Radiation and the single cell: the physicist's contribution to radiobiology

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The Fourteenth Douglas Lea Memorial Lecture†

Radiation and the Single Cell: The Physicist's Contribution to Radiobiology

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When Wilhelm Conrad Roentgen, in the course of performing experiments with discharge tubes, discovered a new kind of ray he became the father of radiation physics. Within a matter of weeks he made an exhaustive study of the absorption of the new rays, which he called X-rays, in a variety of materials; like so many physicists before and since, he then turned to biology! However, when the idea occurred to him of interposing a human hand in the beam, some natural caution, inbred in most physicists, prevented him from using his own; instead he persuaded his wife to put her hand in the beam, and in this way the first radiograph was produced. As historical fact, therefore, Roentgen, a physicist, was also the father of diagnostic radiology. Within two years of their discovery, X-rays were used therapeutically for the first time, when Professor Freund demonstrated the disappearance of a hairy mole to the Vienna Medical Society in 1897. At least one textbook has already named Freund the father of radiotherapy.

No one knows who is the the father of radiobiology—which was once described as being rather like a mule, inasmuch as it was born of crossed ancestry and has no future beyond the present generation. These factors may or may not account for the slow development of radiobiology compared with the rapid strides in the clinical uses of radiation in the early days. During the nineteen twenties and early nineteen thirties, just about every living species was exposed to X-rays in the name of radiobiology, but it is hard to identify much significant progress. This was 'phenomenological' radiobiology—they irradiated and they waited to see what would happen.

Against this background, two young physicists began their researches into the biological actions of radiation which marked the turning point in the fortunes of radiobiology. They were Charles Coulson, and the man in whose memory this lecture series is held, Douglas Lea.

The times of Douglas Lea, 1910–1947

The Douglas Lea Lecture is now an established institution in the Hospital Physicists' Association. This year, I feel that the time is ripe for a return to the original format of the lecture, for more to be said about Lea himself, and his place in the development of our speciality. A whole generation of young physicists have grown up in the HPA who did not meet Lea.

† Based on the lecture delivered to the Hospital Physicists' Association on 11 September 1975.

Douglas Lea was born in Liverpool on 8 February 1910. From Liverpool Collegiate School, he went with scholarships to Trinity College, Cambridge, in 1928. He gained firsts in Part I of the Mathematical Tripos in 1929, and in Part II (physics) of the Natural Sciences Tripos in 1931. Professor Charles Coulson was a fellow student for Part I, while Lea's wife, Eileen, as well as Professor J. S. Mitchell, were his fellow students for the second part of the tripos. He started research in physics at the Cavendish Laboratory at a time when Lord Rutherford's genius pervaded the laboratory, though Lea's discovery in 1937 of the capture of a neutron by a proton to form deuterium, with the emission of gamma rays, was associated more with Sir James Chadwick. Fig. 1 is a photograph of the research group at the Cavendish in 1933; many individuals who were subsequently to influence medical physics and radiobiology can be identified in this group. Lea was elected to a fellowship at

