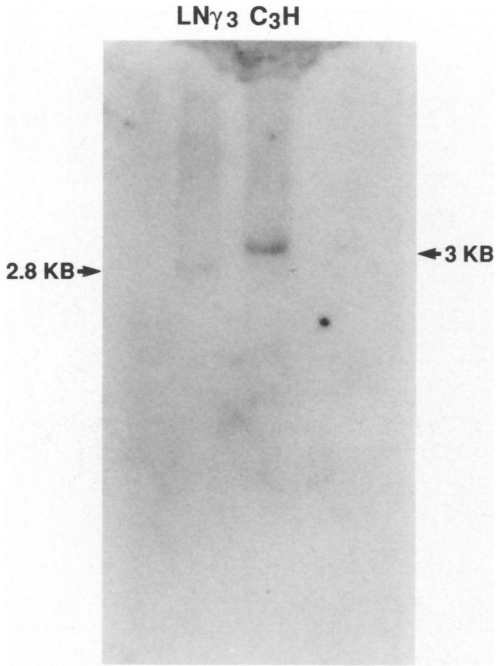

FIG. 26. Northern blot comparing RNA from a transformed cell line induced by γ rays (LNγ3) with normal C3H 10T1/2 cells. The signal is smaller, i.e., has travelled further on the gel from the transformed cells, which is also consistent with a deletion. [Unpublished figure; described in (33).]

ACKNOWLEDGMENTS

The author takes pleasure in acknowledging the contributions of students and colleagues, at Oxford during the early years and for the past two decades at Columbia, for it is in the cut-and-thrust of everyday discussion that ideas are formulated and hypotheses tested. Long-term research support has been provided by the United States Department of Energy (Grant DE-FG02-88ER60631) and by the National Cancer Institute (Grants CA12536, CA49062, CA24232, CA44128, CA18506).

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