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The role of repair in radiobiology

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Summary. Apart from cancer and mutation induction, radiobiological effects on mammals are mostly attributable to cell 'death', defined as loss of proliferative capacity. Survival curves relate retention of that capacity to radiation dose, and often manifest a quasi-threshold ('shoulder').
The shoulder is attributable to an initial mechanism of repair ('Q-repair') which is gradually depleted as dose increases. Another form of repair, which is not depleted ('P-repair'), increases the dose required to deliver an average of one lethal event per cell (dose 'D_0'). Neither form of repair can unambiguously be linked with repair of defects in isolated DNA. An important initial lesion may well be disruption of the complex structural relationship between the DNA, nuclear membrane and associated proteins. One form of P-repair may be restoration of that structural relationship.
Key words. Repair; cell survival; fractionation; sublethal; potentially lethal; relative biological effectiveness (RBE); DNA breaks; DNA synthesis; P-repair; Q-repair.

Introduction

Before nuclear energy became available for both peaceful uses and weaponry, interest in the effects of ionizing radiation on man was centred on its use for medical diagnosis and for the treatment of disease – mainly cancer. Attention had to be paid also to hazards of accidental or unavoidable incidental exposure: for example, to radiologists and radiographers. Some radiobiologists and radiotherapists recognized that cancerous tumours regressed after irradiation because the malignant cells had lost their capacity to reproduce themselves; but concurrent damage to the normal tissues of treated patients was not in those days regarded as the consequence of randomly occurring events in individual cells. Studies on intracellular effects in organized tissues focussed on the induction of chromosomal aberrations (plant cells, tissue cultures), and on the induction of heritable changes in germ cells, mainly in plants and the fruit-fly, *Drosophila*. After 1945, however, considerable effort was made in many countries to learn in general about radiation effects on mammals, for the most part laboratory animals, with the object of extrapolating to man. A good deal of attention was initially devoted to 'LD 50' doses, i.e. those necessary to kill 50% of an animal population within a certain time. A voluminous literature accumulated on biochemical and physiological effects of irradiation. Because death was thought to be attributable to complex changes in metabolism, consequent on irradiation. But an experiment by Quastler[33], conceived in very simple terms, demonstrated that there were basically three modes of death, depending on the dose range. The lowest range, 4–6 grays for most mammalian species, killed 50% of the animals within 15–30 days. Death occurred at 4–5 days after irradiation by doses in the range 10–100 grays ; after greater exposures, death occurred within a few hours.
It gradually emerged that the first two of these three modes of death depend on the loss of proliferative capacity by stem cells in tissues that require continual replenishment of functioning cells. This is the case with haemopoietic' tissue and with the cells populating the surface of the villi in the intestinal tract. One of the most easily evident effects of radiation on cells of all classes is indeed

the loss of their ability to give rise to daughter cells, when they would normally do so; in other words, irradiation causes cells to lose their 'viability', in the sense in which that term is used in microbiology. Destruction of a cell's viability is commonly referred to in radiobiology as 'cell death', or as a 'lethal effect': terms which must be understood to have that restricted meaning, because cells 'killed' in that sense often retain some biochemical functions, like respiration, virtually intact. Throughout this article the terms 'cell death', 'lethal effect' etc. will carry that restricted meaning. Cells retaining their ability to reproduce themselves after irradiation will be called 'surviving cells' or 'survivors'.

Reference was made above to a third mode of death in irradiated animals, occurring within hours after very large doses (>100 Gy). This is attributed to prompt loss of function by the cells of the nervous system. Effects of such large doses are assumed to be outside the scope of this general review.

Once it had become clear – after considerable controversy! – that the death of stem cells was the reason for '30-day (haemopoietic) death' and '4-day (intestinal) death' in mammals, it gradually came to be accepted that damage to normal tissues concomitant with radiotherapy treatment has its origin in loss of proliferative capacity by stem cells: with direct consequences, as in the failure of provision of functioning cells, in tissues requiring constant renewal thereof; or more indirectly, where the failure of stem cells to divide might trigger a series of cellular and therefore tissue malfunctions[27]. It is a valid generalization that 'Radiation Effects on Man and Animals' have their origin in intracellular effects: by inhibition of division in those cells whose normal function is to reproduce themselves; or by somatic mutations, which might be carcinogenic; or by gene mutations in germ cells. The title of this contribution is therefore to be interpreted in a restricted sense: 'The Role of Repair in *Cell* Radiobiology'. Only repair which is relevant to cell survival will be discussed, since cytogenetics[37] and mammalian genetics are dealt with in companion papers.

It may seem obvious today that biological evolution needed, and included, the development within cells of capacity to repair environmental damage to structures the integrity of which is needed for proliferation. 'DNA repair' is currently an important part of cell biochemistry and molecular biology research. That line of work owes its inception to the observation that radiation damage might be repaired. In the first instance this was recognized for the killing of bacteria by ultraviolet light (UV) at wavelengths specifically absorbed by nucleic acids. Considerable progress has been made in identifying enzymes which mediate repair of UV-induced damage to DNA, and correlating their activity with cell survival. Analogous success is wanting, in the case of ionizing radiation. It may well be relevant that energy from the latter, unlike far UV, is deposited in all cell constituents at random; but attempts to elucidate the biochemical mechanisms of repair have been focussed overwhelmingly on DNA alone.

Quantification of 'cell death'/'cell survival'

Description of intracellular repair as an operational phenomenon requires consideration of methods for quantifying radiation-induced cell 'death', i.e. failure to give rise to a clone of similar cells. In practice, it is normally *success* in reproduction that is recognized: for example in vitro, by the appearance of colonies on plates. From colony counting, or other assays suitable for calculating the fraction of irradiated cells that have succeeded in reproducing themselves, a relationship is established between a series of graded doses and the fractions of the irradiated population that have survived those doses. Only seldom can the relationship be expressed in a precise mathematical form; it is much more usual to display it graphically. It is customary, and convenient, to plot surviving fraction on a logarithmic scale, with dose on a linear scale. On a linear scale the graphical representation of data for small surviving fractions (less than 1%, say) would be of no visual help. But, more importantly, it is of great theoretical interest to test whether a survival curve is exponential (a straight line, on a semi-logarithmic plot); or whether a 'shouldered' curve has a well-defined terminal exponential region. The slopes of exponential curves or of terminal regions of curves are highly

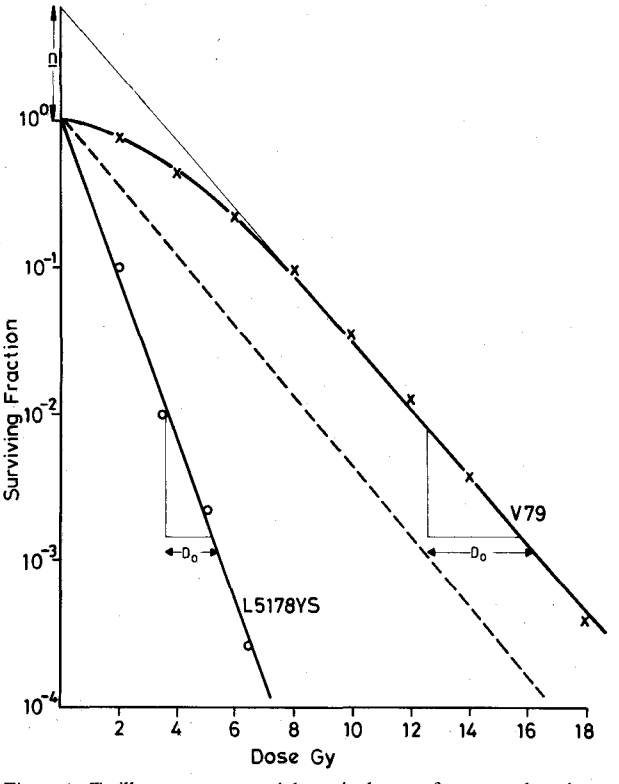

Figure 1. To illustrate exponential survival curve for mouse lymphoma strain L5178Y/S and shouldered survival curve for Chinese hamster lung cells, V-79. (Adapted from fig. 1., Cramp et al.[10])

significant for appreciation of the extent and nature of repair phenomena.