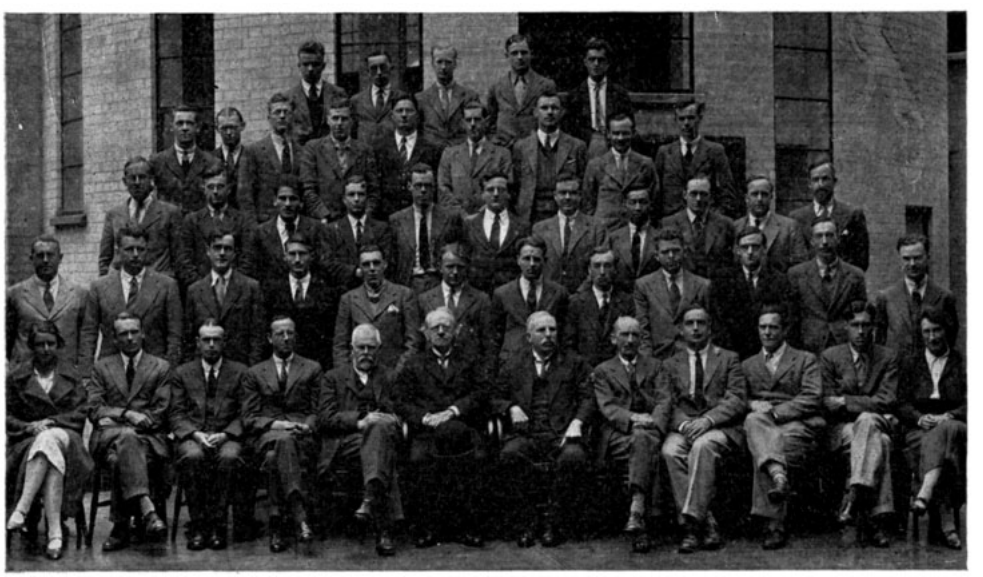

Fig. 1. Photograph of the research group at the Cavendish Laboratory, Cambridge, taken in 1933. (Photograph by courtesy of Mrs. Eileen Lea and the Cavendish Laboratory.)

W. J. Henderson, W. E. Duncanson, P. Wright, G. E. Pringle, H. Miller
C. B. O. Mohr, N. Feather, C. W. Gilbert, D. Shoenberg, D. E. Lea, R. Witty, –. Halliday, H. S. W. Massey E. S. Shire
B. B. Kinsey, F. W. Nicholl, G. Occhialini, E. C. Allberry, B. M. Crowther, B. N. Bowden, W. B. Lewis, P. C. Cho, E. T. S. Walton, P. W. Burbidge, F. Bitter
J. K. Roberts, P. Harteck, R. C. Evans, E. C. Childs, R. A. Smith, G. T. P. Tarrant, L. H. Gray, J. P. Gott, M. L. Oliphant, P. I. Dee, J. L. Pawsey, C. E. Wynn-Williams
Miss Sparshott, J. A. Ratcliffe G. Stead, J. Chadwick, G. F. C. Searle, Prof. Sir J. J. Thompson, Prof. Lord Rutherford, Prof. C. T. R. Wilson, C. D. Ellis, Prof. Kapitza, P. M. S. Blackett, Miss Davies

Trinity College in 1934 and received his Ph.D. in 1935. At this period, a letter he wrote to his close friend L. H. Gray (dated 3 June 1935) can be described as prophetic:

'Do you want to come back to Cambridge? . . . there are advertised as vacant three lectureships and one demonstratorship in the department of physics, caused by the disappearance of Chadwick, the retirement of Searle, and the departure of Feather. I am putting in for the demonstratorship as a matter of form, but there isn't any hope of obtaining it as Shire, who is senior to me, is also applying, apart from anyone else. *I am wondering whether or not to give up*

nuclear physics, and go over permanently to biological work. Nuclear physics is really frightfully overcrowded, there are about ten labs working at once on all the principal problems.'

What a galaxy of talent there was at the Cavendish at that time and what halcyon days for physics; but Lea could already see the writing on the wall. As Eileen Lea, his wife, put it in a letter to me recently, this turning to biology was the result of a deliberate search for an important unexplored field. At this time, Lea's close friendship with C. A. Coulson was a most important factor—two clever men, enjoying friendship and intellectual stimulation. Coulson explained this change from physicist to biologist in the Fourth Memorial Lecture (Coulson 1955).

Both Lea and Coulson had the singular good fortune to be elected simultaneously to fellowships at Trinity College. Both felt that this unique opportunity should be used profitably and wrote to half a dozen leading scientists in Cambridge asking them to identify the chief unsolved problems in their particular field. In the event, they elected to study the influence of radiations on bacteria, because there was plenty of evidence that this was important, but not properly understood. Lea was appointed as a physicist to the Strangeways Laboratory, first with a grant from the British Empire Cancer Campaign and then as a Prophit Student of the Royal College of Surgeons, succeeding L. H. Gray in this studentship.

Once experiments were under way, he wrote enthusiastically to Gray, who by this time had moved to London:

'I can strongly recommend the bacteriological technique—it seems to be very easy.

