• Radiation Biophysics

Specific cellular radiobiology studies are often required to test aspects of the mathematical models developed in the Radiation Dosimetry program. These studies are designed to determine whether specific mathematical expressions, which characterize the expected effect of biochemical mechanisms on observable biological responses, are consistent with the behavior of selected cell lines. Since these tests place stringent requirements on the cellular system, special techniques and culture conditions are required to minimize biological variability. Cells not progressing through the cell cycle are needed in order to study the effects of dose protraction; cells in specific parts of the cell cycle are required in order to determine the changes in biochemical mechanisms that are responsible for the cell cycle effect. The use of these specialized cell populations, which have been carefully characterized, is providing data on the extent of repair following low doses, and on the changes in the types of damage that can be repaired as the cell progresses toward mitosis.

RE-ENTRY OF STARVED PLATEAU-PHASE CHINESE HAMSTER OVARY CELLS INTO THE REPLICATIVE CYCLE

J. M. Nelson, L. A. Braby, N. F. Metting, and W. C. Roesch

Kinetic differences between fed and starved Chinese hamster ovary (CHO) cells have been demonstrated. We have further characterized these differences by subculturing 14-day-old plateau-phase cells and following them as they progressed through their first post-plateau-phase cycles. Deoxyribonucleic acid (DNA) distributions were determined by flow-cytometric (FCM) analysis of fixed trypsinized cells, stained by the acriflavine-Feulgen procedure. About 83% of the starved populations are found in G(1) phase. These cells re-enter the replicative cycle almost immediately, passing into S phase within 11 hours, and into G(2) phase within 18 hours. The remaining 17% initially comprise about 2/3 (~10%) S-phase and 1/3 (~7%) G(2)-phase cells. Both of these subpopulations progress rapidly through mitosis, apparently producing large numbers of DNA-deficient daughter cells as they do so. In contrast, fed cultures consist of a mixture of cycling and noncycling cells. The noncycling cells appear very similar to those observed in starved cultures. The cycling populations, however, progress peristaltically in an exceptionally synchronous manner, reflecting the intermittent feeding regimen. These nonexpanding, yet actively growing fed populations continue to produce nonviable daughters, which are later observed as Feulgen-positive debris.

Manuscripts reporting these FCM findings have been submitted for review. These papers demonstrate the accumulation of nonprogressing cells in both G(1) and G(2) phases, and confirm the existence of nonsynthesizing cells within S phase (i.e., nonprogressing cells that have S-phase DNA content). Also reported is the finding that fed plateau-phase cultures contain a significant growing population, which contributes large amounts of karyorrhectic debris to the system, apparently as a consequence of continuous production of DNA-deficient cells.

REMOVAL OF RADIATION DAMAGE BY SUBPOPULATIONS OF PLATEAU-PHASE CHINESE HAMSTER OVARY CELLS

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Centrifugal elutriation techniques have been applied to many cell systems for separation of asynchronous or mixed populations, according to cellular cross-sectional area. Cells of the same cell-cycle age tend to have similar physical dimensions and densities. Although the correlation between DNA content and cell size is far from perfect, these methods are still suitable for producing populations significantly enriched in cells of a given cell-cycle age.

Our stationary-phase Chinese hamster ovary (CHO) cells are composed primarily of G(1)-phase cells (83%), with the remainder comprising both G(2) and S phases. A deoxyribonucleic acid (DNA) distribution of this starting population is shown in Figure 1a.

