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Can cell survival parameters be deduced from non-clonogenic assays of radiation damage to normal tissues?

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Summary Though dose-response curves for large scale radiation injury to tissues are undoubtedly related to survival curves for clonogenic cells, the relationship between the two sets of curves is not necessarily simple. Sterilization of clonogenic cells occurs near-instantaneously by comparison with the protracted lag period for gross injury to tissues. Moreover, with some types of macroscopic damage, the shapes of the dose-response curves may depend on time of assay. Changes in the area or volume of irradiated tissue may also influence the shapes of these curves. The temporal pattern of expression of large scale injury also varies between tissues, and two distinct groups can be recognized. In rapidly proliferating tissues, the lag period is almost independent of dose, whilst in slowly proliferating tissues, it is inversely proportional to dose. This might be explained by invoking differences in corresponding proliferative structures of the tissues (Three compartmental Type H versus One compartmental Type F proliferative organization). For the second group of tissues, in particular, mathematical modelling suggests a systematic dissociation of the dose-response curves for clonogenic cell survival and for large scale injury. This dissociation, which arises even in the case of single doses, may be even more important when radiation is fractionated. In particular, it may be difficult to disentangle the contributions made to inter-fraction sparing by cellular repair processes and by proliferation-related factors.

We have chosen to address the question of correspondence between the clonogenic cell survival curves on the one hand, and the dose-response relationships for gross lesions or functional impairment on the other.

Even at a superficial level, there are indications that dose-response relationships for macroscopic damage or overall functional deficiency cannot be regarded as faithful reflections of survival curves. Invariably, a temporal dissociation is observed in that impairment, by irradiation, of cellular reproductive potential could be regarded as nearly instantaneous by comparison with the time-scale for development of gross damage which takes weeks, months or even years. With some types of macroscopic endpoints for radiation injury, the shapes of the dose-response relationships may vary depending on the point in time after exposure at which the measurements are made. This is exemplified in Figure 1 where, with time elapsing from kidney irradiation, the mortality curves are gradually displaced towards lower doses and become steeper (Phillips & Ross, 1973).

Spatial considerations also put the two types of dose-response relationships apart. The parameters of any given cell survival curve, if they are representative of properties of individual cells, are entirely independent of the sample size. This is not the case with dose-response relationships for the incidence of macroscopic lesions of a defined severity, where the probabilistic prediction is that an increase in the number of cells exposed to ionizing radiation would be responsible for displacement of the curve towards the lower dose

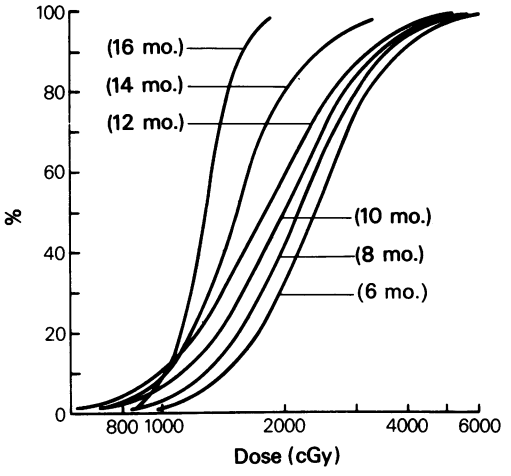

Figure 1 Probit curves for percentage of death *versus* log dose, in kidney-irradiated mice. Death is due to renal failure. Reproduced with permission from Phillips & Ross (1973).

*Deceased.

range and for an increase in the slope. Figure 2 shows a probit plot for the incidence of ulcerative oesophagitis (UO) in mice *versus* single X-ray dose (Michalowski *et al.*, 1983). At 50% incidence level, induction of UO requires a dose higher by ~ 2 Gy when a 12 mm long segment of the oesophagus is irradiated by comparison with the ED50 for twice the length. Moreover, for the larger field the slope is steeper, and the actual incidence tends to exceed the expected one, as calculated on the basis of experimental findings for the shorter segment and represented by the broken line.

It may be seen therefore that both temporal and spatial influences may be important in the translation of radiation sterilization of individual clonogenic cells into large scale effects resulting in functional impairment or gross injury of organized tissues, as observed in the clinic or the laboratory.

