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Cell kinetics and radiation pathology

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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

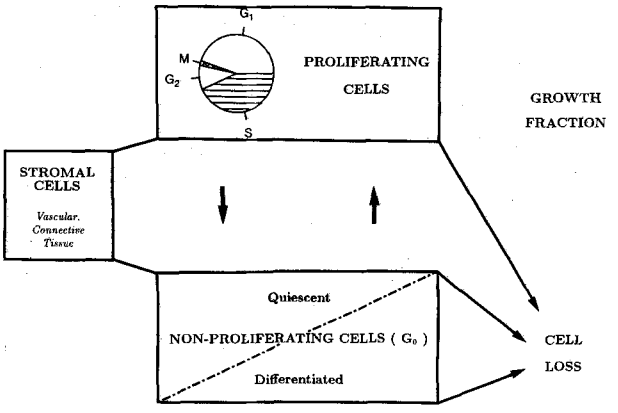

Figure 1. Schema to illustrate the importance of cell cycle time, growth fraction and cell loss in determining the proliferation characteristics of any tissue. Stromal cells are needed to support the parenchymal cells.

layer adjacent to the basement membrane, and as cells lose contact with that basement membrane they progressively differentiate to form a keratin squame at the surface. Similarly, in intestinal epithelium, cell division is confined to the crypts of Lieberkühn; the newly formed cells are carried up the villus to act as a barrier to fluid loss, electrolyte imbalance and bacterial invasion, and are eventually desquamated from the tip of each villus. Other tissues do not show clear demarcations of the proliferative and differentiated zones. Thus in lung, kidney, heart, liver, spinal cord, muscle, etc. it is hard to detect which cells are responsible for making good the small attrition that results from normal wear and tear. The very low level of cell turnover adds to this difficulty since such cells would be rare anyway (less than one in ten thousand will be in cell division at any instant).

Stathmokinetic techniques

The most obvious way to detect proliferating cells is to identify them in the act of division, i.e. during mitosis. However this act is very brief, lasting only a few minutes. Furthermore, mitotic activity may be confined to one part of the day, and the search for mitotic cells may be fruitless if this diurnal rhythm is not recognised and taken account of. It can be misleading to take a single sample at one time of day since that may represent a peak or trough of mitotic activity[4], and the mitotic index $\left((\text{M.I.}) = \frac{\text{nr. cells in mitosis}}{\text{total cells}} \times 100\%\right)$ can differ by a factor of 10. One method of overcoming these difficulties is to use an agent which prevents the completion of mitosis so that mitotic cells can be accumulated as they reach metaphase. A group of metaphase arrest agents, the vinca alkaloids (e.g. colcemid and vinblastine) can be used to poison the mitotic spindle proteins and prevent separation of the two sets of chromosomes, without totally destroying the cell structure. By measuring the rate of accumulation of mitotic figures (fig. 2, top) with increasing hours of exposure to these agents it is possible to calculate the duration of mitosis (T_M), the rate of entry of cells into mitosis and hence the time that would be needed for all the cells notionally to pass through cell division. This gives a measure of the potential doubling time of the population

$$T_{pot} = \frac{\text{M. I.}}{T_M} \times 100\ \text{h}$$

This approach is described in detail, with all its advantages and disadvantages, in a book by Aherne et al.[1].

Autoradiography and flow cytometry

The discovery by Howard and Pelc in 1953[21] that the duplication of DNA in preparation for mitosis is a discrete event in the life of the cell, separated by a premitotic gap G_2 and a postmitotic gap G_1, has opened up many more sophisticated techniques, which allow a more complete description of the proliferation kinetics[8, 30]. Cells actively synthesising DNA can be tagged or labelled with specific precursors of DNA. The first such label was tritium-labelled thymidine. Cells incorporating this can be subsequently identified in sections or smears by their ability to darken photographic emulsion, because the β-rays from disintegrating tritium nuclei interact with silver halide grains to produce a latent image. A technique of continuous labelling can be used to measure T_S and T_{pot}, in a similar manner to the mitotic accumulation (fig. 2b). More recently another technique has been developed using bromine-labelled or iodine-labelled uridine[18, 19]. DNA in which this is incorporated can be stained using a monoclonal antibody which detects the distortion caused by the Br or I replacement of a methyl group in the DNA. This represents a major technical advance for several reasons: It is less hazardous to use these chemically substituted precursors in patients than a radioactive precursor. It requires only a few hours to stain with a monoclonal antibody instead of 1–2 months of exposure of a section to a photographic emulsion. The monoclonal antibody can be counter-stained with a colour reaction for conventional microscopy (e.g. horseradish peroxidase) or a fluorescent tag which then allows it to be detected in a flow cytometer. This makes it possible to look for rare events in a large population of cells because flow cytometers can analyse many thousands of cells each minute[19].

