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CELL CYCLE KINETICS AND RADIATION THERAPY

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ABSTRACT

The application of cell cycle kinetics to radiation therapy is one hope of significantly improving therapeutic results. Three classes of approach are available: the combined use of intracyclic differential sensitivity and cell synchronization; the application of cycle-linked radiation sensitizers and chemotherapeutic agents to affect cycling versus noncycling cells; and the selection of therapeutic intensity on the basis of repopulation rates of tumor and relevant normal tissue before and after perturbation. A survey of how each approach could be used and of the available kinetic data suggests that there is much to be learned about the kinetic properties of tumor and normal tissue before these approaches are likely to bear fruit.

Cell Cycle

Radiation Therapy

Chemotherapy

Synchronization

Tumor Kinetics

Radiation therapy as currently practiced involves the subtle largely empirical art of balancing the recurrence of cancer due to undertreatment against severe damage to local tissues due to overtreatment. Therapeutic results too often fall short of desired success rates; yet the therapist is continually tantalized by the likelihood that a slight shift of therapeutic ratio favoring normal tissue over cancer would have a profoundly beneficial effect. The application of cell cycle kinetics to radiation therapy is one hope for improving the therapeutic ratio; but as I will try to show, kinetic approaches are complex, poorly understood, and presently too elusive to elicit confidence or to be used clinically. Their promise lies in their diversity and in the magnitude of our ignorance about how they work and how they should be used.

Potentially useful kinetic approaches to therapy can be grouped into three classes. The first class takes advantage of intracyclic differential sensitivity, an effect involving the metabolism and biology of the cell cycle; its strategies are based on synchronization of cells over intervals of hours to days. The second class involves the distinction between cycling and noncycling cells; its strategies are based on the resistance of noncycling cells to cycle-linked radiation sensitizers and

chemotherapeutic agents. The third class uses cell repopulation between fractions; its strategies are based on the relative growth rates of tumor and relevant normal tissue before and after perturbation.

Intracyclic Differential Sensitivity

Pattern, Amplitude and Phasing. The radiation sensitivity of cells varies with relative cell age (1). Such age responses are roughly sinusoidal and fall into two patterns depending on whether there are one or two resistant periods per cell cycle (Figure 1). Conceivably the differences in pattern could be used therapeutically. For example, if one were dealing with tumor cells having a single resistant period per cell cycle and normal cells having a double resistant period, then the therapeutic ratio might be improved by synchronizing the cells early in the cycle and treating at the time of the first resistant peak. But realistically, almost all cells that have been studied are of the two-period-per-cycle type; much of the seemingly dramatic difference in pattern disappears when considered in real time because the cells with the single period of resistance generally have very short cycle lengths with little or no G_1 period.

The amplitude of the sinusoidal age response describes the magnitude of selective killing effects. When two populations such as those in Figure 2 differ in their amplitude of age response, a therapeutic advantage is achievable. In general

such strategies will focus on the large-amplitude population. For example, if the normal cell response has the large amplitude, then timing of therapy would be oriented toward the resistant peaks. If the tumor-cell response has the large amplitude, then one aims for the sensitive troughs. Large differences in amplitude have been reported among cell types, with peak to trough survival ratios ranging from threefold to seventyfold (2). Ten to twentyfold differences are present in the one-period-per-cycle response of V79 Chinese hamster cells studied by Sinclair (1) and shown in Figure 3. Amplitude generally increases with dose; in the V79 cells it plateaus at 750 rad, a single dose larger than those usually given in clinical radiotherapy.

In Figure 4 threefold differences are shown by Rockwell and Kallman in the two-periods-per-cycle response of EMT6 mouse mammary tumor cells grown in vitro (3); and here the full effect is manifest at 600 rad. So far as I know, systematic differences in amplitude have not been found for tumor versus normal cells. What is more, much of the reported differences in amplitude could be technical, reflecting methods and quality of synchronization rather than inherent biological differences in age response.

For exploitation of amplitude or any other age response the cells must be synchronized. In examples such as Figure 3 or 4, a first radiation dose to asynchronous cells gives the age-response survival pattern and thus partially synchronizes the survivors into the resistant parts of the cycle. Alternatively,

the cells can be synchronized by chemical blocking agents, but then to capitalize on differential sensitivity one must understand how the phasing of the age response relates to the site of drug action.