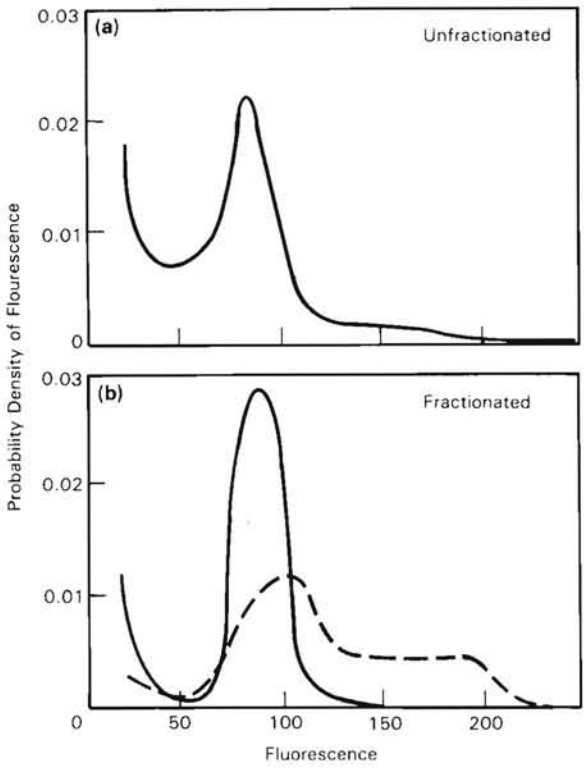

FIGURE 1. Typical DNA Distributions for Unfractionated, Starved Plateau-Phase Cells (a) and for Two Fractions of the Same Sample (b), Separated by Centrifugal Elutriation. The distribution in the upper panel shows about 83% of the population in G(1) phase, 10% in S phase, and 7% in G(2) phase. In the lower panel, the first fraction (solid line) contains smaller cells and contains about 99% G(1)-phase cells. A significant increase in the concentration of larger cells is apparent in a later fraction (dashed line), where the G(2)- and S-phase component represents about 50% of the population. Cells had been fixed with 70% ethanol and stained with propidium iodide.

Starting with such cultures, we are able to produce fractions enriched in G(1) and non-G(1)-phase (i.e., G(2)+S) cells. With care we have even been able to separate G(2)-phase from S-phase cells.

A relatively pure (99%) population of G(1)-phase cells can be obtained in this manner (Figure 1b, solid line). The G(2)- and S-phase cells can also be concentrated, but to a lesser extent. Although specific cases are frequently better, G(2) + S-phase non-G(1)-phase cells are generally enriched from about 17% to around 50% (Figure 1b, dashed line). Removal of radiation damage by cells in each of these fractions was studied in split-dose experiments. Results of a typical experiment of this sort are shown in Figure 2. To date, we have observed no significant differences in cellular repair rate, either among the individual fractions or between the fractionated and unfractionated data sets. This suggests, therefore, that each of the repair processes found in stationary-phase cells is cell-age independent. That is, both processes probably operate concurrently in each cell regardless of the cell's position in the interphase cell cycle.

However, as has long been known, cellular radiation sensitivity does change rapidly and considerably as the cells progress from one phase to the next through the cell cycle. Since the rate of damage removal appears invariant, the change in survival must reflect the efficiency of producing that damage. We should also be able to draw some conclusions regarding damage production from these data. The damage accumulation model (Roesch 1978) considers the production of both single- and two-event types of damage, with the exponential removal of the latter. Each damage process is treated independently; in this model A is a constant associated with the probability of producing two-event damage, while C is a similar constant associated

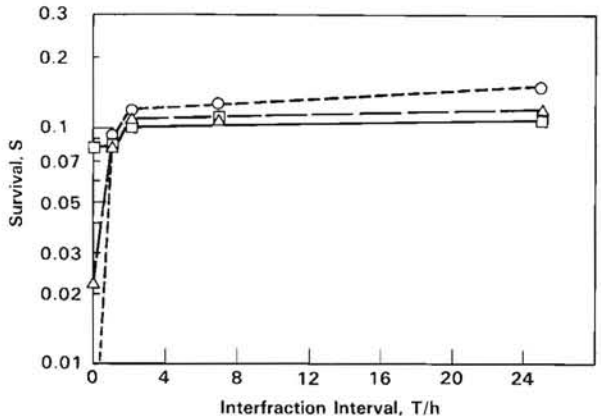

FIGURE 2. Split-Dose Survival of Unfractionated, Starved Plateau-Phase Cells and Two Fractions Derived from the Same Sample by Centrifugal Elutriation, Plotted Against Time Between Irradiations. There is no striking difference among the unfractionated sample (solid line), the G(1)-phase cell fraction (small dashes), and the enriched S- and G(2)-phase cell fraction (large dashes). Differences that are apparent, particularly in the early data, are probably not significant.

with production of single-event damage. Changes in the relative values of A or C would be observed as changes in the shape of the survival curve. As an example, assume, for simplicity, only a single repair process. If there were no change in the probability of producing two-event damage, A, i.e., the dose-squared component, but the probability of producing the single-event damage, C, varied through the cell cycle, we would expect a significant change in asymptote on a semilog survival curve.