The bacteria to the number of, say, 100 are thus spread out over the bottom of the dish, when they have multiplied sufficiently (which only takes 24 hrs) they have so many descendants that each little colony is readily visible with the naked eye, so counting the colonies gives one the number of organisms which have survived the radiation. "Survival" here means retention of the power to reproduce of course.'

Lea at once recognized that until survival curves could be generated with good precision, it would not be possible to make any inferences regarding the mode of action of the radiation. He wrote in the first paragraph of his first paper in the field of biology (Lea, Haines and Coulson 1936):

'The mechanism of disinfection, however, remains obscure. Theories have been proposed, but little attempt seems to have been made to analyse the implications of the various hypotheses and point by point to confirm or disprove them. Moreover, some writers have ignored the fact that the physical processes accompanying the passage of various radiations through matter are fairly completely understood.'

This illustrates the impulse of the physicist to tackle the problem at its most fundamental level. From the experiments that followed, he was able to rule out the possibility that cell killing was due to a conventional chemical process, or a rise of temperature, and by extending his work from α-particles to β-rays, X-rays and γ-rays, he concluded that ionization was the dominant factor.

Lea was acclaimed by his contemporaries to be an intellectual giant. Fig. 2 is one of the few photographs of him available. He was a tall rugged man, with a thick mop of black hair. He was something of an athlete, loved physical exercise, and at one time took up rowing, but was too much of an individualist

to settle into the rhythm of a rowing eight. For much the same reason he was not regarded as a good committee man; in fact he resigned from all committees and told his friend Hal Gray that he did so because he had observed that the surest method of getting a resolution defeated was for him to defend it himself! Perhaps it is as well that his science, as well as his role in the HPA, was in the pre-Zuckermann era!

Fig. 2. Douglas Lea. (Photograph kindly supplied by Mrs. Eileen Lea.)

Physics in medicine and biology

We must view Douglas Lea, his experimental work as well as his attitude to life, against the background of his times. He chose to stay in Cambridge, but meanwhile, in the bigger population centres, events were moving rapidly in the application of physics to radiobiology. By the time of Lea's untimely death in 1947, Gray had moved to London, and while still intimately involved with radiotherapy physics had begun to perform the radiobiological experiments for which he is renowned. Indeed, Gray and Read and their colleagues had begun to dominate the radiobiological scene as a result of a series of classical experiments. They too had developed a simple biological system, commended to them by Mottram, namely the inhibition of root growth of *Vicia faba*. They made a number of important contributions, but in terms of its lasting impact, the most significant was the measurement of the extent to which cell killing by X-rays depended on the presence of molecular oxygen (Gray and Read 1942, Read 1952). The discovery of the oxygen effect, and the eloquent pleading of its

potential implications in radiotherapy, has in many ways dominated the thinking of radiobiologists, possibly out of all proportion to its true importance, for over two decades.

Single mammalian cells

The next highly significant development in radiobiology came from outside the field of radiology. Theodore T. Puck (fig. 3) was born in Chicago in 1916 and received the degrees of B.S. and Ph.D. at the University of Chicago. His subject was Physical Chemistry, but his Ph.D. thesis was supervised by an outstanding physicist and Nobel laureate, James Frank. From his mentor, Puck learned the simple, direct and quantitative approach. In a series of classic papers in the mid nineteen fifties, Puck and his colleagues made a major breakthrough by developing techniques to culture single mammalian cells, and to elucidate their response to various agents including radiation (Puck and Marcus 1956). The first X-ray survival curve is shown in fig. 4. It is difficult to overstate the impact that this development has had in radiobiology and radiotherapy.

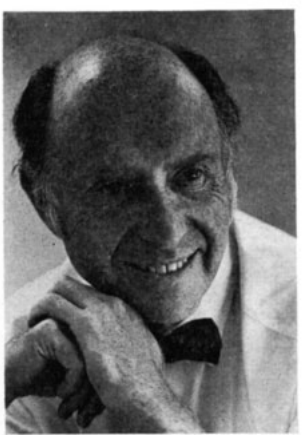

Fig. 3. Theodore T. Puck. Professor of Biophysics at the University of Colorado.