With these labelled precursors, which are relatively non-toxic, it is possible to measure the cycle time of proliferating cells, the duration of the G_1, S, G_2 phases, and the fraction that are involved in the proliferative cycle. Since two phases can be distinguished it is possible to watch cells labelled with ^3H thymidine in the DNA synthesis phase T_S, appearing in mitosis after a lag period (G_2), and then reappearing one full cycle later, after M, G_1, S and G_2 have been traversed. This is done by obtaining a profile of the fraction of labelled mitosis at successive times after labelling[8, 26]. To determine the potential dou-

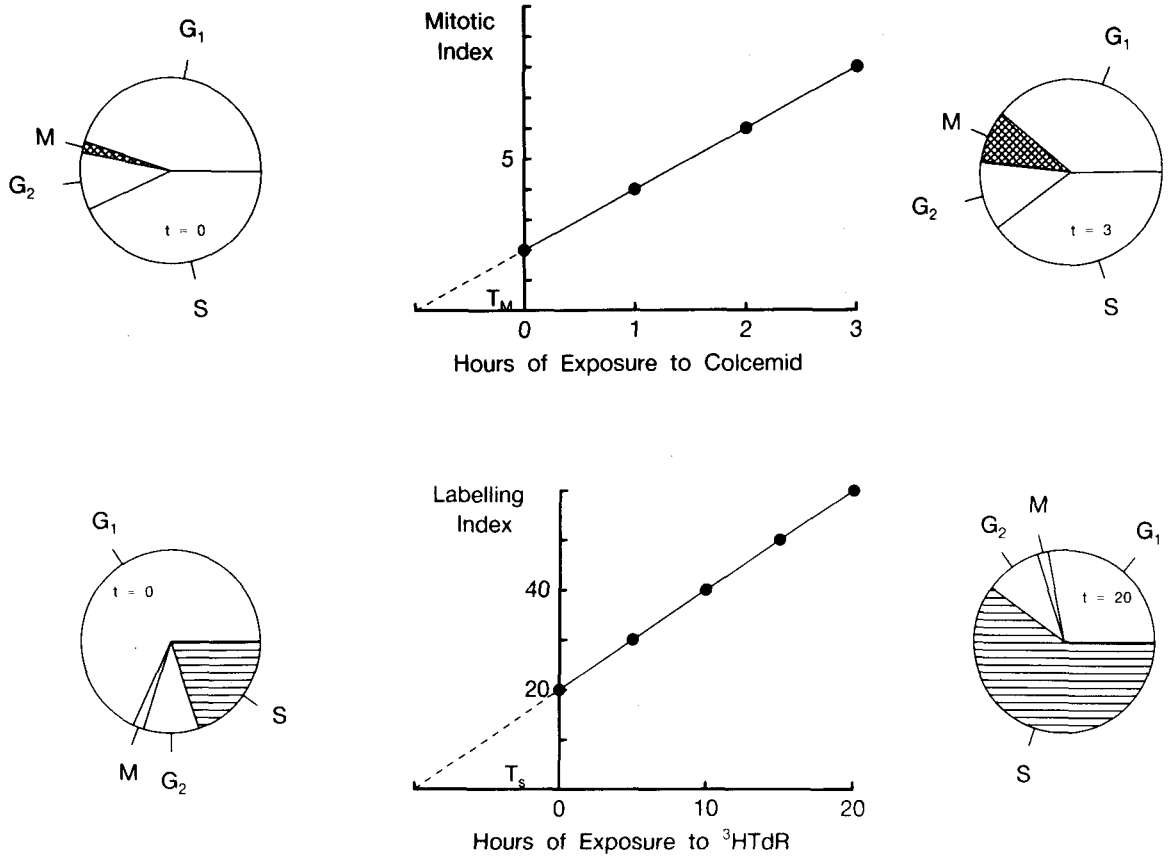

Figure 2. Diagrammatic illustration of how the duration of mitosis and the potential doubling time (1/ cell birth rate) can be determined using colcemid blocking of metaphases (upper panel). In a similar way the duration of DNA synthesis and rate of passage through S can be measured using continuous exposure to tritiated thymidine.

bling time T_{pot} it is only necessary to derive the duration of the DNA synthesis phase T_S, and the instantaneous fraction of cells in S. This can be done with a single sample using flow cytometry[3]. The S phase is 10–20 times longer than T_M and hence larger numbers of labelled cells are present, which improves the statistical accuracy of these measurements. The changes in labelling index throughout the day are also 'damped out' relative to the mitotic index, with the diurnal fluctuations being only twofold in skin, compared with tenfold for mitoses[4].