Figure 5 combines the radiation age-response, the phases of the cell cycle, and the site of action of some representative blocking agents. The radiation age-response is loosely coupled to the phases, with maximum sensitivity at mitosis and resistance rising during the S (DNA synthetic) phase. Drug effects are more directly tied to the phases of the cycle, with cell killing occurring predominantly in S and cell blocking predominantly at the G₁-S boundary and at mitosis (4). Use of blocking agents to synchronize cells might greatly sharpen the therapeutic ratio of a subsequent treatment with radiation; but there are many complications to this approach, including systemic effects of such drugs, uncertainty about the kinetics as the block is released, and the interaction of chemical and radiation effects on cell survival (4).

Figure 6 shows an example of the complex pattern of sensitivity found in combination treatment of mouse leukemia cells with 5FU and x-rays (5). Such patterns are poorly understood at present, but they do differ in at least one nontumor system--normal bone-marrow colony-forming cells. Extension to other tissues, other tumors, and other combinations may someday make this a formidable approach to cancer therapy.

Cell Cycle Time. To make the transition from relative age to real time, we now consider the duration of the cell cycle. Under idealized steady-state conditions, stable difference in the mean cycle times of the critical normal and tumor populations leads directly to strategies of selective killing of the cancer cells. The upper panel of Figure 7 shows two age responses with identical patterns and amplitudes but with cycle lengths in the ratio of 2:3. The resistance ratio of the two responses shown in the lower panel is a compound sinusoid with much structure and dramatic peak to trough ratios. Regardless of which response is the normal, one could in principle optimize the therapeutic ratio from such a diagram. As a rule the interval between peaks and troughs of therapeutic ratio is a quarter of the shorter cell-cycle time--and hence may be only a few hours. The construction of such a diagram in an actual therapeutic situation would require estimates of each cycle time to within plus or minus 10 percent, a difficult feat with current kinetic methods.

The cell cycle times of tumor and normal tissues overlap throughout a broad range. The shortest cycles are found in intestinal crypts, hair follicles, and exceptionally aggressive experimental tumors. The longest measured cycles are in corneal epithelium, skin, and epithelial human tumors.

Since current methods of selecting tumors and analyzing cycle times are biased toward short cycles, we probably have a distorted view of the range and distribution of cell cycle times.

For several reasons it will be unrealistic to plan therapy on the basis of unperturbed steady-state estimates of the cell cycle time. Each radiation exposure is followed by a G₂ block which effectively prolongs the cycle. The duration of the block in V79 cells as a function of dose and cell age is shown in Figure 8 as measured by Sinclair (6). The impact of this block on the timing of peaks and troughs of sensitivity is shown in Figure 9 taken from Young and Fowler (7) and based on Sinclair's data. The location of peak and trough and the interval between them varies with dose. Obviously, if one were selectively killing V79 cells in the presence of yet another cell population, differences of a few hours would have major effects on the therapeutic ratio.

Typically, G₂ block in a variety of cell systems lasts for about one hour per 100 rad (8). Differences amongst cell types may relate to cycle length (9), but consistent differences between tumor and normal tissues are yet to be described. Such differences are worth exploring because G₂ phases generally are longer in tumors than in normal tissues (10,11). This is the only consistent cell cycle difference between the two that has so far been uncovered, and to my knowledge it has not been exploited for therapy.

Another complication of cell cycle time is variability. Regardless of how cells have been synchronized, they progressively disperse and typically return to the asynchronous state within one or two cycles. Thus the sinusoidal responses already described are actually decaying in amplitude with time. Such damping works against the therapist by forcing him to treat quickly, but it can also work for him when the rates of damping differ greatly between tumor and critical normal tissue.

Flow microfluorimetry (or impulse cytophotometry) of DNA per cell is the most direct and powerful way to measure synchronization and desynchronization in perturbed cell populations. The upper block of panels in Figure 10 shows a time sequence of experimentally determined distributions of DNA content of CHO cells after their release from block in early G_1 (12). At 2-1/2 hours after release the cells peak in early S, then later they are in mid-S, in G_2 and G_1 , and on into the second cycle with much broadening of the peaks due to desynchronization. The lower panel of Figure 10 is a computed simulation of the sequential DNA distributions of the CHO cells (13). Good estimates of the rates of dispersion as well as the transit times of the synchronized cells can be obtained from such simulations.