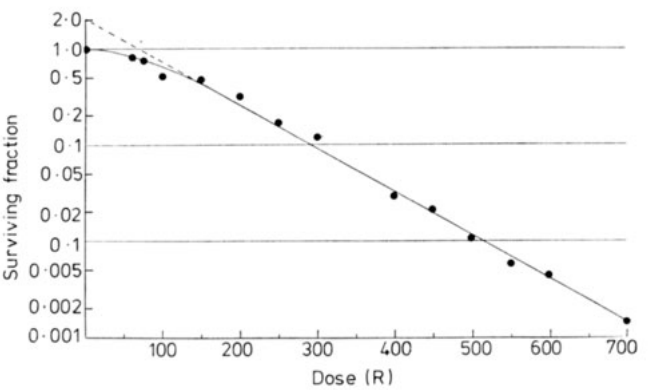

Fig. 4. The first survival curve for mammalian cells exposed to X-rays (from PUCK, T. T., and MARCUS, P. I., 1956, *J. Expt. Med.*, **103**, 653).

The cell culture technique developed by Puck and Marcus (1956) has allowed major steps forward in radiobiology, and has transformed the thinking of the radiotherapist. When the technique was first described, it generated great excitement in the radiobiological world, but the initial enthusiasm was not shared by everyone. Some were sceptical that cells growing in a petri dish, in very artificial conditions, could ever be a realistic model for radiation therapy studies. The fears and reservations of the sceptics were voiced with characteristic eloquence by Dr. Spear in the MacKenzie Davidson Memorial Lecture in 1957. He said that:

'An isolated cell *in vitro* does not necessarily behave as it would have done, if left *in vivo* in normal association with cells of other types. Its reactions to various stimuli, including radiations, however interesting and important in

themselves, may indeed be no more typical of its behaviour in the parent tissue than Robinson Crusoe on his desert island was representative of social life in York in the mid-seventeenth century.'

This was a damning and witty charge, but countered with equal brilliance by the late Dr. David Gould, then Professor of Radiology at the University of Colorado. He pointed out that the *in vitro* culture technique measured the reproductive integrity of cells, and that there was no reason to suppose that Robinson Crusoe's reproductive integrity was any different on his desert island from what it would have been had he remained in York; all that Robinson Crusoe lacked was the opportunity! The opportunity to reproduce to the limit of their capability is afforded to cells cultured *in vitro* when they find themselves in the petri dish, with temperature and humidity controlled, and with an abundant supply of nutrients.

At the time it required faith and optimism to believe that survival curves determined *in vitro* could be applied to the complex *in vivo* situation. Such faith and optimism were vindicated by the subsequent development of ingenious techniques to measure survival curves *in vivo*. In the first of these, the dilution assay technique, designed to produce a survival curve for lymphocytic leukaemia cells in the mouse, Charles Wilson, a member and past President of the HPA, was a co-author with Dr. Hewitt (Hewitt and Wilson 1959).

The *in vitro* cell culture technique captured the imagination of many radiobiologists who had trained initially as physicists, and much of the best work in the field has been performed by them. Elkind demonstrated the repair of sublethal damage, Barendsen and Todd independently catalogued the effectiveness of a wide variety of different radiation types from neutrons to heavy ions, while Sinclair demonstrated the age response function for X-rays and for neutrons (Elkind and Sutton 1960, Barendsen, Beusker, ver Groesen and Budke 1960, Todd 1967, Sinclair and Morton 1966). The appeal of the technique is that it is both quantitative and amenable to changes of physical conditions.

The current scene

So what has been the impact on clinical radiotherapy of more than forty years of experimental radiobiology in which physicists have played such a significant part? This has been in two areas. (a) The thinking of the radiotherapist has been transformed, and the teaching of aspiring therapists has been revolutionized, particularly in the United States, where now it can almost be described as a laboratory-based medical discipline. (b) The development funds, and much of the current excitement and interest in the field of radiation therapy revolves around the introduction and clinical trial of high LET radiations—a development based almost entirely upon radiobiological grounds. Table 1 lists the existing and projected high LET facilities in the world, which in sum represents a great deal of human effort.