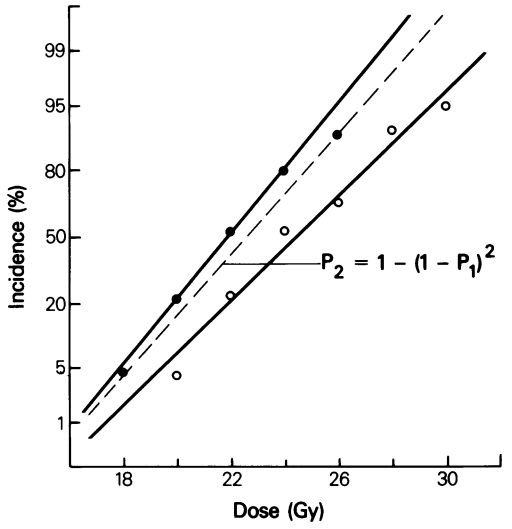

Figure 2 Probit curves for the incidence of ulcerative oesophagitis *versus* dose in thorax-irradiated mice for field length of 12 mm (○) and 24 mm (●) (continuous lines). For explanation of theoretical curve (broken line) see **text.** From Michalowski *et al.* (1983).

Kinetic patterns of radiation response

In recent years, a number of studies have focussed on the detailed kinetics of the expression of radiation injury by different tissues, and in particular, on the relationship between the time at which damage of a given severity is observed, the radiation dose which has been given, and the steady-state kinetics of cell renewal in the tissue prior to irradiation. These studies have led to the recognition of two distinct groups of tissues for which the relationships appear to be very different.

The first group of tissues, characteristically those which express injury early rather than late, present a pattern which has become familiar to radiobiologists and to cell kineticists. These tissues, of which bone marrow, epidermis and gastro-intestinal epithelium are typical examples, express radiation damage at a rate which is almost uninfluenced by the radiation dose (see Figure 3), provided only that the dose is above a threshold to induce a particular gross lesion. Moreover, the time at which this level of injury appears is directly proportional to the turnover time of the mature cells of the tissue concerned.

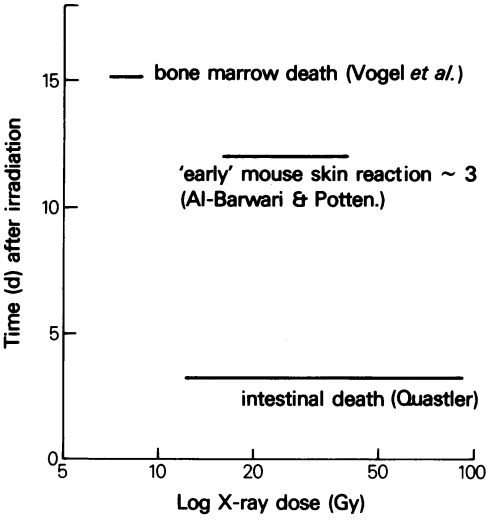

Figure 3 Time after irradiation to express a fixed level of damage as a function of dose for bone marrow (Vogel *et al.*, 1957), epidermis (Al-Barwari & Potten, 1979) and for small intestine (Quastler, 1945).

The second group of tissues, typically late-responders to irradiation, follow a pattern which is qualitatively quite different from that of the preceding group. Organs or tissues of this type include the central nervous system, the kidney, the lung and the vascular endothelium. Figure 4 shows the relation of latency to dose for radiation damage to mouse lung, and demonstrates a pattern which is typical of this group of tissues. As may be seen, the lag period is very sharply shortened with increasing dose, especially at lower doses. Only at higher doses do the falling curves begin to level off. Evidently, different mechanisms would seem to be implicated in the expression of radiation damage by these different tissues.

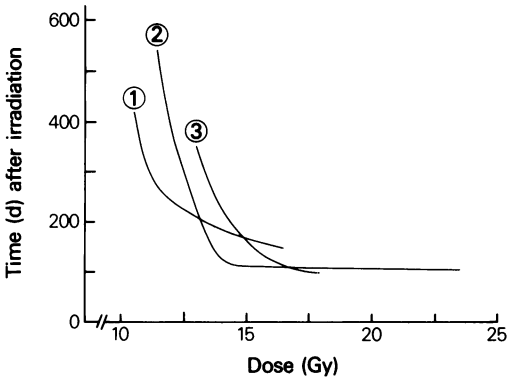

Figure 4 Time after thoracic irradiation to death of 50% of mice as a function of dose. Data of (1) Hill (Personal Communication 1982), (2) Collis & Steel (1982) and Siemann *et al.* (1982) (3).