In unperturbed asynchronous populations, the transit times and standard deviations of transit can be taken from conventional curves of fraction of labeled mitoses (FLM). Very likely the dispersion of truly synchronized cells is the same

phenomenon as the damping of FLM curves due to desynchronization of cells labeled in S, although it may be that truly synchronized cells sense the need to disperse or have abnormal kinetics secondary to the synchronizing event. Direct comparisons of FLM curves and fluorimetry on the same cell system would readily settle this question.

If FLM curves are valid indicators of damping, then wide differences in desynchronization rates exist. Computer analyses of FLM data provide estimates of the standard deviation and coefficient of variation of cell transit through each phase and through the entire cycle. These cycle-based or phase-based statistics are straightforward estimates of desynchronization when comparing populations with similar cycle and phase lengths, but when transit times are dissimilar the statistics are difficult to interpret. For example, two populations with equal coefficients of variation of cycle time but unequal cycle lengths will have different rates of desynchronization. For some applications dispersion per cycle or per S phase is of interest; in other situations the most useful descriptor of desynchronization would be the dispersion that takes place over a stated time interval. By disregarding phase-specific differences in dispersion and by assuming statistical independence of the aging process throughout the cycle, a simplified estimate of time-based dispersion σ_t can be derived from dispersion per cycle σ_c :

$$\frac{\sigma_t}{\sqrt{t}} = \frac{\sigma_c}{\sqrt{T_c}}$$

where t is the interval over which the dispersion is being estimated and T_c is the mean cell cycle time. A useful special case is the hourly dispersion σ_d , when $t = 1$.

Figure 11 shows the behavior of hourly dispersion as a function of cell cycle time for 49 computer-analyzed well-behaved FLM curves. These curves are culled from the literature and have been fitted by Gordon Steel (14), by our own program (15), and by several other methods (16,17). The data base and its other kinetic properties have been partially described (11). The clear regression of hourly dispersion on cycle time suggests that the control of progression loosens as the cycle lengthens. Very likely the sensitive peaks and troughs also lengthen with cycle length and hence from the therapist's viewpoint the ideal parameter might be dispersion per resistant peak duration, an ordinate that perhaps would eliminate much of the regression in Figure 11. But it is interesting to note that tumors and normal tissues have an overlapping distribution whereas ascites cell tumors lie slightly below. In general, ascites cells have unique kinetic attributes; in this case the data suggest that the fluid environment of ascites cells may minimize dispersive effects.

The therapeutic impact of dispersion is summarized in Figure 12 taken from Gray (13). The three panels show the effect of coefficients of variation of cycle time (and hourly dispersion) of 19 percent (0.78 hours), 31 percent (1.27 hours) and 62 percent (2.53 hours) on cells initially fully synchronized

in G_1 . The curves, which give the oscillations of the fraction of cells in each phase, are realistic indicators of what a therapist might have to work with if, for example, he was optimizing the killing of cells in the S phase.

There are other complications of cycle time, such as diurnal variation (18), and the kinetic distinctions between oxygenated versus hypoxic or clonogenic versus nonclonogenic cells, but to me the most interesting complication is the change in cycle time as therapy progresses. Cell depletion secondary to treatment causes reactive hyperplasia involving recruitment of noncycling or G_0 cells and shortening of the cell cycle times. In solid tumors, the cycle shortening is sometimes present and sometimes not; at best it is a twofold effect (19). In skin, greater than a fivefold shortening of the cycle has been measured after irradiation (20), and significant shortening occurs in gut and other rapidly turning over tissues (21). What little is known of these effects in normal tissue suggests that they involve tissue-specific feedback controls driven by the depletion of functioning cells.

The contrasting effects of cell depletion on the cycle time of tumor, rapidly turning-over tissue, and slowly turning-over tissue are shown in Figure 13. Cells are reproductively killed by radiation in much the same way in all three cases, but the depletion of what might be called the functioning fraction depends on the turnover time. In rapidly turning-over tissue the functioning fraction lags slightly behind and then parallels

the survival curve. The rapid depletion of such normal cells elicits a brisk corrective kinetic response in cycle time. The tumor-cell cycle may be changing as well, although not as dramatically and not on the same time scale as the normal tissue. When such rapidly responding tissues are dose-limiting and intracyclic sensitivity is to be used throughout therapy, one must understand the full kinetics of the corrective responses. Slowly turning-over tissues are much more likely to be dose-limiting, and in these tissues cell depletion lags greatly behind cell survival. Although this simplifies the dynamics of the intracyclic response, it seriously compromises these tissues in other ways, as will be shown below. It should be emphasized that Figure 13 is largely conjectural because we are presently limited to data on only a few tumors (19) and a few normal tissues (9) soon after single doses.