The Radiotherapeutic Research Unit of the Medical Research Council at Hammersmith Hospital was first in the field in the post-war years, and it is impossible to overstate the importance of the pioneering work done by them. The careful and extensive preclinical measurements in both physics and radiobiology allowed the safe introduction of neutrons into clinical use; everyone who follows owes them a debt, and once again it was a physicist, this time Dr. J. Fowler, who did much of the experimental work, and maintained enthusiasm

Table 1. Existing and projected high LET facilities

Particle	Facility		Energy (MeV)
Neutrons	Cyclotrons	Hammersmith (Edinburgh)	15
		Seattle	22
		NRL	35
		TAMVEC	50
		Tokyo	35
	14 MeV $d^+ \rightarrow T$	Rijswijk	
		Manchester	
		Glasgow	
		Hamburg	
Pi mesons		Berkeley	
		Nimrod (Harwell)	
		Los Alamos	
		TRIUMF	
		Zurich	
		Stanford	
Heavy ions		Berkeley (BEVALAC)	

Table 2. Cyclotron-produced neutrons for radiotherapy (using the $d \rightarrow Be$ reaction)

Facility	Location	Deuteron energy (MeV)	Treatment distance (cm)	Depth for 50% dose (cm)	Dose rate (rad min^{-1})
Hammersmith	London, U.K.	16	125	8	40
University of Washington	Seattle	22	150	10	20
Naval Research Laboratory	Washington, D.C.	35	125	11·6	60
TAMVEC	College Station, Texas	50	125	15	70

for the work throughout (Fowler 1967). The clinical trial of neutrons received a significant boost when Hammersmith was joined by the three big U.S. cyclotrons, the characteristics of which are listed in table 2. These machines all have higher energies than that at Hammersmith, and therefore better depth doses, particularly in the case of TAMVEC and NRL, where the percentage depth doses rival those of a 4 MeV linear accelerator. At first it was feared that the higher energy machines would be characterized by a larger oxygen enhancement ratio (OER) because of the longer range of the recoil protons produced and the lower average LET at which their energy is deposited. However, it turns out that at the higher energies, spallation products are of increasing importance; as well as recoil protons, the neutrons produce densely ionizing α-particles by

interacting with atoms of carbon and oxygen (fig. 5). Microdosimetric measurements made at the NRL cyclotron are shown in fig. 6; although responsible for only a modest fraction of the dose, these heavy recoils dominate the biological

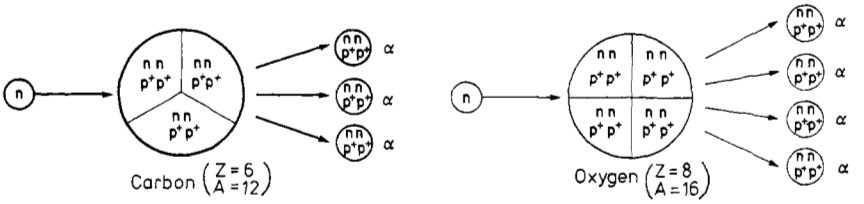

Fig. 5. Illustrating the production of spallation products. As the neutron energy rises, the probability increases of a neutron interacting with a carbon or oxygen nucleus to produce three or four α-particles respectively.

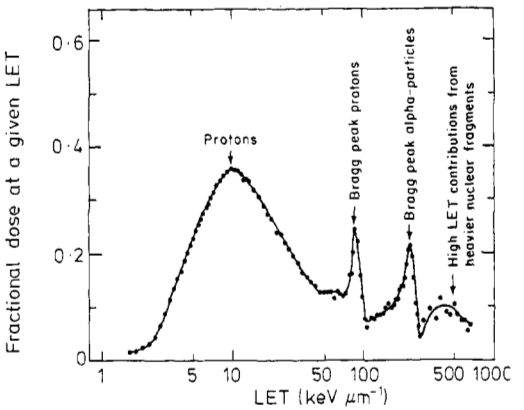

Fig. 6. Microdosimetric data for the neutron beam generated at the Naval Research Laboratory Cyclotron (35 MeV d⁺ → Be). A small proportional counter was used to measure the fraction of the absorbed dose associated with each increment of linear energy transfer. The contribution from the secondary recoil protons, the α-particles produced by spallation, as well as heavier nuclear fragments can be clearly seen (from Hall, E. J., Roizin-Towle, L., and Attix, F. H., *Int. J. Radiat. Oncology, Biol. & Physics*, 1975, **1**, 33–40).