As shown in Figure 3a, a 50% change in the value of C leads to a change in survival of about an order of magnitude. Similarly, if C were to remain constant while A was varied, the relative amount of damage being removed would vary accordingly. As shown in Figure 3b, a pronounced change in the intercept would be observed after the survival curves are normalized to the same final amount of damage, i.e., to the same survival level. Such effects were not observed in these experiments. However, it is possible that a relatively small change in C, coupled with a somewhat larger change in A, might be hidden within the resolution of the data. Similar changes would be reflected in the survival curve when two repair processes are considered. If the rate of both of the processes and the amount of damage available for repair by one of them were held constant while the amount repaired by the other varied, the form of the survival curve would change (see Figure 4). The experimental data

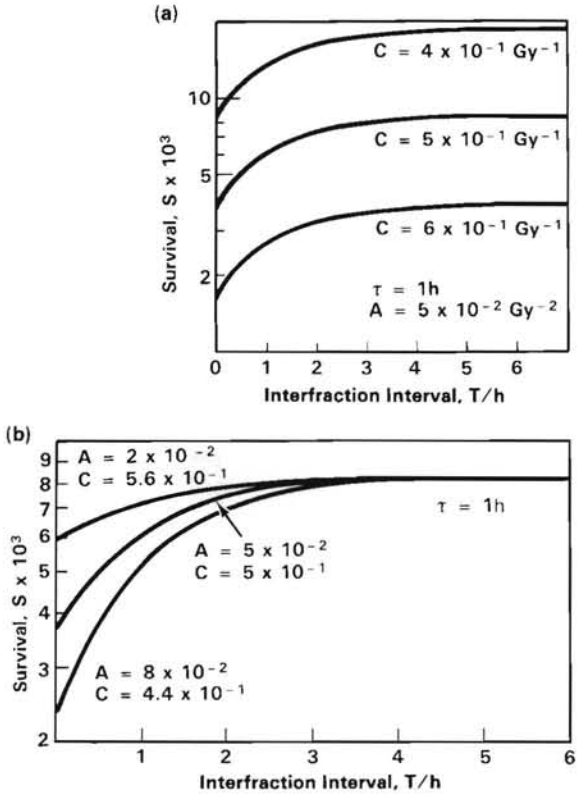

FIGURE 3. Hypothetical Split-Dose Survival Curves, Predicted by the Damage Accumulation Model (Roesch 1978), That Would Result If the Single- or Two-Event Factor Varied. To simplify the situation, only a single repair process has been assumed. (a) shows the effect of changing, the probability of producing the single-event damage (C) while holding the probability of producing the two-event damage (A) constant. In (b), A has been allowed to vary. In this case, the value of C has also been varied in order to bring the final survival to the same level.