Noncycling Cells

The effects of intracyclic differential sensitivity do not apply to out of cycle or G_0 cells. Thus the proportion of cells in G_0 limits a population's susceptibility to cycle-linked drug effects and to strategies based on synchronization. This immunity to therapy can work for or against the therapist. The G_0 tumor cell resists chemical radiosensitization by BUDR (22) and is a major factor in the nonresponsiveness of solid tumors to all cycle-linked chemotherapy (23). The G_0 normal cell protects bone marrow, liver, kidney, and many other

tissues from chemotherapy and radiosensitization. In radiotherapy the out-of-cycle normal cells can be a major problem because they are vulnerable to radiation but are not protected by repopulation.

The G_0 commitments range greatly in normal tissues, from essentially zero in skin and intestinal crypts to essentially 100 percent in liver and other facultatively or slowly turning-over tissues. In microscopic foci of tumor the G_0 commitment is small but increases rapidly with subsequent growth. By the time a solid tumor is macroscopic, the commitment stabilizes with some 25 to 80 percent of cells out of cycle.

The G_0 cells are dramatically responsive to depletion of the population and are probably controlled in much the same way as the feedback mechanisms that change the cell cycle. How G_0 commitment and recruitment might work during therapy is shown in Figure 14. In rapidly turning-over tissues the few G_0 cells that are present are soon recruited into cycle. Tumors probably respond more slowly but before therapy ends they too are likely to have recruited all their surviving cells into cycle. Slowly turning-over tissues, the ones most likely to be dose-limiting, have a large G_0 commitment and recruit late in treatment or even after treatment has ended. These relationships provide an important rationale for chemotherapy, for as Schabel and colleagues have shown (24), solid tumors become much more susceptible to cycle-linked therapy after their cell number

is reduced by nonspecific therapy. In a similar way Brown et al. show improvement of therapeutic ratio when radiosensitization with BUdR is continued during fractionated radiotherapy (22). Slowly we are learning more about the biology of G_0 and we may someday understand enough about tissue-specific and tumor-specific G_0 control to take full advantage of this effect in therapy.

Repopulation

The multiplication rate of surviving cells is the product of the growth fraction and the reciprocal of the cell cycle time, and hence involves the same kinetic factors that have already been discussed. The issue now is not synchronization or recruitment but rather the relative rates of replacement of surviving normal and tumor cells. When normal tissue repopulates more rapidly than tumor, the therapeutic ratio is improved by long intervals between fractions, low dose rates and even split courses. When the critical normal tissue repopulates more slowly than tumor, the therapy must be as intense as other factors allow. Most likely the relative rates of repopulation will change as therapy progresses; and thus the intensity of optimum therapy will also have to change. To obtain maximal repopulation rates for normal tissue, the therapist must force a functional impairment of the tissue. For slowly turning-over tissue, it is conceivable that optimal therapy would involve a deliberate insult a month or more before definitive therapy in order to activate repopulation in the critical dose-limiting cells. Such strategies oriented

toward maximizing normal regrowth are incompatible with those oriented toward the protected status of normal G_0 cells.

The accelerated repopulation of clonogenic tumor cells is shown dramatically in the data of Hermens and Barendsen in Figure 15 (25). After 2000 rad, the clonogenic rhabdomyosarcoma cells show a roughly tenfold shortening of their doubling time and at their peak response appear to be doubling twice a day. Figure 16 shows the comparable response of jejunal and colonic epithelium as measured by Withers using the split-dose approach to colony assay in vivo (26). In jejunum after a delay of almost three days, the data suggest two logs of repopulation with a doubling time of four to eight hours. The response in colon is later and less brisk, with doubling times roughly similar to those of the repopulating rhabdomyosarcoma cells. Withers' results using similar techniques give a doubling time of 22 hours for skin (27), 42 hours for stomach, and 70 days for testis (28). Such experiments need corroboration and extension to many tumors and to other normal tissues of relevance to the therapist, but it is obvious already that the patterns will not be simple.