response and account for the low OER of the big U.S. cyclotrons (fig. 7). Recent measurements at the University of Maryland cyclotron show that if the energy of the bombarding deuterons (or protons) is raised still further, the OER falls towards unity. It would be an ironic twist of fate if it turned out, in retrospect, that the cyclotron energies chosen for the clinical neutron trials correspond to the *maximum* possible value for the OER obtainable with neutrons! Radiobiological measurements with neutrons have, virtually, been completed now and the much more difficult and time-consuming phase of clinical evaluation has begun.

Meanwhile sources of negative pi mesons are barely ready for experimental study, although the first cancer patient was treated at Los Alamos late in 1974. The dose delivered by a beam of pions to tissue-like medium increases slowly with depth in the beginning, but gives rise to a sharply defined maximum near the end of their range. During the first few centimetres of their absorption in

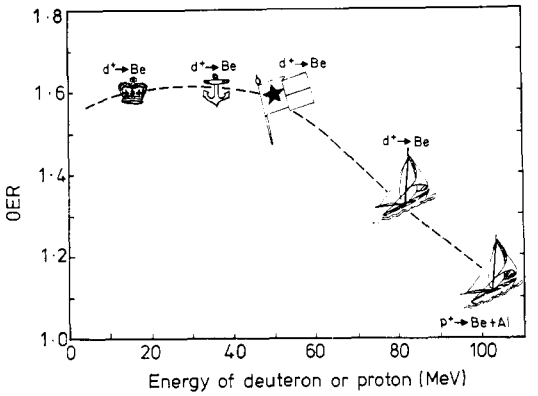

Fig. 7. Oxygen enhancement ratio (measured with *Vicia*) as a function of deuteron or proton energy for cyclotron-produced neutrons (Institutions, left to right, MRC, NRL, TAMVEC, University of Maryland).

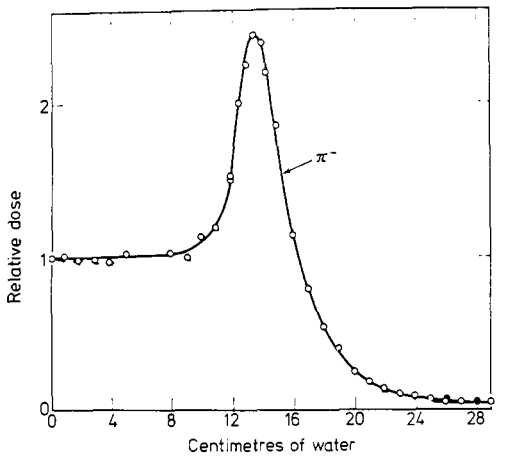

Fig. 8. Depth–dose distribution of a 65 MeV pi meson beam in water (from RAJU, M. R., LAMPE, E., and CURTIS, S. B., 1971, *Phys. Med. Biol.*, **16**, 599–610).

unit density material, pions behave like 'over-weight' electrons and are sparsely ionizing. As they come to rest, they are captured by nuclei of the medium which disintegrate into short range densely ionizing fragments; this constitutes the so-called 'star' production. This concentration of energy deposited near the end of the range of pions (fig. 8) is the reason for the enormous interest that has been shown in them, because it offers the exciting possibility of being able to concentrate dose within a designated tumour volume, while minimizing the dose to surrounding normal tissue. Not only this, but the biologically effective dose in the tumour volume is further enhanced because of the high relative biological effectiveness and reduced oxygen enhancement ratio characteristic of the high LET component of the radiation. This is what pion therapy is all about, and the justification for the large sums of money spent on its development. The accelerator at Los Alamos was the first to be used to treat cancer patients with pions. It was built by the United States Atomic Energy Commission (now

ERDA) at a cost of $57 million. It is used principally for high energy physics research, but a fraction of the beam is diverted into a specially constructed biomedical facility. Its enormous size and spectacular location high on a mesa in New Mexico are evident from the aerial photograph reproduced in fig. 9.