Figure 1 shows an exponential survival curve for the sensitive variant of mouse lymphoma strain L5178 Y/S, irradiated by X-rays[10]. Also in figure 1 is a survival curve for the cell line V79, originating from embryonic hamster lung. It shows a pronounced shoulder, merging into a straight line, i.e. an exponential region. In current radiobiology literature frequent use is made of the so-called 'linear-quadratic' expression for such curves,

$$\log \text{surviving fraction} = -\alpha D - \beta D^2 \quad (1)$$

where D is the dose, α and β are constants. Equation 1 does not provide for an exponential region at the higher doses. The equation is a good description of the relationship between survival and dose in the shoulder region; but there is good evidence that, when experimental artefacts are avoided,[6] shouldered survival curves taken to low survival levels do terminate exponentially[3]. Equation 1 can in general not be fitted to survival data including surviving fraction of $10^{-3.5}$, and less, when precautions are taken to avoid artefacts that are easily encountered when such low levels of survival are handled[20].

Significance of the slope of an exponential region in a survival curve

Exponential survival is to be expected if there is a given probability of cell killing by a single energy deposit or 'hit'. Suppose that dose D_0 is needed to deliver an *average* of one lethal event per cell in a total population of N_0 cells. Because energy is deposited at random by ionizing radiation, the dose will be distributed among the population in accordance with the Poisson distribution; so a fraction e^{-1} of the population will have experienced no hits, and will be survivors. It follows that, after any dose D, the fraction that has experienced no hits, i.e. the surviving fraction S, is given by

$$S = N/N_0 = (e^{-1})^{D/D_0} \quad (2)$$

Equation 2 cannot describe a shouldered survival curve such as that shown in figure 1; but it is useful to describe the terminal region thereof by the equation

$$S = ne^{-D/D_0} \quad (3)$$

where n is the number at which the back-extrapolation of the terminal exponential region intersects the zero-dose axis, and is commonly known as the extrapolation number. The parameter D_0 has precisely the same significance for the terminal region of the shouldered curve as it does for one that is exponential over the whole range: namely, the dose that will deliver an average of one hit per cell in the irradiated population. Therefore a decrease in slope (slope $= D_0^{-1}$) signifies an increase in the dose required to deliver an average of one lethal hit per cell, and vice versa.

Significance of the parameter n (extrapolation number): Q-repair

At the time of writing this topic is somewhat controversial. For some years, most radiobiologists believed that the shoulder to a survival curve manifested the requirement for an accumulation of energy deposits within the cell nucleus before the cell would lose its proliferative capacity: either there were multiple, redundant targets, any surviving one of which would be sufficient for successful cell division; or else each target (one or more per cell) required more than one hit for inactivation. Indeed, what is now referred to as the extrapolation number used at one time to be called the 'hit' or 'hitness' number. Shouldered survival curves are sometimes described as giving evidence of the need for 'damage accumulation' before cells are killed. On that view, the shoulder region represents the range of doses within which the majority of cells have not yet experienced enough hits to be killed: they are said to have sustained 'sublethal damage'[5]. On such interpretations, the parameter n should relate to a real 'target number' or 'hit number'. But there is a considerable body of evidence to the effect that n can have no such real meaning. Extrapolation numbers to survival curves are well known to be variable, from day to day for any one cell line. They depend on methods of growth of the cells, and/or quite subtle details of their handling before irradiation. Variation of n through the cell cycle, from 1 to a very large number, is a well-known phenomenon (e.g. fig. 2)[40]. Difficult as it is to conceive of pre-irradiation conditions, or changes in the stage of DNA synthesis, bringing about changes in target or hit numbers, the attachment of any real meaning to n, in the 'damage-accumulation' sense, becomes impossible in the light of changes brought about by *post*-irradiation handling. Figures 3 and 4 show two examples[17]. To suppose that target or hit numbers, or, in any other sense, the amount of damage that needed to be accumulated, could be changed *retrospectively* is hardly conceivable! Clearly a conceptually different interpretation of shouldered survival curves is required.

In his discussion of shouldered survival curves for bacteria, Powers[30] put forward an idea, subsequently adopted and developed by several authors[1,16,32], the basis of which is presented graphically in figure 5. It is postulated that cells for which such curves are valid have an intrinsic capacity for repair which is depleted as dose increases. The initial slope of the survival curve is therefore less than it would be, if the repair capacity were absent; as it lessens with increasing dose, the slope of the curve increases, until, after full depletion, the curve attains the

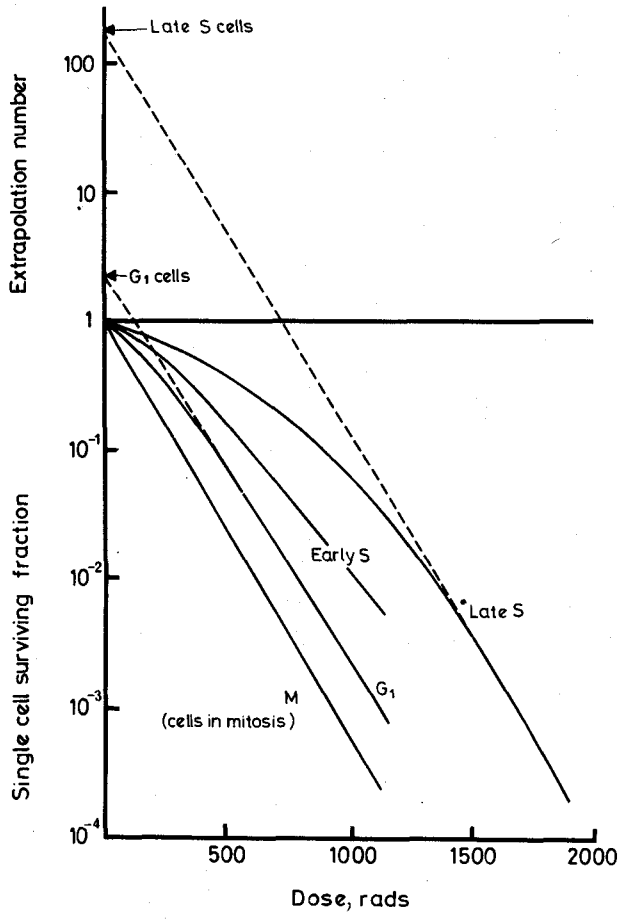

Figure 2. Survival curves for synchronized V-79 cells in different stages of the cell cycle. (Adapted from fig. 9, Sinclair and Morton[41])

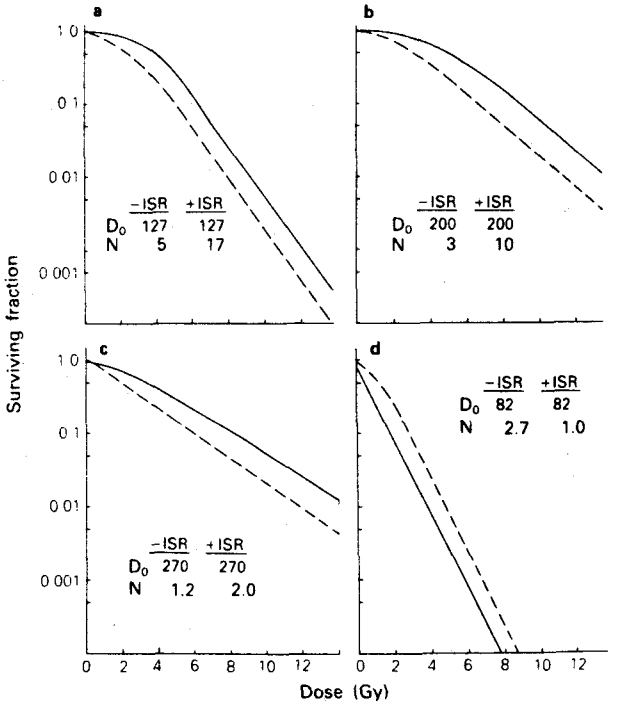

Figure 4. Each panel shows the survival curve for the parenchymal cells of a specific organ (mammary (**a**); thyroid (**b**); liver (**c**); bone marrow (mouse (**d**). In each panel the dashed line represents cell survival of cells removed immediately after irradiation while the solid line represents the survival of cells that are allowed to remain in situ after irradiation before removal for assay. (From fig. 1, Gould et al.[17])