Conclusion

By now the message should be clear: cell cycle effects impinge on therapy in many ways, so many that to optimize therapy in kinetic terms will be an enormous conceptual and biological task. Conceptually we are dealing with many cell types, eight or more dynamic properties of the cell cycle, multiple modalities

of treatment, and several other important noncyclic aspects of therapy such as repair of sublethal damage, reoxygenation, and cell mobility. Problems of this complexity cannot be handled intuitively or with blackboard logic. They must be modeled, and one can readily predict that with this many variables the modeling will have to be done with great sophistication. There are now dozens of computer models of cancer therapy, with a wide and healthy diversity of biological assumptions and mathematical techniques (29).

But much more importantly before we can establish the credibility of such models we need a far better understanding of the key biological phenomena. We must know the cell kinetic properties of a wide variety of tissues and tumors--not just unperturbed and not just after a single perturbation, but throughout entire treatment courses. We must identify and understand the specific dose-limiting cells for each radiotherapeutic region of the body. We must extend such studies to man. And to do these things we must have new techniques, particularly more accurate and more practical methods of kinetic and clonogenic analysis.

The near-term prospects for kinetic optimization are rather pessimistic, but I believe the long-term chances are too good to pass up. Unless cancer is cured by some other means, we may well be using kinetics routinely in the years to come. What form might this take?

- Perhaps an all radiotherapeutic approach but with doses and dose intervals that change greatly over the course of therapy.
- Perhaps combined therapy in which each fractionated episode includes a relatively nontoxic blocking agent to synchronize the cells followed by radiation at a carefully defined interval.
- Perhaps with an armamentarium of chalones and antichalones such that the therapist is in full control of the kinetic variables for all the relevant tissues.

But first there is much science to be done.

References

- (1) W.K. Sinclair, Cell cycle dependence of the lethal radiation response in mammalian cells. Current Topics in Radiation Research Quarterly 7, 264-285 (1972).
- (2) G.F. Whitmore, S. Gulyas and J. Botond, Radiation sensitivity throughout the cell cycle and its relationship to recovery. In Cellular Radiation Biology, pp. 423-441, The Williams and Wilkins Company, Baltimore, 1965.
- (3) S. Rockwell and R.F. Kallman, Cyclic radiation-induced variations in cellular radiosensitivity in a mouse mammary tumor. Radiat. Res. 57, 132-147 (1974).
- (4) F.A. Valeriote, Cell kinetics and tumor therapy: An overview. In The Cell Cycle in Malignancy and Immunity (CONF-731005), NTIS, Springfield, VA. (in press)
- (5) T. Vietti, F. Eggerding and F. Valeriote, Combined effect of x-radiation and 5-fluorouracil on survival of transplanted leukemic cells. J. Nat. Cancer Inst. 47, 865-870 (1971).
- (6) W.K. Sinclair, Sensitivity to mitotic delay and stage in the cycle. Current Topics in Radiation Research Quarterly 7, 323-327 (1972).
- (7) J.M. Young and J.F. Fowler, The effect of x-ray induced synchrony on two-dose cell survival experiments. Cell Tissue Kinet. 2, 95-110 (1969).
- (8) S. Okada, Radiation effects on cell progress through the life cycle. In Radiation Biochemistry, Volume 1, (K.I. Altman, G.B. Gerber, and S. Okada, Eds.), pp. 189-246. Academic Press, New York, 1970.