Kinetic models of radiation response

A commonly accepted explanation of the constant, dose-independent rate of expression of radiation injury by the first group of tissues was put forward by Patt & Quastler (1963) and was based on the organization of the cell populations. The epithelial lining of the alimentary tract, the epidermis, the bone marrow and its derivative blood-circulating cells, as well as testicular epithelium are schematically represented as each comprising three compartments: stem cells characterized by infinite potential for cell division but incapable of function; transit cells which acquire functional capacity while their proliferation becomes increasingly limited; and the mature, fully functional but irreversibly postmitotic cells. We call such three compartmental cell populations type H for hierarchical.

Since cell reproduction is by far the most radiosensitive activity of mammalian cells, and cell division is necessary to express cellular radiation damage, irradiation results in selective injury to the first and second compartments, and thus leads to temporary interruption of the cellular input to the third, mature cell compartment whose cells remain intact. Therefore, according to theoretical computations, (Wheldon *et al.*, 1982) the depletion of the functional cells which follows irradiation continues to proceed linearly at the physiological rate, and the time of onset of any fixed level of injury is largely independent of dose and determined by the time of transit through the mature compartment.

An important radiobiological characteristic of the hierarchical structure is the existence of the transit compartment which differentiating stem cells must traverse, typically undergoing a number of obligatory divisions, before their progeny acquire functional competence. Hence, radiation-sterilized stem cells, capable of only one or two divisions, can make little contribution to maintenance of tissue function. The greater the number of obligatory divisions within the transit compartment, the less will be the contribution made to tissue function by "non-viable" stem cells with only "sub-clonogenic" proliferative ability.

One way of accounting for the alternative pattern of response to irradiation, characterized by the marked influence of radiation dose upon the rate of development of radiation damage, is to consider radiobiological properties of a cell population of which the parenchymal cells of the liver are an archetype. All cells of such a population are functionally competent while retaining their proliferative potential, with a majority of hepatocytes usually residing in the G_0 phase of the reproductive cycle. In normal circumstances the rate of cell loss is very low, and a correspondingly small percentage of cells is triggered into the cell cycle at any given time. However, an incidental, simultaneous loss of a large number of cells uncovers the proliferative capacity of the remainder which, as is the case after partial hepatectomy, parasynchronously enter the reproductive cycle and keep dividing until the loss is made good. We call such one compartmental populations flexible, or type F.

Mathematical modelling and computer simulations of radiation response of a type F cell population showed that, in contrast to type H populations, the rate of depletion following large doses of radiation is not constant but increases with time ("the avalanche phenomenon"). Also, at a given level of population size, the instantaneous rate of depletion is higher the higher the dose. The dose-dependent rate of depletion of a type F tissue leads to a pronounced dose-dependence of the time to reach any fixed level of injury, with higher doses resulting in earlier functional impairment. At any dose level the time-scale of expression of injury is shorter than anticipated from the rate of cell turnover in the unirradiated tissue (Wheldon *et al.*, 1982).

A distinguishing feature in the radiation response of the type F model tissue is the role played by sub-clonogenic proliferation. The relative importance of non-viable cells for type F responses results from the absence of a transit compartment in type F proliferative organization. Hence, even a single successful division contributes usefully to maintenance of tissue function. This necessitates a shift of emphasis from almost exclusive concern with clonogenic potential to study of dose-dependence of the actual proliferative performance of all cells capable of division. Already the classical

paper of Puck & Marcus (1956) clearly shows that the proliferative performance of both clonogenic and sterilized cells is increasingly impaired with increasing dose. This amounts to an additional dose-dependent component in the gross response of the type F proliferative organization which is distinct from, and not necessarily parallel to, the dose-response relationship for clonogens.

Factors influencing large scale responses to single doses

Any list of factors bearing on the ultimate severity and timing of injury caused by irradiation should also include the functional reserve of the tissue and the demand for its function. To give but one example, when kidneys of rats are irradiated and the animals then kept on a low protein diet, they develop less severe renal lesions and survive twice as long as the identically treated rats fed the standard diet (Mahler *et al.*, 1982). A truncated list of possible factors influencing large scale effects of single doses is presented in Table I.