Flow cytometers can also be used, in a simple way to determine the fraction of cells in G_1, S and G_2 by simply staining the DNA with a fluorescent dye, e.g. ethidium bromide. Measuring the fluorescence intensity of each cell is then a measure of the DNA content of that cell. Diploid cells in G_1 have a 2n DNA content, those in G_2 a 4n content (i.e. tetraploid cells) and those actively synthesising DNA have intermediate values. Tumour cells are often aneuploid but they still have two peaks of fluorescence, with the G_2 peak having twice the fluorescence of the G_1 peak.

In slowly proliferating tissues many of the cells are 'out of cycle'. These cells, described as being in a G_0 phase, usually have a G_1 content, and can be stimulated or recruited into cycle when needed. The cell cycle time and labelling indices are very different from one tissue to another. In general, epithelial surfaces, exposed to the greatest contact with the environment, have the highest turnover of cells. Nevertheless T_C can vary from 12 h in intestinal crypts and their follicles to 100 h in the basal layer of skin, and to extremely long and as yet unmeasured value in bladder, kidney tubules, lung epithelium, etc.[10].

Differences between tumours and normal tissues

All tumors are, by definition, expanding masses. This results from cell production exceeding cell loss. It can happen because of a shortening of the intermitotic time with no increase in the growth fraction, because of an increased growth fraction with no change in T_C, or an increase in the life-span of the post mitotic cells and hence a decrease in the rate of cell loss. All three changes and combinations of them have been observed in malignant tumours. The cell cycle time of rodent tumours is often 12–24 h, but in human tumours it is longer, 24–120 h. The growth fraction in rodent tumours is often 40–60%, but in well-differentiated human tumours the normal tissue elements of the stroma may themselves constitute more than 50% of the total mass and the

growth fraction of the tumour cells may appear to be quite low. In autoradiographs it is often difficult to distinguish between normal and malignant cells and most published labelling indices include both. In flow cytometers they may be distinguished by their ploidy and may be separately analysed. The fraction of new cells lost from the tumour during each cycle can also vary widely. The cell loss factor, ϕ[29] is calculated from the discrepancy between cell production (T_{pot}) and actual volume doubling time T_D

$$\text{Cell loss factor } \phi = \frac{1 - T_{pot}}{T_D}$$

It is negligible in some fast growing sarcomas (where all cells seem to remain within the tumour volume) but can be 0.90–0.95 in some carcinomas (i.e. 90–95% of cell production does *not* remain in situ to contribute to tumour growth). In all normal adult tissues production and loss are exactly balanced and $\phi = 1.00$. If the rapid production in most epithelia is not exactly balanced by loss the tissue volume will increase. Decreasing values of ϕ characterise a progressive increase in tumour growth rate, even if the growth fraction and cell cycle time had not changed from those of the parent tissue. The factors influencing the changes in T_C, GF and ϕ are still poorly understood in spite of many cell kinetic studies and the great upsurge of interest in oncogene control of growth factors.

Cellular response to radiation

With the exception of lymphocytes and germinative cells, which die in interphase, all cells die a mitotic or post-mitotic death. This occurs at the first mitosis after high doses and at the second or third mitosis after lower doses. The average lethal dose is only a few gray, whereas the dose needed to cause immediate biochemical death is hundreds of gray. Thus cell proliferation is one of the most sensitive parameters to measure radiation injury inflicted upon cells. The standard radiobiological test of radiation sensitivity is to expose cells to radiation, and then plate them in suitable culture medium, and count what fraction of them are capable of producing a colony or clone of survivors. Clones containing fifty or more cells spread flat on a petri dish are macroscopically visible when stained with an appropriate dye. These represent 5 or 6 successful divisions (1:2:4:8:16:32:64) and are taken as an indication that the cell has reproductively survived.

A number of clonogenic assays are now available for tissues in situ, as summarised in a recent book by Potten and Hendry[25]. They consist of recognising the offspring of rare isolated survivors, in a sea of heavily irradiated dead cells.

Tissue dysfunction

It is generally believed that the functional damage that appears in all normal tissues after irradiation can be traced back to the loss of reproductive ability in the proliferative compartment of that tissue. The functional deficit may be apparent as soon as the abortive mitoses begin to occur. More usually, the tissue has a functional reserve and a considerable depletion in cell numbers can be tolerated before functional impairment is detectable. A delay, before tissue damage is evident, occurs because the differentiated quiescent compartment is still capable of functioning. Once these cells have lived out their normal life span the failure to replace them from the proliferating compartment will then precipitate tissue dysfunction. A prime example of this is bone marrow, where stem cells have a turnover time of about 36 h. However, the differentiated granulocytes and platelets have a turnover time of several weeks, and the erythrocytes of several months. Thus, loss of function, expressed as bleeding or susceptibility to infection is manifest after 3–4 weeks, and anaemia not for several months.