- (9) J. Denekamp, Changes in the rate of proliferation in normal tissues after irradiation. In Proceedings of the Vth International Congress of Radiation Research, 1974. (in press)
- (10) R. Baserga and F. Wiebel, The cell cycle of mammalian cells. Intern. Rev. Exptl. Pathol. 7, 1-30 (1969).
- (11) M.L. Mendelsohn, The cell cycle in malignant and normal tissues. In The Cell Cycle in Malignancy and Immunity (CONF-731005), NTIS, Springfield, VA. (in press)
- (12) P.M. Kraemer and R.A. Tobey, Cell-cycle dependent desquamation of heparon sulfate from the cell surface. J. Cell Biol. 55, 713-717 (1972).
- (13) J.W. Gray, Cell cycle analysis from computer synthesis of deoxyribonucleic acid histograms. J. Histochem. Cytochem. 22, 642-650 (1974).
- (14) G.G. Steel, The cell cycle in tumours: An examination of data gained by the technique of labelled mitoses. Cell Tissue Kinet. 5, 87-100 (1972).
- (15) M. Takahashi, J.D. Hogg, Jr., and M.L. Mendelsohn, The automatic analysis of FLM curves. Cell Tissue Kinet. 4, 505-518 (1971).
- (16) P.J. Brockwell, E. Trucco, and R.J.M. Fry, The determination of cell-cycle parameters from measurements of the fraction of labeled mitoses. Bull. Math. Biophys. 34, 1-12 (1972).
- (17) P. Dombernowsky and N.R. Hartman, Analysis of variations in the cell population kinetics with tumor age in the L1210 ascites tumor. Cancer Res. 32, 2452-2458 (1972).

- (18) J.N. Izquierdo and S.J. Gibbs, Turnover of cell-renewing populations undergoing circadian rhythms in cell proliferation. Cell Tissue Kinet. 7, 99-111 (1974).
- (19) A.F. Hermens and G.W. Barendsen, The importance of the proliferation kinetics and the clonogenicity of tumor cells in relation to volume responses of experimental tumours after irradiation. In Proceedings of the Vth International Congress of Radiation Research, 1974. (in press)
- (20) T. Yamaguchi and J. Tabachnick, Cell kinetics of epidermal repopulation and persistent hyperplasia in locally β -irradiated guinea pig skin. Radiat. Res. 50, 159-180 (1972).
- (21) J. Leshner and S. Leshner, Effects of single-dose partial-body x-irradiation on cell proliferation in the mouse small intestinal epithelium. Radiat. Res. 57, 148-157 (1974).
- (22) J.M. Brown, D.R. Goffinet, and J.E. Cleaver, Preferential radiosensitization of mouse sarcoma relative to normal skin by chronic intra-arterial infusion of halogenated pyrimidine analogs. J. Nat. Cancer Inst. 47, 75-89 (1971).
- (23) H.E. Skipper and S. Perry, Kinetics of normal and leukemic leukocyte populations and relevance to chemotherapy. Cancer Res. 30, 1883-1897 (1970).
- (24) F.M. Schabel, Jr., The use of tumor growth kinetics in planning "curative" chemotherapy of advanced solid tumors. Cancer Res. 29, 2384-2389 (1969).

- (25) A.F. Hermens and G.W. Barendsen, Changes of cell proliferation characteristics in a rat rhabdomyosarcoma before and after x-irradiation. Europ. J. Cancer 5, 173-189 (1969).
- (26) H.R. Withers, Regeneration of intestinal mucosa after irradiation. Cancer 28, 75-81 (1971).
- (27) H.R. Withers, Recovery and repopulation in vivo by mouse skin epithelial cells during fractionated irradiation. Radiat. Res. 32, 227-239 (1967).
- (28) H.R. Withers, N. Hunter, H.T. Barkley, Jr., and B.O. Reid, Radiation survival and regeneration characteristics of spermatogenic stem cells of mouse testis. Radiat. Res. 57, 88-108 (1974).
- (29) J. Aroesty, T. Lincoln, N. Shapiro and G. Boccia, Tumor growth and chemotherapy: Mathematical methods, computer simulations, and experimental foundations. Mathematical Biosciences 17, 243-300 (1973).

Footnote

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Figure Legends

Figure 1. Pattern of radiation age-response. A cell's relative age ranges from zero when the cell emerges from mitosis to one when it subsequently divides. Cells fall into two roughly sinusoidal patterns depending on whether there are one or two periods of resistance per cell cycle.

Figure 2. Amplitude of radiation age-response. A therapeutic advantage can be gained when two populations, depicted as the solid and broken line, differ in the amplitude of their age response.

Figure 3. Radiation age-response of V79 Chinese hamster cells in vitro. Note the consistent pattern of one resistant period per cycle. The amplitude increases with radiation dose and reaches a maximum peak to trough difference of twentyfold in cell survival. The cells are in mitosis at zero hour. Reproduced from Sinclair (1).

Figure 4. Radiation age-response of EMT6 mouse mammary tumor cells in vitro. These cells have two resistant periods per cycle and a maximum peak to trough difference of threefold in cell survival. Reproduced from Rockwell and Kallman (3).