Table I

Large scale effects of single doses of radiation on normal self-renewing cell populations are determined by:

- intrinsic radiosensitivity of clonogenic cells (survival curve parameters)
- dose-dependence of the rate of proliferation of surviving and non-viable clonogenic cells
- proliferative organization of the population (type H versus type F)
- turnover time of *functional* cells under physiological (steady-state) conditions
- sensitivity of the homeostatic control of population size responsible for regenerative proliferation (repopulation)
- functional reserve
- demand for function

Factors influencing large scale responses to multiple doses

Those factors which influence tissue response to single doses of radiation will, in general, also play a part in the gross response to multiple doses. However, fractionated exposures may also bring into play additional variables some of which are tentatively listed in Table II. First among these are the cellular repair processes which are the focus of these proceedings. We would like, however, to draw attention to additional factors usually grouped together under the broad heading of "effects of proliferation". In particular, regenerative (i.e., non-steady-state) proliferation, if it comes into play within the time-scale of the treatment schedule, will have at least three radiobiological consequences. Firstly, it gives rise to increased cellular birth rate, and hence to increased inter-fraction sparing. However, this may be at least partly offset by a reduced capacity for repair of PLD in cycling cells. Thirdly, regeneration entails changes in the age-structure of the population which, in turn, result in an altered shape of the single dose survival curve. The time-scale over which steady-state proliferation gives way to regenerative proliferation is governed by the steady-state kinetics of the cell lineage in question and the sensitivity of the homeostatic monitor of cell number.

Table II

Large scale effects of multiple doses of radiation on normal self-renewing cell populations are determined by:

- cellular repair of non-lethal damage
- rate of maintenance (steady-state) proliferation
- timing and rate of regenerative proliferation (repopulation), i.e., increase in cellular birth-rate accompanied by reduction in the amount of PLD repaired and
  changes in proportion of cell cycle phases leading to modified radioresponsiveness of the population
- differential lengthening of cell cycle phases (mitotic delay)
- cell cycle phase-dependent "killing"
- decay of radiation-induced parasynchrony (redistribution)

Since these proliferation-related factors influence the timing and severity of gross damage, their dependence on the radiation dose becomes an important question. In particular, there is no *a priori* reason why each of these factors must necessarily follow a dose-response relationship which parallels that for reproductive integrity of clonogenic cells; this applies both to the relationship to total dose, and to the fractional dose at each exposure. Should any one of these relationships differ from the corresponding relationship for clonogens, a systematic difference in the shapes of the appropriate curves for clonogenic cells, and for functional impairment or gross injury, is to be anticipated. In practical terms, the possibility of total dose-dependence, and of fractional dose-dependence of these proliferation-related factors means that the radiobiological consequences of proliferation are not necessarily the same for different treatment regimes, even when these are given in the same overall time.

Attempts have been made to deduce the clonogenic cell survival curve from isoeffect data obtained by means of a series of multifraction experiments (see Withers, 1983). The assumptions implicit in this approach are that the shape of the survival curve for the first exposure is faithfully reproduced at each subsequent irradiation, and that the total amount of intervening proliferation of clonogenic cells, as well as their post-treatment proliferation are independent of fractionation regime. From the acceptance of these assumptions it follows that with sparsely ionizing radiations the downward bending of the quadratic survival curve tends to be more pronounced among the clonogenic cells of late reacting normal tissues than among those responsible for early reactions. However, this general deduction does not seem to be supported by the shape of the directly generated survival curves for the parenchymal cells of the thyroid, mammary gland and liver (Gould *et al;* these proceedings).

Moreover, the survival curves for normal plateau-phase fibroblasts, a cell type ordinarily associated with late responses (fibrosis), are purely exponential (Arlett & Priestley; these proceedings). It seems therefore safer at present to view the fractionation experiments as addressing the practical question of the degree to which the interfraction sparing depends on fraction size and number, rather than as uncovering the intrinsic radiobiological properties of the clonogenic cells.

Should it prove to be the case that the dose-response relationships for large scale radiation damage are dissociated from underlying survival curves, this would be of more than academic interest. In particular, a better understanding of such a dissociation could pave the way for *post*irradiation manipulations at the cell population level aimed at modulation of gross radiation injury or functional impairment.

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