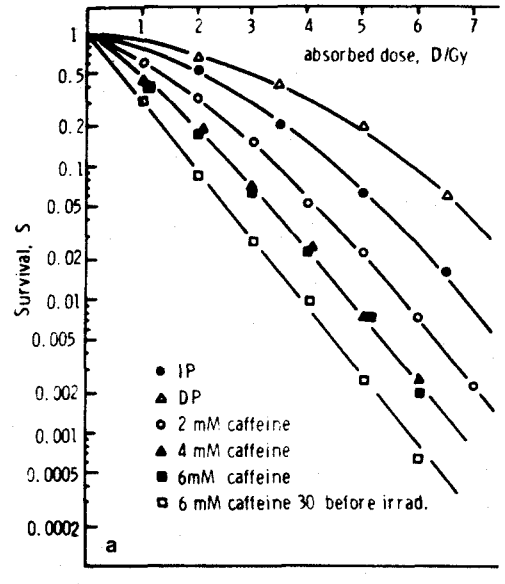

Figure 3. Survival curves for V-79 cells in G_1 phase treated after irradiation by caffeine at various concentrations. (From fig. 7, Iliakis and Nüsse[22])

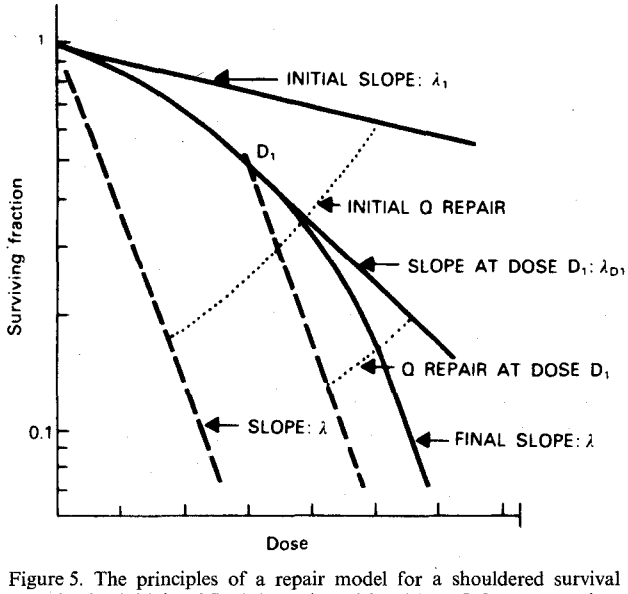

Figure 5. The principles of a repair model for a shouldered survival curve having initial and final slopes λ_1 and λ. With no Q-factor operating, curve would be exponential with slope λ. With Q-factor present in the unirradiated cells, the fraction of otherwise lethal lesions initially repaired is λ_1/λ. After dose D_1 the slope of the curve is increased to λ_{D_1}.

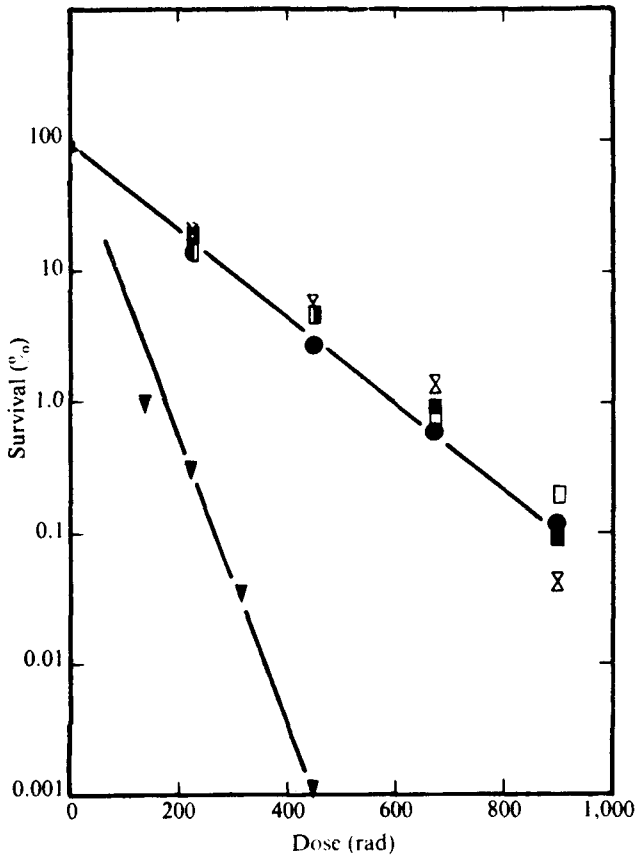

Figure 6. Survival curves for skin fibroblasts from human donors: upper curve, from four normal subjects; lower curve, from a patient with Ataxia telangiectasia. (Adapted from fig. 1, Taylor et al.[42])

slope it would have had if that particular mode of repair had been absent from the unirradiated cells. An example of cells lacking this postulated repair capacity is provided by some lines of freshly cultured human skin fibroblasts (fig. 6)[41]. But, as explained below, those cells evidently have capacity for one or more other modes of repair.

From his work on the change in the radiation response of mammalian cells through the cell cycle (fig. 2) Sinclair[39] concluded that there was some factor, varying through the cell cycle, which 'could repair radiation damage before it was expressed'. He called the postulated factor 'Q' and that name was adopted in later publications supporting the 'repair-depletion' interpretation of shouldered survival curves[1-3]. Accordingly it is postulated that Q-factor is the agent of Q-repair; possibly the damage so repaired is of a specific form, namely Q-lesions.

According to that interpretation of shouldered survival curves, the parameter n has nothing whatever to do with the accumulation of damage to target structures; its value depends on the concentration of Q-factor and on whatever biochemical support is available for the efficacy of the repair it achieves. The concept of 'sub-lethal damage', in the sense in which that phrase is still commonly used, cannot be accommodated by the repair-depletion model. On the other hand, observations such as those illustrated by figures 3 and 4 can readily be accounted for. Evidence on the effects of caffeine also on UV-irradiated cells supports the hypothesis that its presence inhibits repair (fig. 3), particularly Q-repair. An interesting feature of fig. 3 is that the curve on the extreme left is fully exponential and at the same time clearly parallel to the terminal region of the curve for untreated cells. This lends support to the argument that shouldered curves do, in general, terminate in exponential regions.

Gould et al.[17] found it to be true of parenchymal cells of several kinds that the value of n was less when tissue was excised immediately after irradiation, for survival curve determination, than when it was left in situ for 24 h (fig. 4). This 'in situ repair', as they termed it, was evidently the converse of the differences in n reported by Durand and Sutherland[12] for cells irradiated as single cells, or as 'spheroids' (small collections of cells in three-dimensional contact with each other). The larger value of n for the latter depended on a property that was lost gradually, over hours, after the spheroids were dispersed into single cells. Perhaps Q-factor is lost from cells when three-dimensional contacts with others are disrupted; or perhaps Q-repair is more effective when such contacts exist.