Figure 3 illustrates how the death of animals after whole body or localised irradiation will occur at different times for different tissues. High doses deplete the intestinal epithelium and mice die of dehydration and infection within 4–7 days. Those appearing to survive 10 Gy will die of bone marrow depletion by one month. A similar biphasic pattern of deaths was seen in man after the nuclear bombing of Hiroshima and Nagasaki. After the recent Chernobyl accident, workers on the site also developed functional symptoms of gut and bone marrow damage on the classic timescale although this did not lead to deaths in most of the victims.

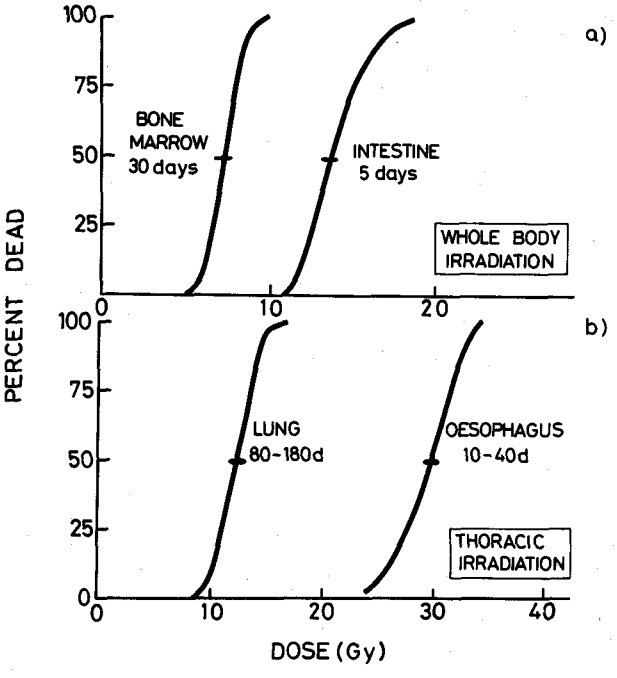

Figure 3. Lethality curves for animals treated with whole body or localised irradiation. The early deaths only occur after high doses, but survivors may succumb to late tissue injury after lower doses. (Reproduced from Denekamp[9])

Two phases of death are also seen after localised thoracic irradiation (fig. 3b). Oesophageal damage causes death by starvation within 6 weeks, but the surviving animals may succumb to pulmonary fibrosis and respiratory insufficiency at about 6 months. In each instance the time course for organ dysfunction is well correlated with the proliferation kinetics of the target cells[17, 23, 34].

Tumour control

The ability to cure cancer patients with radiotherapy is obviously limited by the side effects on normal tissues. Local control of a solid tumour is also a result of killing (in the reproductive sense) clonogenic tumour cells. Moderate doses of radiation may easily kill down to 1% or 0.1% of the original population, but this will only result in a partial shrinkage followed by regrowth of the tumour mass (fig. 4). Permanent local control requires the eradication of every clonogenic tumour cell. The rate of shrinkage and the time of regrowth depends upon the original proliferation characteristics of the tumour in a quite complicated way[6, 8]. In summary, tumours with high cell loss factors (e.g. many carcinomas) shrink rapidly, as cell production ceases but 'normal' cell loss continues. Tumours with low cell loss factors (e.g. undifferentiated sarcomas) shrink more slowly, and may even continue to grow for a few days after large doses of radiation. Tumours with a large component of connective tissue may show extremely slow or no shrinkage, even if every clonogenic cell has been eradicated.

Therefore the time at which a clinician can judge whether he has achieved permanent control varies from tumour to tumour. Some tumours, if they are to recur locally after treatment will do so within 12 months, e.g. glioblastoma, Ca bronchus. Many others may recur up to 3–5 years, e.g. carcinoma of the cervix and the head and neck region. A few sites, with indolent tumours, may show local recurrence even after 10 years (e.g. breast and some prostatic cancers). Thus, although long-term palliation may provide an important benefit to patients, the real success of any new treatment can only be determined after the appropriate interval, when any failures will have recurred at that site. Figure 4 illustrates that no cures are achieved until some of the tumours have had the last clonogenic cell sterilized. Beyond a threshold dose the proportion of tumours controlled will increase sharply with dose, depending only on the intrinsic sensitivity of the tumours cells and the heterogeneity from one patient to another. Thus a relatively small increment of dose could give a very large increase in cure rate. It is this fact that makes it worthwhile to attempt to devise less toxic treatments, so that normal tissue damage is spared, and tumour control rates increased, e.g. with tumour radiosensitizers or normal tissue radioprotectors.