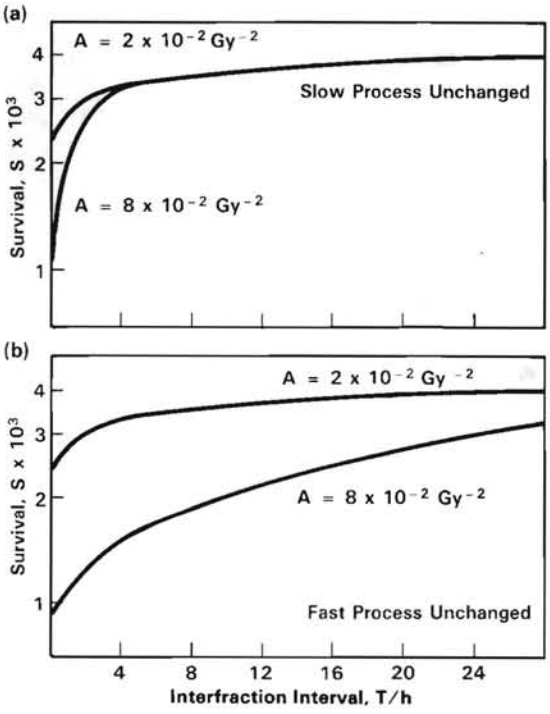

FIGURE 4. Hypothetical Split-Dose Survival Curves for a System Having Two Repair Times That Would Result If Only the Two-Event Factor Were Varied. In (a) only the probability of producing damage that would be removed by the faster repair process is allowed to change. In (b), only the probability associated with the slower process is allowed to change.

are inconclusive; however, they do suggest that production of one or another sort of damage probably dominates during specific phases of the cell cycle, while the capacity for removal of all types of damage remains relatively constant. This work will continue during the next year.

REFERENCE

W. C. Roesch. 1978. "Models of Radiation Sensitivity in Mammalian Cells." In Third Symposium on Neutron Dosimetry in Biology and Medicine. Ed. G. Berger and H. G. Ebert. Commission of the European Communities, Luxembourg, pp. 1-27.

KINETICS OF C3H 10T1/2 MOUSE CELLS

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The 10T1/2 cell line has been brought to PNL because of its usefulness as an in vitro biological system for transformation assay, and because a fast repair process in response to irradiation has been observed in these cells (Malcolm and Little 1979). The cell line was originally established from embryonic C3H mouse tissue and characterized by Reznikoff, Brankow, and Heidelberger (1973). Its full name is C3H/10T1/2 Clone 8, American Type Culture Collection (ATCC) number CCL226. Before using the cells in radiobiological studies, it was important to observe the growth kinetics of our cultures and verify their similarity to those reported by other laboratories in which they are used, so that our subsequent measurements could be compared with and added to the body of scientific work already compiled.

Several frozen vials of 10T1/2 cells were received in January 1986 from the ATCC and were immediately stored in liquid nitrogen. One of the vials was thawed and the contents were used to initiate monolayer culture. The optimal freezing protocol* was determined for our cultures, and then suitable numbers of passage 10 cells were grown up and frozen for eventual experimental use.

To become familiar with the cell morphology and cloning characteristics, we conducted an experiment in which a number of flasks were seeded with about 350 cells each. Each day, starting with the second day after plating, a flask was stained and the number of clones plus the number of cells in each clone were counted. There are several types of cell morphologies within the population, some initial cause for concern. However, the plating efficiency was determined to be an average of 28.6 ±2% for days 5 to 8 after plating (shown in Figure 1), which is very close to Reznikoff's value of 27.8% and within the range of 25% to 30% reported in the literature today. The generation time at the period of fastest replication was found to be 15.5 hours, also virtually the same as that reported by Reznikoff.

Flow cytometry (FCM) studies were begun to assess cell cycle characteristics. Again, a number of flasks were seeded, but this time with a large number of exponentially growing cells. Daily, a flask of cells was fixed and stained from FCM. Figure 2 shows deoxyribonucleic acid (DNA) histograms of days 1, 3, 4, and 11. Cell cycle fractions were analyzed using a program developed by Dean (1985). Notice that on day 1, 85% of the cells are in G1 phase. This may indicate that the plating efficiency of G1 cells is greater than that of the other fractions. Unfortunately, the original seeding culture was not also sampled for DNA content, so there is no baseline for comparison. Day 3 showed the largest percentage of cells in G2M phase (22%); day 4 showed the highest fraction of S phase (52%). By day 11, cell cycle activity had slowed to the point at which 88%

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*Ten percent dimethyl sulfoxide (DMSO) was added to the culture medium; prepared vials were frozen for 2 hours in a -76°C freezer and then transferred to a liquid nitrogen dewar.