Effect of dose-fractionation, and its association with Q-repair

From early days of radiotherapy it was recognized that damage to patients' normal tissues was much reduced if the total dose was delivered in fractions, with one or more days between them, rather than all at once. The cellular basis for this phenomenon was demonstrated first with the alga, *Chlamydomonas*[23], and then with mammalian cells (cultured from Chinese hamster embryonic ovaries) grown in vitro[13]. The sparing effect of dose fractionation is associated with shoulders to survival curves, as shown by figure 7. Experiments of Elkind and Sutton[13] demonstrated that if cells surviving a given dose D_1 were allowed a radiation-free interval, before further irradiation, those survivors responded as if they had not previously been exposed: further graded doses elicited a secondary survival curve almost exactly repeating the one constructed with graded doses each following directly on the one before. In other words, the secondary survival curve was again shouldered. After dose D_1 followed by a sufficiently long interval, there were almost exactly n times as many surviving cells as there would have been with no fractionation interval.

In accordance with the then rather generally held view that a shouldered survival curve implied acquisition by the cells of 'sub-lethal damage', the sparing effect of dose fractionation became generally known as 'repair of (or recovery from) sub-lethal damage' – 'SLD repair'. A radiation-free period of the order of hours is generally

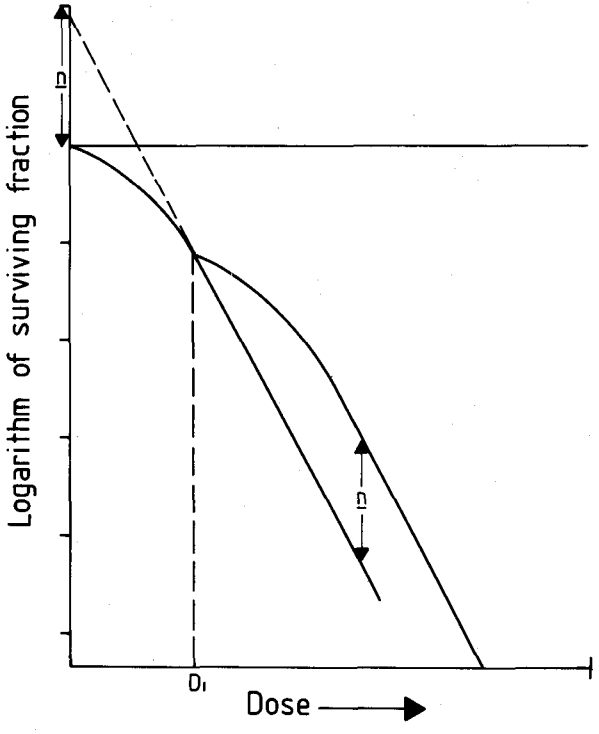

Figure 7. To illustrate that cells surviving dose D_1, then allowed a radiation-free interval, will respond to further radiation with the shoulder to the survival curve fully restored.

required for manifestation of the fully shouldered secondary survival curve, so it was assumed that such a time-span was needed for repair of all the postulated sub-lethal damage that the surviving cells had sustained. Those concepts, however, have no place within the repair-depletion interpretation illustrated by figure 5. Q-repair is regarded as an intrinsic capacity of cells that have not yet been irradiated, and it can act as soon as radiation lesions have been inflicted. The sparing effect of dose-fractionation is presumed, rather, to come from reconstitution or re-synthesis of Q-factor during a radiation-free interval, in cells which are still survivors, but in which all capacity for Q-repair has been depleted.

It is not always appreciated that these two postulated mechanisms for the sparing effect of dose fractionation are mutually exclusive. Shoulders to survival curves are sometimes regarded as giving 'evidence of repair': yet at the same time it is still supposed that the repair in question occurs during, and indeed requires, a radiation-free interval. According to the repair-depletion model, however, dose fractionation, and also low dose rates, are comparatively sparing in their effects because they provide time needed for restoration of Q-factor. Q-repair itself probably proceeds promptly after the cell has experienced a Q-lesion. If reappearance of Q-factor within the cell concomitantly resulted in some relatively slow repair of Q-lesion, during a radiation-free interval, there should be more survivors of dose D_1 (fig. 7) at the end of that interval than at the beginning. Such a phenomenon has never been observed in numerous split-dose experiments.

Repair of 'potentially lethal damage': 'PLD repair': 'P repair'

It would clearly be semantically acceptable to regard any form of repair that results in an increase in cell survival as 'repair of potentially lethal damage'. But use of that phrase in such a general way has led to considerable confusion, because there is evidence of modes of repair operating over the whole of the dose range used for constructing survival curves, whereas Q-repair, by hypothesis, operates only over the shoulder region. Repair which is *not* depleted in a dose-dependent manner must, of course, decrease the terminal slope of the survival curve. Hahn and Little [19] found that if cells in vitro were irradiated in the stationary phase of growth, the final slope of the curve was less when the replating of single cells was delayed for a time.

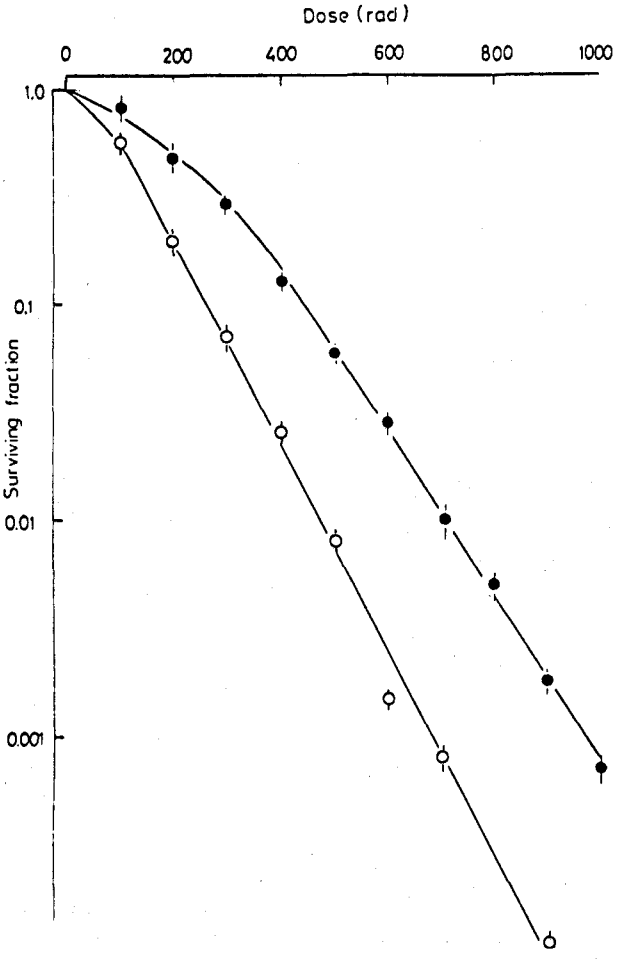

Figure 8. Dose response curves for inactivation of X-irradiated plateau phase cultures of normal human skin fibroblast. ○ = plated immediately after irradiation; ● = plated 6 h after irradiation. (Adapted from fig. 3, Cox [7])

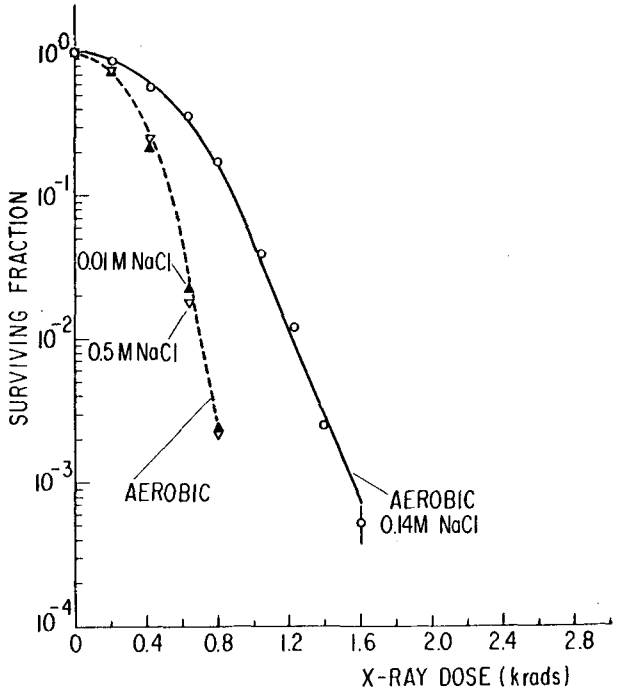

Figure 9. Survival curves for V-79 cells held in anisotonic or isotonic buffered slaine after irradiation. (Adapted from fig. 9, Utsumi and Elkind[43]).