Factors influencing cellular radiosensitivity

The pathological side effects of radiation in different tissues depend largely upon the reserve capacity of that

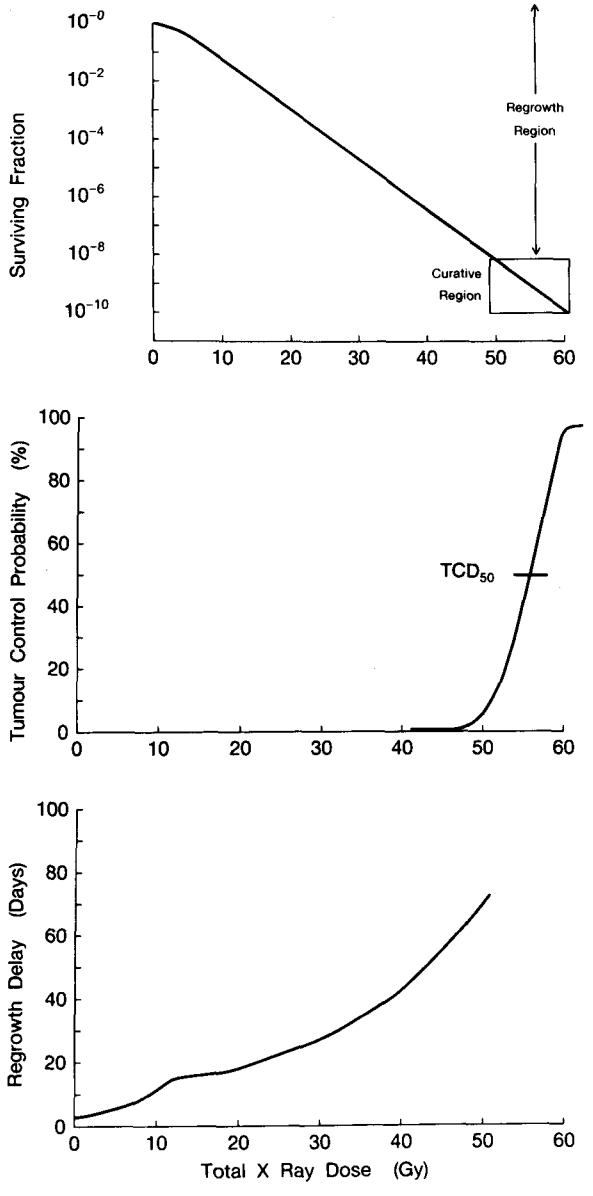

Figure 4. Diagram to illustrate the relationship of tumour cell survival to local control (middle panel) and to regrowth delay, for a tumour containing 10^8 clonogenic cells. Only when the last clonogen has been eradicated in some of the tumours will any local control be achieved, i.e. at 10^{-8} surviving fraction. At lower doses the surviving cells lead to regrowth of the tumour (lower panel).

tissue and its ability to initiate a rapid phase of regeneration to replenish the population before the differentiated cells fall to a nadir. Superimposed upon that, however, are the factors influencing the intrinsic radiosensitivity of the proliferative cells. These factors relate mainly to the cells' chemical and biochemical state, and their ability to repair damage initially inflicted in the DNA. A lethal radiation dose (to a cell) causes thousands of DNA strand breaks, but as long as each break is opposite an undamaged strand the cell has very competent enzymatic repair systems to excise the damaged section and replicate the original template. Even strand breaks which are

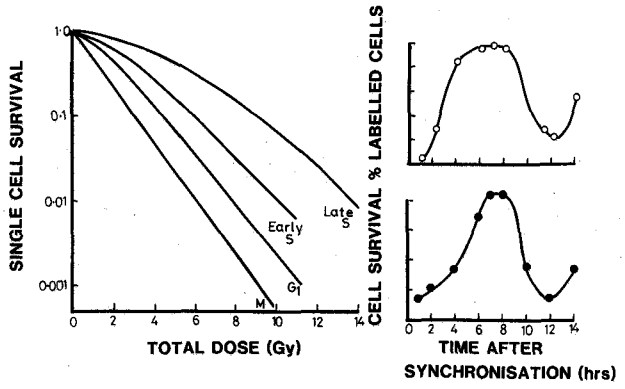

Figure 5. Survival curves for synchronised cells treated at different stages of the cell cycle. V79 cells are most sensitive in mitosis and least sensitive in late S. The right hand panels illustrate that the maximum resistance after a fixed dose of 7 Gy matches the time at which maximum labelling of cells in DNA synthesis is seen with ^{3}HTdR. (Redrawn from Sinclair[27])

within a few base pairs on opposite strands may be reparable. But strand breaks immediately opposite each other, or in a site where damage has also been done to the supporting protein framework, may be either misrepaired or not repaired at all. The consequence of misrepair will of course vary, depending on whether the lesion occurred in a critical segment of DNA or in a repetitive non-critical region and whether the chromosome remains intact or suffers gross rearrangement. The capability of each cell to effect this biochemical repair varies depending upon its position in the cell cycle. This is illustrated in figure 5. Cells are generally most sensitive at mitosis, when the chromatin is in its most supercoiled configuration, subject to torsional stresses, least accessible to enzymes and just at the time when the two sets of chromosomes need to be equally distributed between the daughter cells. The time of maximum radioresistance varies from one cell line to another[9, 27, 28]. Generally it occurs somewhere during the S phase when enzymes involved in DNA duplication are most abundant.