These new developments in radiotherapy may or may not lead to improvements in cure rates. The improvements may be spectacular or barely worth while; across the board or limited to a few specific types of tumours. But whatever the outcome may be, the trial of neutrons and pions is based on sound physical and biological principles.

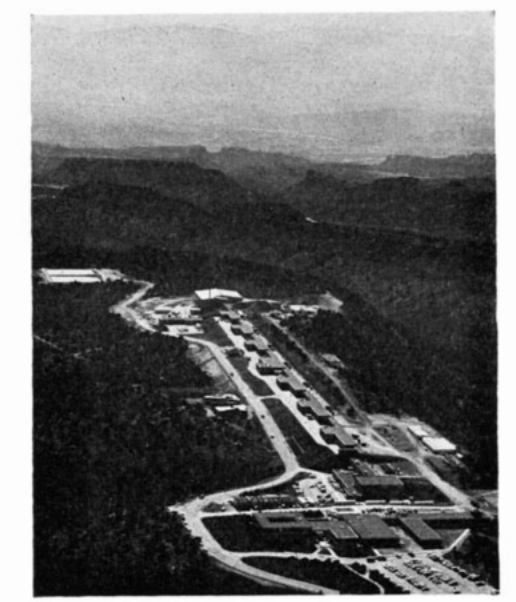

Fig. 9. The Clinton P. Anderson Los Alamos Meson Physics Facility. This machine, built by the USAEC (now ERDA) at a cost of $57 million, is over half a mile in length. Its enormous size and spectacular location high on a mesa in New Mexico are both evident from this aerial photograph. Pions produced by this accelerator are used experimentally for the treatment of cancer. (Photograph by courtesy of Los Alamos Scientific Laboratory, New Mexico.)

In vitro transformation

The single-cell culture technique, used with such success to produce survival curves for cell killing, has more recently been adapted to study transformation to a neoplastic state. Borek and Sachs (1966) first demonstrated the *in vitro* transformation of mammalian cells by X-rays. The technique has been further developed into a quantitative system to elucidate the shape of the dose–response relationship for X-ray transformation by Borek and Hall (1973). The cells are prepared from fresh explants of hamster embryos, and minced up and made into a cell suspension before being seeded into petri dishes. They are irradiated as single cells and then allowed to grow into colonies. Examples of stained colonies are shown in fig. 10. In the normal colonies, the cells are orderly and show contact inhibition. There is a low incidence of transformed

clones, identified by their piled-up morphology, random cell orientation and loss of contact inhibition—characteristics not seen in the untreated control cultures. The neoplastic nature of these transformed clones has been confirmed in a number of ways; their ability to grow in low serum concentration or in soft agar, their agglutinability by plant lectins, and ultimately their ability to produce tumours (usually fibrosarcomas) when injected back into animals.

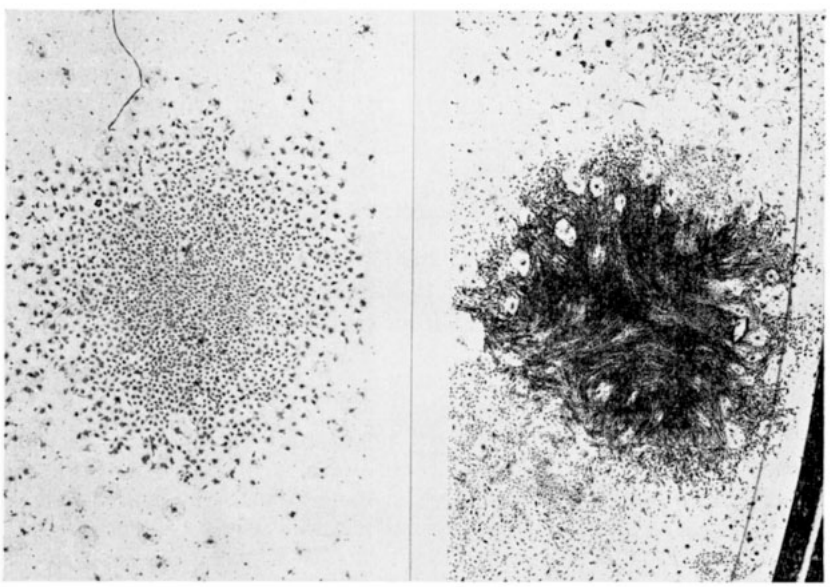

Fig. 10. Colonies formed from hamster embryo cells. On the left is a normal colony; note that the cells are orderly in appearance and show contact inhibition. On the right is a colony arising from a transformed cell; note the random cell orientation and loss of contact inhibition. (Photograph by courtesy of Dr. Carmia Borek.)