Those authors referred to the phenomenon as 'repair of potentially lethal damage' ('PLD repair') and, to some extent, that phrase has come into use particularly in the context of a change in slope of the terminal region of the survival curve: i.e. a change in D_0 (fig. 8)[7]. Such observations made considerable impact because the PLD repair occurred only in cells in the stationary phase of growth at the time of irradiation. It seemed, therefore, to have relevance in the radiotherapy of cancerous tumours, if the phenomenon occurred also in vivo. Tumour cells that were in the stationary phase might be selectively 'radioresistant' because of PLD repair.

Effects in the opposite sense have also been described in terms of PLD repair. Some treatments applied after irradiation disclose the cells' liability to an increased radiation response, manifested as a decrease in dose to give an average of one lethal event per cell. An example is afforded by the holding of cells for a period in anisotonic conditions[11,41] (fig. 9). Such results are interpreted as showing an inhibition of the cells' normal capacity for PLD repair.

Confusion exists in the radiobiological literature because of the lack of any agreed definition of 'PLD repair': particularly since the phrase has sometimes been used also to imply that mode of repair which engenders survival curve shoulders and is therefore, by hypothesis, depleted with increasing dose. It was suggested[1] that all modes of repair which remain in operation throughout the dose range used in constructing a full survival curve should be classified as 'P-repair'. The operation of P-repair will increase the dose needed for an average of one lethal event per cell; inhibition of P-repair normal to the cell will reduce that dose.

Radiation-sensitive mutants

Wild-type cells of all classes have been found to give rise to radiation-sensitive mutants; UV-sensitive bacterial strains were the first to be identified. Variant cells are categorized as radiosensitive when survival is less, after a given radiation dose, than it is for the wild-type parent strain handled in precisely the same way. Such a decrease in survival may originate from deficiency in P-repair or in Q-repair: in other words, the parameter D_0 may be less for the mutant (fig. 10)[21]; or the 'shoulder width' (parameter n) may be less (fig. 11)[43], with no difference in D_0; or both parameters may be less.

Clearly mutants such as those illustrated by figure 11 have as much capacity for P-repair as their wild-type parents; but capacity for Q-repair has been reduced to zero.

Radiosensitive cells have been isolated from patients suffering from certain hereditary diseases which render their normal tissues very sensitive to ionizing radiation; the first association between sensitivity of cells cultured from such patients and the radiosensitivity of their tissues was

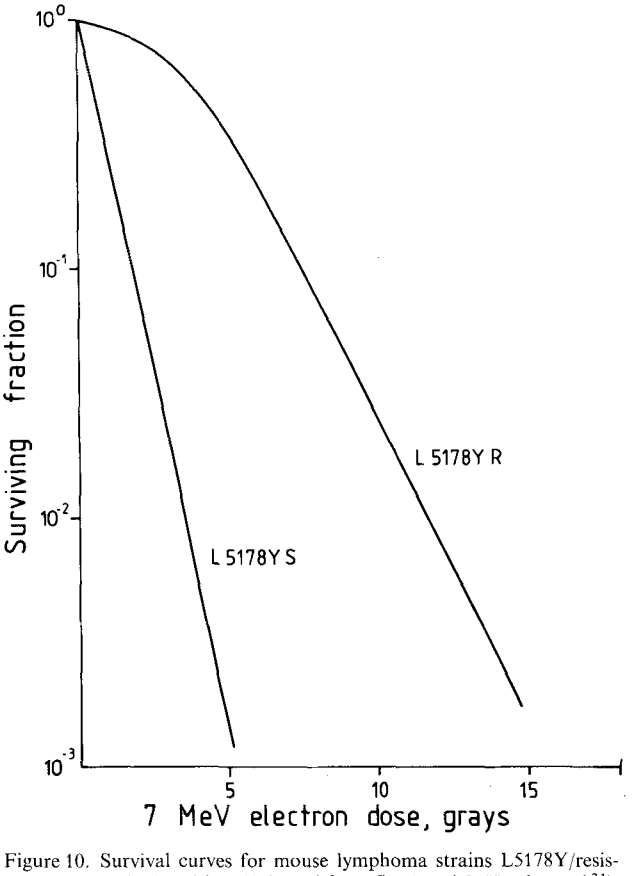

Figure 10. Survival curves for mouse lymphoma strains L5178Y/resistant and L5178Y/sensitive. (Adapted from figs 1 and 2, Hesslewood[21]).

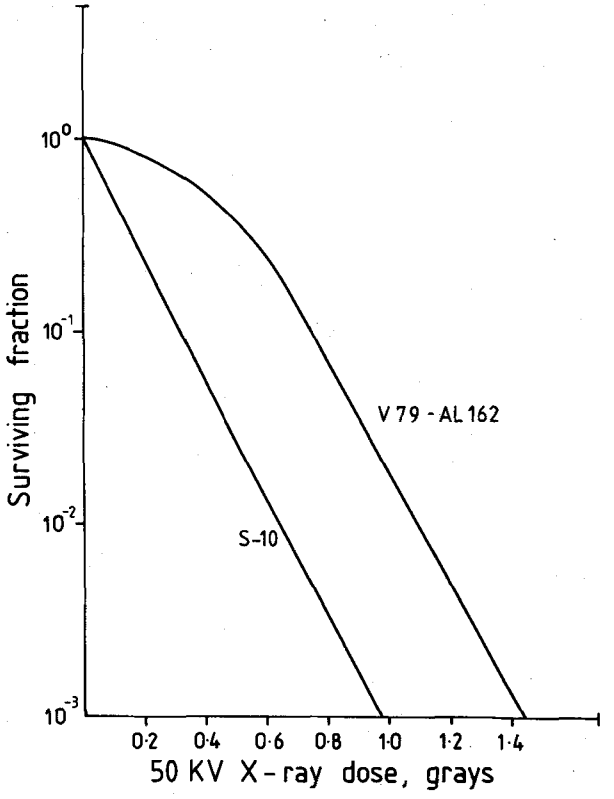

Figure 11. Survival curves for V-79 AL162 and 'radiosensitive' mutant S10. Curves reconstructed from survival curve parameters given by Utsumi and Elkind[43].

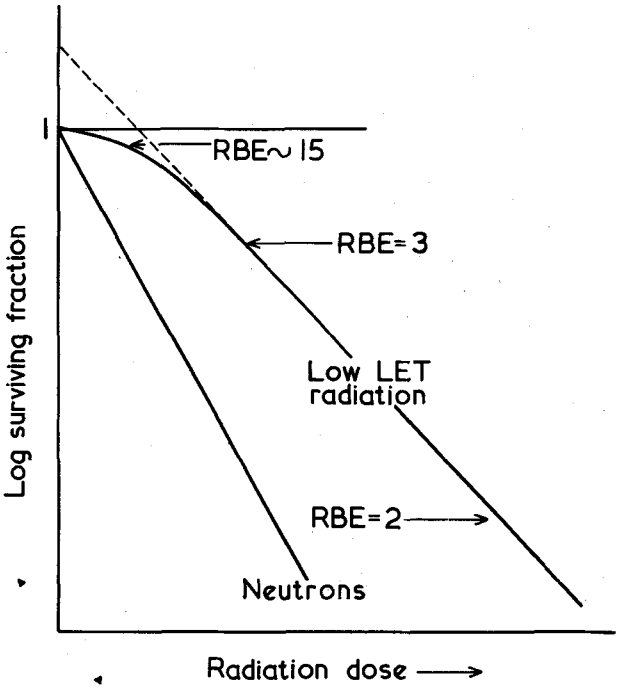

Figure 12. To illustrate dependence of Relative Biological Effectiveness on level of survival.

made with patients suffering from Ataxia telangiectasis (A–T)[41]. Survival curves for skin fibroblasts from normal subjects and from A–T patients showed that values of D_0 for the latter were less, by factors of two to three (fig. 6). Attempts to identify the deficiency in repair capacity of A–T cells are discussed under 'Biochemical investigations'.