Mitotic delay

Cells which have been irradiated show a delay in their progression around the cell cycle. Those in the premitotic G_2 phase show the maximum delay. Those in S show a moderate delay, those in G_1 very little and those in mitosis seem to continue through mitosis in a normal time. The nett result is an accumulation of many cells at the G_2/M boundary, followed by an overshoot. The timing of this was originally described as being a function of dose, approximately 1 min. per rad, or 1 h per gray[13]. Studies of skin, when compared with cells in culture, suggest it is more likely to be a constant fraction of the cycle time, rather than a constant amount of time[10]. The delay per gray varies from 1 h in a rapidly proliferating culture to almost 10 h in unstimulated skin. When expressed relative to the cell cycle time it is seen to be approximately 10% of the cycle time per gray.

Redistribution

The arrest of cells in different phases and the differential cell killing in different phases have an important influence when a series of treatments are used (fractionated therapy). The interval between treatments can have a major effect, since the next dose may be given when the majority of survivors are at maximum sensitivity or at maximum resistance. Redistribution of the cells into different phases, at a rate which depends upon the cell cycle time, will vary from tissue to tissue. It may be very fast in intestine, so that the population returns to its original overall sensitivity within a day. In a slowly proliferating tissue the resistant survivors will remain for days or weeks in that resistant stage and may not redistribute into more sensitive phases before the treatment has finished.

Hypoxic radioresistance

The radiosensitivity of all cells depends upon the level of oxygenation at the time of irradiation; cells in the absence of oxygen are 2.5–3.0 times more resistant than those which are well oxygenated.

Hypoxic cells occur naturally in tumours and hence give a therapeutic disadvantage. This could conceivably be overcome by deliberately making the normal tissues hypoxic for the period of each irradiation. This hypoxic radioprotection can be more readily achieved in some normal tissues than in others[31]. It does not relate to cell kinetic parameters, but rather to the vascular architecture of the tissue and the degree of oxygenation of the different blood supplies. However, a fine structure may be superimposed upon this, since the level of thiols changes around the cell cycle which alters the oxygen depletion level needed for radioprotection but only in a minor way[5, 24, 28].

The presence of radioresistant hypoxic cells in tumours, however, is a very common feature and results from the imbalance between tumour cell proliferation and the vascular proliferation needed to maintain a network of capillaries. Tumour cells produce angiogenic factors (TAF) which can stimulate normal endothelium in blood vessels to migrate towards the source of TAF, and to proliferate[14]. Neo-vasculature is a feature of all solid tumours, but it is often poorly organised, with little structural integrity and with a disorganised array of convoluted vessels. Tumour cells are pushed centrifugally away from vessels as they divide and may eventually exceed the maximum diffusion distance for oxygen, and become radioresistant because of it. The thin-walled vessels are also susceptible to collapse as interstitial pressure changes occur, and this may lead to whole vessels and their dependent sleeve of tumour cells becoming temporarily (or permanently) hypoxic. Long-term hypoxia will lead to cell death, but if an oxygen supply is re-established (reoxygenation) the surviving hypoxic cells can recover and regrow the tumour.

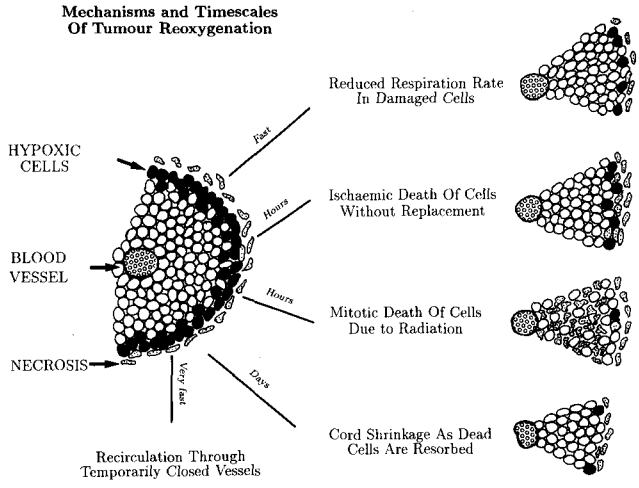

Figure 6. Cells distant from blood vessels become starved of nutrients, particularly oxygen, and are radioresistant. Prolonged hypoxia leads to cell death and necrosis. Five different mechanisms are illustrated whereby hypoxic cells (marked black) are reduced, after a first dose of radiation leading to apparent reoxygenation and consequent increased radiosensitivity. These processes occur at different rates in tumours with different proliferation patterns.