The shape of the dose–response curve for transformation by X-rays is shown in fig. 11. Doses as low as 1 rad can be detected, though at this level the technique becomes tedious since the incidence of transformed clones is only one in 8000. Between 1 and 75 rad the incidence of transformation increases with dose, reaching a plateau of about 1% for doses from 100 to 300 rad. For still higher doses, the transformation incidence falls, in spite of the fact that cell killing is inherently accounted for in the technique, since only surviving clones are scored. This implies that cells transformed by radiation are more susceptible to cell killing by radiation.

The contrast between normal and transformed cells shows clearly in pictures taken with the scanning electron microscope. Normal cells show orderly growth and contact inhibition, in contrast to the random piled-up appearance of the neoplastic cells. The surface of the normal cells is smooth, while transformed cells appear to have a highly structured surface with what are called 'blebs' very much in evidence. The only time when the surface of a normal cell looks like a transformed cell is during mitosis; this is a very interesting fact, since uncontrolled division is the hallmark of malignancy.

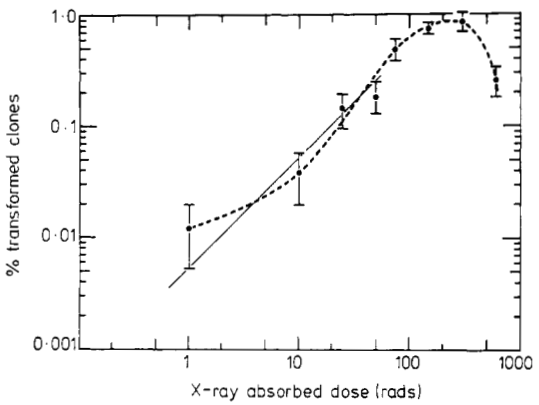

Fig. 11. Dose response for X-rays. Incidence of hamster embryo cell transformation following *in vitro* exposure to X-rays. For doses at which more than one experiment was performed, the data were pooled; the mean value together with the standard deviation are plotted in the figure. The broken line is fitted by eye to the mean data points; the full line has a slope of +1, and passes through the error bars of each datum point (from BOREK, C., and HALL, E. J., 1973, *Nature*, **243**, 450–453).

This relatively new technique opens the door to many new studies in carcinogenesis, which were difficult to tackle *in vivo* because of the very large number of animals required; dose fractionation, dose rate, combination with chemical carcinogens, high LET radiations, to name but a few. I like to think that it is the sort of technique that would have appealed to Douglas Lea. He would most certainly have used it with great effect, because it is both quantitative, and can be used at low doses. One can imagine the letter he might have written to a close colleague, bubbling over with excitement at discovering a new methodology—as he did in 1935 when first shown the bacterial colony assay method. He might have written that it is relatively simple, repeatable, quantitative, and addresses a most relevant and urgent question of the time. What more could a physicist want in his wildest dreams?

What has been the contribution of the physicist to radiobiology at every stage? To be quantitative; to work with simple systems and to deduce basic principles that have a general application. This is the legacy that we have inherited from men like Douglas Lea. It is clearly difficult to follow in the footsteps of one who walked with such majestic strides, but it is evidently our duty to try.

It is a pleasure to record my gratitude to a number of people whose help was invaluable in the preparation of this paper. Mrs. Eileen Lea gave me a better understanding of the life and work of her husband, and also kindly provided me with a number of original documents and photographs. Professor J. Mitchell generously gave me several original letters of Douglas Lea. Professor J. Boag kindly donated copies of correspondence between Lea and Gray dating from the nineteen thirties. I am grateful to all of these individuals, and many more besides.

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