Relationship between repair and greater effectiveness of densely ionizing radiations

Whereas shouldered survival curves are common for cells irradiated by X- or γ-rays, this is often not the case when more densely ionizing radiations are used (e.g. neutrons, α-particles). With those, survival curves have lower extrapolation numbers, often down to $n = 1$, i.e. exponential curves. Terminal slopes are almost always steeper. Radiations of different ionization density are usually specified in terms of their 'Linear Energy Transfer' (LET), which is the greater, the more densely ionizing the radiation. The phrase commonly used to compare effects of radiations of differing LET is 'Relative Biological Effectiveness' (RBE): conventionally quantified by the ratio of doses required to give the same effect, the least effective usually being taken as standard. As figure 12 demonstrates, RBE is usually not a constant parameter, it varies with the level of survival. It is not self-evident

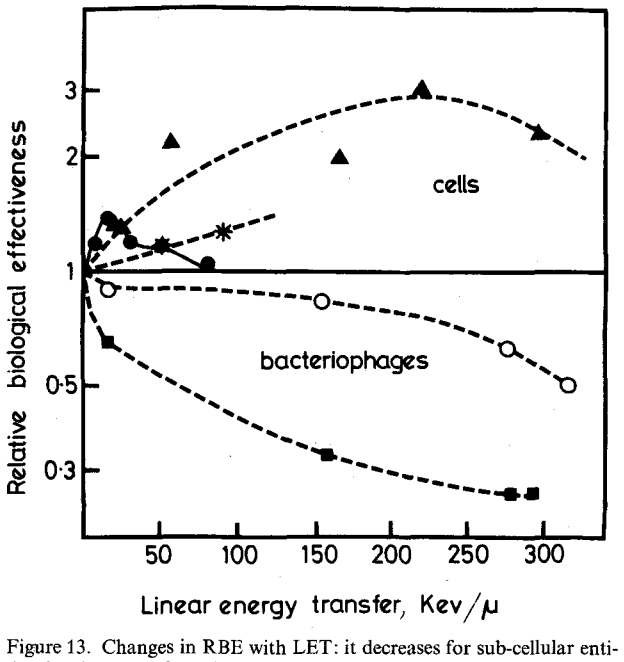

Figure 13. Changes in RBE with LET: it decreases for sub-cellular entities, but increases for cells of most classes.

that densely ionizing radiation should be more damaging than X- or γ-rays. When single macromolecules are irradiated, like enzyme molecules, or the nucleic acid cores of viruses, the reverse is true (fig. 13). This is because a single 'hit' or energy deposit within the macromolecule is sufficient to inactivate it. With densely ionizing radiation, several energy deposits may be made together with-

in the molecular volume, so some of the energy is wasted. Increasing RBE with increasing LET, with regard to radiobiological effects on cells, has therefore been a topic of great theoretical interest. Some hypotheses have, implicitly or explicitly, involved the concept that shoulders to survival curves, or quasi-thresholds seen in other dose-effect relationships, reflect the need for an accumulation of sublesions before the effect is realized [24, 25]. But that approach cannot accommodate increases in RBE seen when no such quasi-threshold exists (fig. 14) [38]. An alternative view is that repair capacity becomes less effective as ionization density is increased, perhaps by virtue of the energy depositions supernumerary to the one actually required for inducing an otherwise reparable lesion.

Repair-deficient mutant cells provide a means of testing the hypothesis that increasing RBE is attributable to decreasing repair capacity. If that is inherently defective, there is less of it to be damaged by densely ionizing radiation, so there should be less increase in RBE than there is for fully repair-proficient cells. It is predictable, therefore, that when effects of an increase in LET on wild-type cells and their radiosensitive mutants are compared, the latter will evince smaller increases in RBE. Table 1 [4, 7, 14, 21, 29] shows how that prediction is verified by results of such comparisons for cells of all classes.

We conclude that the phenomenon of 'increasing RBE with increasing LET', for damage to cells, is plausibly accommodated by the hypothesis of increasing damage to repair capacity with increasing LET. There is some experimental evidence to that effect; but the hypothesis will be properly testable only when the processes of both Q- and P-repair have been elucidated.

Radiations compared	Repair-proficient and deficient strains	Values of RBE	Reference
	Bacteria		
Fast neutrons and X-rays	*E. coli* B/r	1.4	4
	E. coli B_{9-12}	1.0	
	Yeast		
α-particles and γ-rays	A 288 C	3.2	29
	g 218/6b	1.9	
	Drosophila embryos		
Pions at peak LET and X-rays	Repair proficient	⩾1	14
	Deficient (mei 9^{L1})	1.0	
	Human skin fibroblasts		
α-particles and γ-rays	Normal	4.0	7
	A–T	2.0	
	Mouse lymphoma		
Fast neutrons and X-rays	L5178Y/R	2.1	21
	L5178Y/S	1.3	

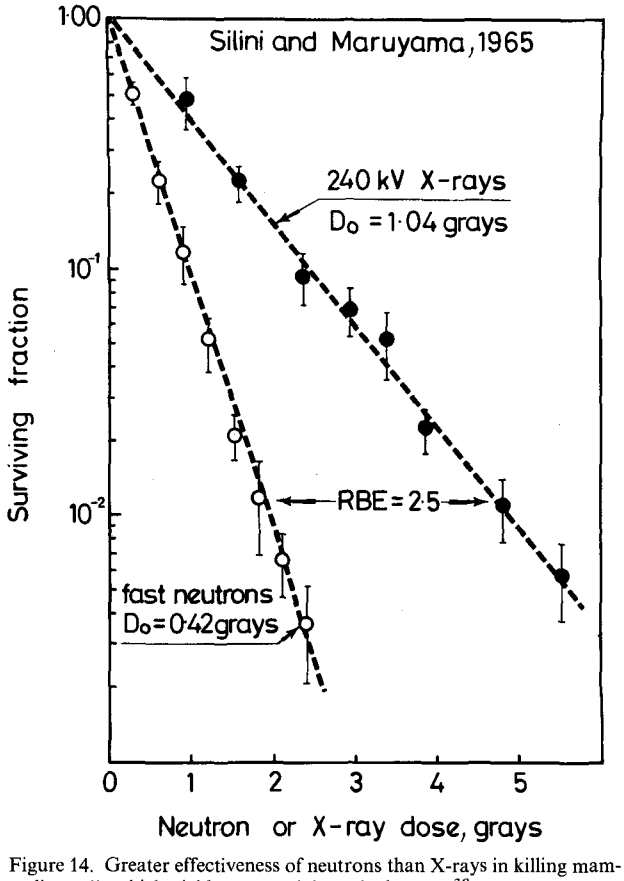

Figure 14. Greater effectiveness of neutrons than X-rays in killing mammalian cells which yield exponential survival curves [39].

Biochemical investigations

Elucidation of mechanisms of action of cytotoxic agents depends on the forging of links between operational phenomena and their biochemical background. In that respect, great strides have been made in cell photobiology. Irradiation by germicidal UV (i.e. at wavelengths specifically absorbed by nucleic acids) generates products in DNA which can be lethal. These have, to a considerable extent, been identified and measured. It is known which enzymes are involved in several modes of repair or bypass of harmful products. It is noteworthy that the isolation of UV-sensitive mutants has been an important factor in this work, in that deficiencies in certain repair-enzymes in the mutant strains have been correlated with their sensitivity. In contrast with photobiology, however, radiobiology lacks clear identification of repair mechanisms that can be unambiguously associated with increased ability of cells to survive irradiation.