Figure 6 illustrates the mechanisms of reoxygenation. These differ in different tumours, for reasons that may relate to their cell kinetic patterns. Carcinomas, derived from high turnover epithelia, retain a high cell turnover and hence a high cell loss factor as a characteristic feature. They shrink rapidly after irradiation and show extensive reoxygenation. Soft tissue sarcomas, derived from stable, non-renewing stromal tissues show low cell loss factors, slow shrinkage and slow or poor patterns of reoxygenation. The timing of treatments, when tumour cure is the objective, should obviously be directed to allow maximum reoxygenation, and hence sensitization, of these radioresistant tumour cells. Techniques are not yet available to determine whether the daily intervals used in routine radiotherapy already maximise this, or whether this advantage would be lost if tumours were treated more frequently than once a day.

Repair of sublethal injury

As was shown in figure 5 the response of cells when treated with irradiation is usually not linear with dose. At low doses the cells seem relatively resistant to the lethal effects of ionizing radiation, but beyond a quasi threshold dose the response seems to be approximately exponential. Many experimenters describe this shouldered curve in terms of the quasi threshold dose D_Q, the extrapolation number n, and the final slope D_0.[2]. If treatments are given in two increments instead of as a single dose the cells need more radiation to kill them. This is attributed to the cells' ability to repair sublethal lesions before the final lethal lesion is inflicted.

Recent analyses of data obtained from multifraction studies of normal tissues in vivo have led to the readoption of a much older model, the linear quadratic (LQ). This also gives a non-linear response when plotted as log SF versus dose. The emphasis of this analysis centres on the slope of the *initial* low dose region, defined by the α term, and the gradual curvature away from this as the dose squared β term becomes predominant. The dose at which the linear and quadratic terms are exactly equal is defined as the α/β ratio [12, 16]. Below the α/β dose cell kill is mainly linear with dose and is not greatly affected if the dose is subdivided into smaller subfractions. Above the α/β dose the quadratic term predominates. This quadratic or β term involves the interaction between two individual dose-dependent events and is greatly modified by further subdividing the dose.

The important feature of the LQ model is that the α/β ratio indicates at what dose levels further sub-fractionation of the dose gives much extra sparing of damage. At 10% of the α/β dose the reparable quadratic term is contributing less than 10% of the cell kill and hence subfractionating in this range of dose per fraction can only influence 10% of the injury. At the α/β dose however, doubling or halving the fraction size would have an enormous influence on the efficiency of cell kill since half the damage is fractionation dependent. An interesting difference has emerged between the response of rapidly proliferating and slowly proliferating tissues indicating that their α/β values are about 10 Gy and 2–3 Gy, respectively [33]. This is illustrated with examples from skin and kidney in figure 7. The sparing effect which results from

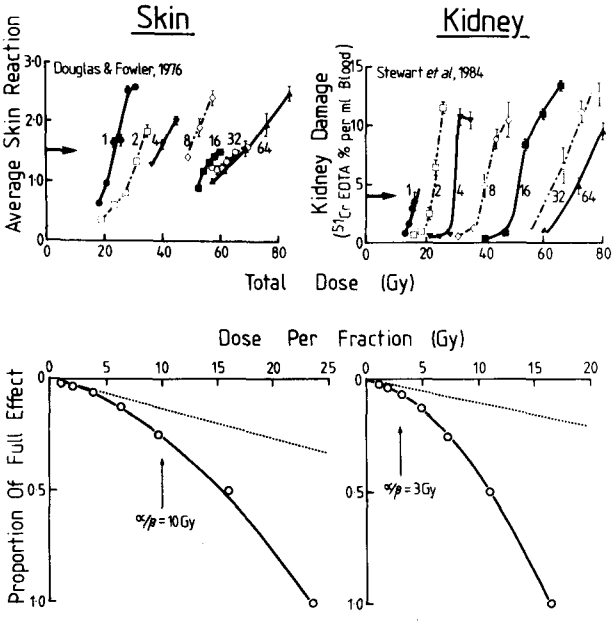

Figure 7. Illustration of the fractionation dependence of the response of two different normal tissues. Skin, an acutely reacting tissue shows sparing as the dose is subdivided up to 16 fractions, but little sparing beyond that. This leads to a very broad shouldered underlying survival curve (lower panel) with an α/β value of 10 Gy. By contrast, kidney, a late reacting tissue shows further sparing if the 16 fractions are subdivided into 32 and then into 64 fractions. This suggests an early departure from linearity with an α/β value of 3 Gy. The lower panels are constructed by assuming a single dose gives the full effect, each of the 2 fractions gives half the effect, etc. (Data derived from Douglas and Fowler [12]; and Stewart et al.[32])

repair in these two tissues is similar from 1–16 fractions but beyond that the pattern differs. Sparing with further fractionation is seen in kidney, even going from 32 to 64 subfractions[32]. By contrast little additional sparing is seen in skin by subfractionation after the dose has been divided into 16 fractions[12].