Figure 5. Radiation age-response with phases of the cell cycle and sites of action of representative agents which block cell cycle progression.

Figure 6. Combined effect of x-rays and 5-fluorouracil (5FU) on asynchronous leukemia cells in AKR mice. Depending on the timing of the two agents, there is a complex pattern ranging from simple additivity of effect to 100-fold synergism. Reproduced from Vietti, Eggerding and Valeriote (5).

Figure 7. Cell cycle time and radiation age-response. In the upper panel the curves marked A and B are the age responses of two populations with cell cycle times in the ratio of 2:3. The comparative resistance or survival ratio of the two populations is shown in the lower panel. Complex changes in amplitude are present in the compound response, but the intervals between major peaks and troughs are determined largely by the population with the shorter cycle time.

Figure 8. Division delay in V79 Chinese hamster cells irradiated in vitro. Delay was measured in terms of the peak incidence of mitoses at the first division after exposure. Reproduced from Sinclair (6).

Figure 9. The timing of peak and trough of resistance of V79 Chinese hamster cells after synchronization by irradiation in vitro. Curve A is the time after a priming dose when a second dose of equal size irradiates the surviving cells at their first trough when they are maximally sensitive. Curve B gives the timing of the subsequent peak of resistance. The two times and the interval between them change with dose. Reproduced from Young and Fowler (7).

Figure 10. Flow microfluorimetric measurement of dispersion of synchronized cells. The upper block of panels shows distributions of DNA content for CHO cells at various times after release from a G_1 block (12). Such distributions are readily obtained by flow microfluorimetry; they show the progression and dispersion of cells as they proceed through DNA synthesis, mitosis and again into DNA synthesis. In the lower block the same distributions have been simulated by a computer model which when properly fit to the experimental data provides estimates of the rates of progression and dispersion of the cells. Reproduced from Gray (13).

Figure 11. Hourly dispersion and cell cycle times for 49 normal tissues, tumors, ascites tumors, and tissue culture populations based on computer-analyzed, well-behaved FLM curves.

Figure 12. The effect of dispersion on residual synchrony. In this simulation by Gray (13) cells are synchronized in G_1 and then allowed to cycle with transit times of 9.1 hours for G_1 , 4.1 hours for S, 3.0 hours for G_2 , and 0.5 hours for M. The three panels show the percent of cells in G_1 , S and $G_2 + M$ based on different coefficients of variation of cell cycle time. In panel A, an hourly dispersion of 2.53 hours effectively damps the synchrony within 5 hours and offers little hope of using intracyclic differential sensitivity. In panel C the hourly dispersion is 0.78

hours and significant oscillations are available to the therapist for at least 75 hours. Panel B is intermediate with an hourly dispersion of 1.27 hours and about 25 hours of reasonable synchrony.

Figure 13. Cell survival, cell turnover and cell cycle time (T_c) in rapidly and slowly turning-over tissue and tumor. In this hypothetical situation the arrows represent fractions of radiotherapy. On the left the critical normal tissue has a rapid turnover; thus the functioning fraction of cells closely tracks the surviving fraction and there is a prompt shortening of the normal cell cycle time. On the right a slowly turning-over normal tissue shows little change in functioning fraction or in cell cycle time.

Figure 14. The commitment of G_0 cells during a full course of radiotherapy. This oversimplified and hypothetical diagram shows the contrasting G_0 commitment of rapidly turning-over tissues, tumors, and facultative or slowly turning-over tissue.

Figure 15. Effect of 2000 rad on the volume and clonogenicity of rhabdomyosarcoma. Between days 4 and 8 post-therapy the clonogenic cells increase their number 100-fold, accelerate their growth rate tenfold, and at the peak response are doubling approximately twice a day. Reproduced from Hermens and Barendsen (25).

Figure 16. Split-dose recovery curves for jejunal (upper curve) and colonic (lower curve) clonogenic cells after a primary exposure of 660 and 700 rad respectively. For jejunum the 100-fold rise between days 2-1/2 and 3-1/2 is interpreted to be repopulation of clonogenic cells and implies a doubling time of 4 to 8 hours. In colon the response begins 4 days after the priming exposure and has a doubling time of about half a day. Reproduced from Withers (26).

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