Attention has, to an overwhelming extent, been focussed on radiation-induced changes in DNA and their repair, a topic which is reviewed in a companion paper [18] and which has been extensively covered recently by George and Cramp [15]. Those authors point out that the majority of studies aimed at following repair of specific defects induced in nuclear DNA have been based on doses to the cells considerably greater than the range usable for following cell survival. Such discrepancies engender doubts as to the validity of accounting for repair, observed operationally, by modes observed at the biochemical level. One technique in current use, however, – 'neutral elution' – can yield measurements of defects induced in DNA by doses within the survival curve range. It has been used

mainly to study induction and repair of DNA double-strand breaks (dsb's). These, some radiobiologists believe, are the principal DNA lesions which, when unrepaired or 'misrepaired', are responsible for cell death. From the two first [33, 34] of a series of papers by Radford on results of his use of the neutral elution technique with mammalian cells, he seemed to consider *repair* of dsb's irrelevant, since he concluded that 'the level of cell killing reflects the *induced* frequency of DNA dsb and is independent of irradation conditions and cell type'. This was based on the following observations:

1) For a range of conditions used before and during irradiation, there was good correlation between numbers of dsb's induced and numbers of lethal events [34].
2) For seven strains of mammalian cells of widely differing radiosensitivity, the correlation between numbers of dsb's induced and numbers of lethal events was roughly the same for all [35].

It is an important aspect of the technique used by Radford that cells were held ice-cold until the elution of DNA had been completed, so that enzymic repair of induced dsb's could not affect the results. Prise et al. [31] did similar experiments, with the same precautions. For hamster V79 cells exposed to X-rays in two conditions giving widely differing survival curves, they confirmed that numbers of lethal events were correlated with numbers of induced dsb's. Such a correlation was seen also when radiations of high LET (fast neutrons and alpha-particles) were used; but the number of lethal events per dsb was 2.5 times as high as for X-rays. That discrepancy could clearly not be attributed to greater *reparability* of dsb's induced by the less densely ionizing radiation, since enzymic repair was not thought to occur during the time the cells were held before the DNA was eluted.

A more serious objection to the conclusion of Radford [35], quoted above, comes from the ample evidence that survival of cells of all classes can be greatly affected by the way they are treated *after* irradiation. As shown by figures 3, 4, 8 and 9, both Q- and P-repair can be increased or diminished. Whatever radiation-induced lesions may be responsible for cell death, it is clear that their effectiveness depends to a large extent on post-irradiation conditions and therefore, presumably, on biochemical steps that either 'fix' the damage or allow its repair. These are at least as important as the numbers of lesions *induced.*

On the plausible assumption that sensitive mutant cells are deficient in repair capacity, some enlightenment might be looked for in studies aimed at pinpointing the deficiencies. Unfortunately those have not often been identified operationally: does failure in repair result in reduction in the dose required to deliver an average of one lethal event per cell, i.e. in the value of D_0; or is the increased sensitivity attributable to reduced shoulder width, i.e. less capacity for Q-repair; or both? Examples of pairs of strains for which the deficiency is evident are the mouse lymphoma strains L5178Y R and S, and freshly cultured skin fibroblasts from normal subjects and from patients suffering the disease Ataxia telangiectasia. Figures 6 and 10 demonstrate that the deficiency in the more sensitive strain is of the P-repair type. Several techniques have been used to compare DNA strand breakage repair in the two pairs, but effectively no difference has been found (see George and Cramp [15] for details). Kemp et al. [26] examined the time course and extent of dsb repair in wild type CHO cells and six sensitive mutants. They found the mutants to be defective, but the extent of the defects did not correlate with the 'different levels of sensitivity'. That lack of correlation might be due in part to differences between mutants in respect of the operational signs of repair deficiency; it should be noted, however, that in those experiments the cells used to follow repair of dsb's were exposed to an X-ray dose well in excess of the survival curve range.

Changes in 'radiosensitivity' during the cell cycle have provided a rationale for studying concomitant changes in capacity for repair as the cells progress through the various stages. But careful delineation of the corresponding survival curves has shown that [22, 40] they differ *only* in respect of shoulder width, i.e. Q-repair (fig. 2) [40]. If that is a rapid process, it is not surprising that examination of DNA repair processes over many tens of minutes, or even hours, should have failed to yield conclusive results [15].

In that connection, it is of interest to note that Radford [36] has reported one phase of repair in dsb's in irradiated V79 cells for which the half-time was only 2–3 min. This was seen in stages of the cell cycle for which survival curves are shouldered, but not with the exponentially killed mitotic cells. However, it would be premature to associate this fast repair with the postulated Q-repair, because it was seen in cells which had been irradiated by doses of 12 Gy and more. As figure 1 shows, all Q-repair should by then have been depleted. For that reason, incidentally, Radford's results cannot be accepted, as he claimed, as evidence conflicting with the repair-depletion interpretation of shouldered survival curves.

The attention currently given to radiation-induced DNA strand breaks, and their repair, was preceded by interest in the inhibition by radiation of DNA synthesis: the first intracellular biochemical consequences to be observed. As a function of time after irradiation, the extent of inhibition is commonly described as biphasic, the initial prompt inhibition being the more severe. The extent of prompt inhibition has in some instances been correlated with sensitivity to the lethal effect; in others, for example with A–T cells, the reverse is true. According to Painter [28], 'there is no simple correlation between inhibition of DNA synthesis and cell killing by ionizing radiation'. For that reason, 'repair', in the operational sense, can hardly be associated with resumption of 'normal' DNA synthesis. Indeed, it has for many years been known that resumption of DNA synthesis in irradiated

cells occurs also in cells that have lost their ability to proliferate; so that very heavily irradiated cells may become 'giant' cells by virtue of DNA synthesis that proceeds although capacity to divide has been lost.

Nevertheless, it is perhaps appropriate to associate the concept of repair with an early step in the overall process of DNA synthesis, namely the bonding of the newly-synthesized single strand to its parent template. The use of very short pulses of radioactive label permitted distinction between new single-stranded and double-stranded DNA; and the ratio of one to the other depended on the cell line[9]: the larger the ratio, the longer was the time the cells needed for firm bonding to occur. In all cell lines, irradiation perturbed the pattern of that ratio, i.e. the time taken for firm bonding was increased[9,10]. An important observation with *unirradiated* cells was that the fraction of single-stranded DNA was larger in radiosensitive variants than in their repair-proficient parent strains; and that radiation perturbed the pattern of bonding to a larger extent, and for a longer time, in the more sensitive strain. Both those observations are illustrated by figure 15[10]. The fraction of single-stranded DNA present in growing unirradiated cells correlated fairly well with their radiosensitivity[8], when the latter was defined in terms of D_0, i.e. the dose required to deliver an average of one lethal event per cell. Conditions which enhanced radiosensitivity, by that definition, concomitantly increased the instability of the newly synthesized DNA. It was suggested that radiation acted to perturb the complex interaction between DNA, enzymes and structural proteins required for successful DNA synthesis[15]. The fate of irradiated cells might then depend on the relationships between the times needed respectively for restoration of the normal configuration and for progress through the steps leading to cell division. If the latter is delayed, as when cells in stationary stage are held for a time, before being placed in growth conditions[22], the balance is pushed in the direction of allowing more cells to divide successfully (fig. 8). In that connection, it is interesting that Cox[7] found very little, if any, evidence of 'PLD-repair' with A–T cells. These have been shown[41] to have as much as 40% of their DNA in single-stranded form[10]. As described above, post-irradiation treatments may result in failure of inherent P-repair capacity, as, for example, when irradiated cells are exposed to anisotonic conditions (fig. 9). As was suggested by Utsumi and Elkind[42], the effect of that treatment might well be attributable to weakening in the structural relationship of DNA to the nuclear envelope and associated protein matrix.