This difference in fractionation response between skin and kidney has been shown to be a general feature of rapidly and slowly proliferating tissues, although the biological basis for it is unclear[15, 16, 33, 36]. Slowly turning over tissues (low α/β value) are very sensitive to changes in the size of each dose fraction within the clinical range of 2–3 Gy, whereas rapidly proliferating tissues and tumours (high α/β values) are insensitive to changes in fraction size. This is the rationale for hyperfractionation, i.e. the use of very many, very small fractions (e.g. 60–80 × 1 Gy) since it will spare late reacting tissues but still be quite effective on tumours. Acute reactions are likely to become dose limiting with this approach.

Repopulation

In the examples shown in figure 7, the time between each pair of fractions was only a few hours. This may allow complete biochemical repair of the reparable DNA lesions but does not allow for much proliferation in the surviving cells. If a longer period of time is allowed between treatments the cells surviving the first dose can proliferate and help to restore the tissue population to its original level. This will occur within hours to days for intestine and other rapidly proliferating tissues[35]. In skin it has no significant influence on the response unless the interval between two treatments is at least a week or the overall time is 2 weeks or more[7]. In slowly proliferating tissues the sparing from repopulation is even more trivial and sometimes cannot be detected even if treatment times are extended over several weeks or months[9].

The repopulation that serves to rescue the tissue can take two forms: the normal rate of proliferation, or an accelerated rate which can be induced as a compensatory response to tissue injury. The normal rate is that present prior to irradiation. This has superimposed upon it the delay resulting from arrest at the G_1/S and G_2/M boundaries. The accelerated proliferation can be much faster. Even intestinal crypt cells can shorten their cycle time from 12 h to about 6 h[22]. This occurs within a day or two of the first irradiation. Skin, which has a normal cell cycle time of 100 h, can shorten this to about 18 h[11]. It seems likely that the time at which accelerated compensatory proliferation occurs relates to the time at which the tissue begins to fail in its normal function. This in turn relates to the turnover time of the differentiated cells, and their rate of replacement by the proliferating sub-population. A failure of replacement leads to a failure of the differentiated function and this is the likely stimulus for an alteration of the production rate. The time at which acceleration has been demonstrated correlates quite well with the time at which tissue dysfunction, and histological disruption become apparent[8]. Thus prolongation of cancer treatment to allow the normal tissues to benefit from the sparing effects of proliferation can be dangerous. Such sparing will be seen in the early reactions that result in rapidly proliferating tissues but will be absent in the late responding deeper tissues, which may be more life threatening if they fail in their function. The realization that this is so has led to a questioning of the common clinical practice of allowing a split or rest period part-way through therapy to allow the normal tissue reactions to subside. During this interval of 2–3 weeks the slowly proliferating tissue will *not* repopulate, but tumour cells may proliferate wildly as they reoxygenate and are recruited back into active division.

Conclusion

Radiation pathology is a general term describing the damage that occurs in tissues after irradiation. After the very low doses, received by the normal working population, no major pathology is seen. There is a hazard of cancer induction if DNA damage that has been inflicted in an individual cell is repaired in such a way that the DNA remains intact but rearranged. This radiation carcinogenesis is however a low risk compared with many chemical carcinogens in the environment and in cancer chemotherapy.

The treatment of cancer by radiation is now commonly accepted as one of the most effective forms of treatment. It can kill tumour cells effectively, but the dose that can be given is limited by the normal tissues that are inevitably included in the beam. Cell function is maintained for some time even after very large doses. However normal tissues show a loss of function and structure because the proliferating subcompartment of each tissue is depleted as the radiation injured cells fail to divide and die. The time at which the cell deficit is detected varies from hours in some tissues to months or years in others. It depends upon the normal rate of cell turnover. The apparent sensitivity of each tissue therefore depends upon the time at which the assessment is made. Lung and kidney would appear very resistant at 1–3 months post irradiation, but would seem very radiosensitive at 6–12 months as their latent damage is expressed.

The ultimate expression of radiation pathology is the death of the whole animal as the essential organ function fails. The time of this death is only comprehensible if the time sequence and the proliferation kinetics of the target cells are taken into account. It must be recognised that it is initial damage to the clonogenic cells, not to the differentiated cells per se that is important.

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Modifiers of radiosensitivity

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Key words. Radiosensitivity; radioprotection; chemical modifiers; biochemical modifiers; physical modifiers; physiological modifiers; synchronisation therapy; tumour radiosensitizers; thiol depletion; tissue hypoxia; blood flow modification.

Introduction

Biochemical and chemical modifiers of radiation response have developed partly because of the interest in differentially sensitizing tumour cells or protecting normal cells. The important concept for their cancer therapy application is that a therapeutic gain is being sought which has an absolute prerequisite of differential effects