Conclusions

In operational terms, processes of repair of radiation damage to mammalian cells are divisible into two classes: those which operate without diminution, and therefore increase the doses required for an average of one lethal event per cell (P-repair); and one operating over only a limited dose range, so that its initial effect is to confer a 'shoulder' on the cell survival curve, or a 'quasi-threshold', when other tests of damage are applied (Q-re-

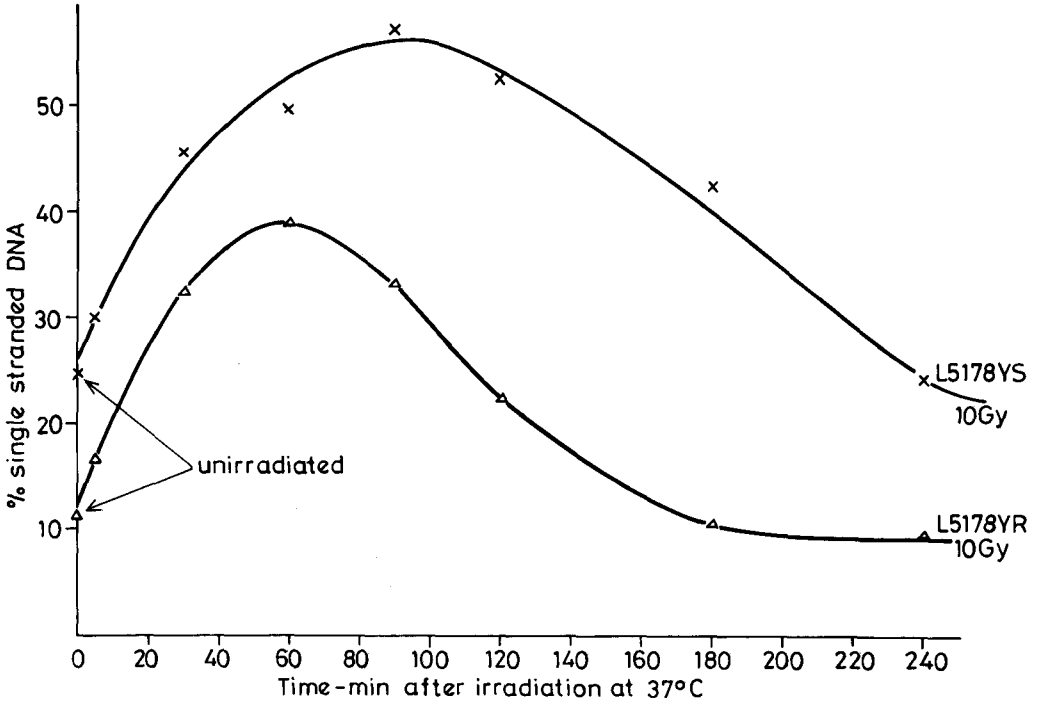

Figure 15. Plot of single-stranded DNA arising from a 30-s pulse of tritiated thymidine against time after 10 Gy. L5178YR = the radiation resistant mouse lymphoma line and L5178YS = the radiation sensitive line[9]. Each point arises from an average of four measurements.

pair). Cells may be fully competent in respect of capacity for P-repair, while lacking operational signs of capacity for Q-repair. Q-repair probably occurs very rapidly after a damaging event. The time-scale should not be confused with that observed for *reconstitution* of *depleted* Q-factor. The increased cytotoxic action of radiation almost always observed with radiations of high ionization density may plausibly be attributed to damage to both types of repair; but confirmation awaits identification (not yet to hand) of the biochemical steps involved in both forms of repair.

Examination of defects in DNA in isolation, notably strand beaks, has not led to satisfactory correlation with phenomena observed at the cellular level; in this respect, results showing changes in cell survival brought about by post-irradiation treatments have not been sufficiently taken into account.

One radiation-induced lesion that may be lethal is disturbance of the normal structural relationship between DNA, the nuclear membrane to which it is bound and associated protein complexes. A sign of the disturbance is an increase in the time required after irradiation for newly snythesized single-stranded DNA to be bonded to the template. Restoration of the correct structural relationship might represent one form of intrinsic P-repair, a concept which accords with several different lines of experimental observations.

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Cell kinetics and radiation pathology

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Key words. Cell proliferation kinetic techniques; cellular radiosensitivity; repair of sublethal injury; repopulation; radiation pathology; tumour cells; tissue dysfunction.

Introduction

The proliferation pattern of any normal or malignant tissue can be described by the cell cycle time, the growth fraction and the cell loss factor of the constituent cell types. These parameters describe the rate of cell turnover; the balance between cell production and loss determines whether the tissue is constant in size, is growing or shrinking. These characteristics of tissue growth influence the response to radiation in a variety of ways – both in terms of intrinsic radiosensitivity and in terms of the pattern of appearance of injury, i.e. the radiation pathology. Tissue pathology, expressed as a loss of tissue function, results from the death of proliferating cells that would normally maintain the supply of differentiated cells.

Cellular radiosensitivity is influenced by the cells' position in the cell cycle, and also by the balance of endogenous redox chemicals. Cell function is quite radioresistant in most cells, but cell division (and the ability to produce a clone of offspring) is exquisitely radiosensitive. The sensitive targets seem to reside in the DNA. The cell can tolerate many molecular breaks in single strands of the DNA, because of efficient repair enzymes, but it is much more difficult to achieve high fidelity repair of two adjacent breaks on opposite strands of the double helix. Chromosome damage can result and lead to an uneven distribution of the genetic material between the daughter cells at a subsequent division. The concentration of oxygen (a chemical radiosensitizer) and of thiols, especially glutathione (chemical radioprotectors) in the vicinity of the DNA have a marked effect on the intrinsic radiosensitivity. The thiol content varies around the cell cycle [24] and the oxygen tension depends on the intercapillary distance. This distance is normally small – giving adequate oxygenation to most normal tissue cells – but is excessive in tumours because of the imbalance between tumour cell proliferation and expansion of the vascular network. Hypoxic cells therefore occur commonly in tumours, but rarely in normal tissues, and these cells are 2.5 to 3 times more radioresistant because of their hypoxia [20].

The response of cells and tissues to large single doses is characterised by a quasi-threshold region, where little effect is seen, and a steeper dose response at high doses. If repeated small doses, each within the quasi-threshold dose range, are given, the cells do however show radiation damage. For cancer therapy, which is almost always given as a series of 'fractionated' doses, it is the response to repeated low doses that is important.

The radiosensitivity of a cell population changes with time after a first dose of radiation because of four processes; biochemical repair, redistribution around the cycle, reoxygenation of hypoxic cells, and repopulation by cell division in the surviving cells. These factors are all influenced by the cell proliferation kinetics [10].

The time at which injury is expressed after irradiation is also dependent upon the cell kinetics because cell death is not expressed until mitosis. Thus rapidly dividing cells express their damage quickly, e.g. in the intestine, and slowly dividing cells show no apparent sign of radiation injury for months, until the next mitosis, e.g. in liver, kidney, lung and most visceral organs. A detailed insight into the cell kinetics of the different cell types in each tissue is therefore necessary to comprehend the pattern and expression of radiation pathology.

Cell proliferation kinetic techniques

In order to understand the proliferative behaviour of a tissue it is necessary to know what fraction of the cell population is actively engaged in the replacement of ageing cells (the growth fraction), how rapidly these cells are dividing (i.e. the intermitotic or cell cycle time, T_C), the lifetime of the differentiated cells and the rate of loss of cells from the population (fig. 1). In many tissues the proliferating and differentiated cells are visibly different and the gross tissue structure may reflect this distinction. For example in skin, cell division is confined to the cell