

# RADIOSENSITIVITY OF MAMMALIAN CELLS

## III. EFFECT OF SUBOPTIMAL GROWTH

### TEMPERATURES ON RECOVERY FROM

### RADIATION-INDUCED DIVISION DELAY

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**ABSTRACT** We investigated the effect of suboptimal growth temperatures on recovery from radiation-induced division delay in Chinese hamster cells. It was found that no recovery occurred during the time that either log-phase or synchronized populations were incubated at 4°C and that injury sustained at low dose rates was cumulative over a period of 6.2 hr at low temperature. Postirradiation conditions influencing recovery from the induced division delay period are different from those affecting survival, suggesting that biochemical damage leading to division delay may be different from that leading to cell death.

## INTRODUCTION

Recent studies have indicated that radiation-induced damage leading to division delay in mammalian cells is qualitatively different from that leading to the death of the cell. Cellular division delay apparently results from radiation effects on the translation of functional proteins (Walters and Petersen, 1968 *b*; Doida and Okada, 1969), while lethality probably involves damage to the genome. Since, at moderate doses which may be lethal to most of the population, the irradiated cell recovers the ability to traverse the life cycle and divide, repair obviously must occur. Although the detailed characteristics of this type of repair remain to be established, it is known that Chinese hamster cells in culture recover the ability to divide under conditions which inhibit normal DNA and RNA synthesis but are unable to recover in the absence of protein synthesis (Walters and Petersen, 1968 *b*). These results are different from those obtained in similar studies of the effect of inhibition of specific macromolecular synthesis on the repair of potentially lethal damage as measured by the ultimate survival of the cell (Phillips and Tolmach, 1966; Elkind et al., 1967 *a*; 1967 *b*; Weiss and Tolmach, 1967; Sinclair, 1968). These findings have led us to investigate the possibility that additional differences between damage leading to division delay and ultimate death may exist. This paper reports the results of a

study of the effect of temperature on recovery of the ability of irradiated Chinese hamster cells to divide. The data indicate that no recovery occurs when irradiated cells are incubated at 4°C and that injury accumulates quantitatively at 4°C in cells exposed at low dose rates to <sup>60</sup>Co gamma irradiation.

## EXPERIMENTAL METHODS

### *Cell Cultivation*

Chinese hamster cells were maintained free of pleuropneumonia-like organisms (PPLO) as suspension cultures in F-10 medium (Ham, 1963), supplemented with 10% calf and 5% fetal calf sera, penicillin, and streptomycin. Cell growth was synchronized by treatment of suspension cultures with 10 mM thymidine for 12 hr, followed by suspension in normal medium (Petersen and Anderson, 1964). Cells were counted with an electronic cell counter, as previously described (Walters and Petersen, 1968 *a*). Counting errors and analysis of reproducibility have been presented elsewhere (Walters and Petersen, 1968 *a*).

### *Irradiation of Cultures*

Conditions for irradiation were the same as in our previous experiments (Walters and Petersen, 1968 *a*). Cells at densities of approximately  $2 \times 10^5$  cells/ml were exposed in jacketed spinner flasks immediately after equilibration at the desired temperature. A General Electric Maxitron therapy unit, operated at 250 kvp, 30 ma, with Thoraeus II filtration (2.6 mm Cu half-value layer [HVL]), was used for X-ray exposures at a dose rate of 60 rads/min. For the low dose rate experiments, a 6 c <sup>60</sup>Co gamma source was arranged to deliver 32 rads/hr. In all cases, the cells were agitated during exposure.

### *Temperature Control of Cultures*

Temperatures of the cultures were adjusted and maintained with a circulating water bath. The cultures were rapidly cooled to 4°C with a Forma-Temp, Jr., circulating refrigerated bath (Forma Scientific, Inc., Marietta, Ohio). The time required for cooling and subsequent rewarming was never more than 3 min from adjustment to equilibration.

## RESULTS

The time course of the radiation-induced division delay in Chinese hamster cells partially synchronized with excess thymidine has been reported in detail (Walters and Petersen, 1968 *a*). The duration of the division delay period and subsequent recovery of the ability to divide were unaffected by the synchronization procedure. To establish that all cells survived for a minimum of one postirradiation division, synchronized cultures were used for the initial studies of temperature effects on recovery of the ability to divide after X-irradiation. Synchronized cells were exposed to 200 rads while in G<sub>2</sub> and cooled to 4°C at various times after irradiation. The cells were incubated at 4°C for 2.5 hr, a time just in excess of the induced delay time, then warmed to 37°C. An example of the data obtained is illustrated in Fig. 1 in which cells were cooled immediately after irradiation.

A number of significant features characteristic of this type of experiment emerge

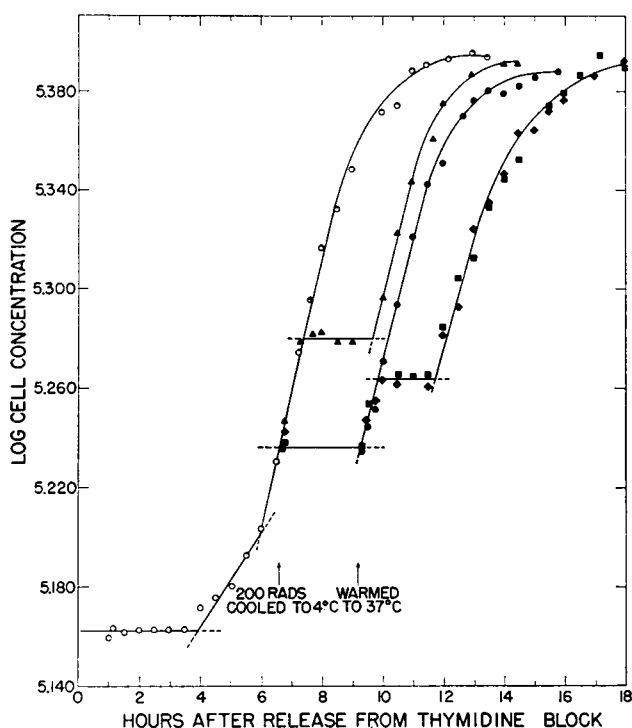


FIGURE 1 Effect of incubation at 4°C on recovery of the ability of irradiated cells synchronized with excess thymidine to divide. The times of respective treatments are shown by the arrows. Each culture was treated as follows: (○) untreated control; (▲) irradiated with 200 rads but not cooled to 4°C; (●) unirradiated cells incubated for 2.5 hr at 4°C; and (■ and ◆) cells irradiated with 200 rads and immediately cooled to 4°C for 2.5 hr.

from the data shown in Fig. 1. Cells that were not cooled continued dividing for 0.9 hr after irradiation. After a delay period of 2.2 hr, division resumed at the control rate; and, when compared with the control, *all* irradiated cells completed the first postirradiation division. In unirradiated cultures incubated for 2.5 hr at 4°C, division ceased immediately upon cooling, resumed promptly upon returning the culture to 37°C, and gave a quantitative yield. These data are consistent with the results of Tobey et al. (1967), who showed that cooling the cells of this Chinese hamster line to 4°C for prolonged periods (up to 6.0 hr) prevented mitotic cells from completing division but did not affect viability. The pattern of cell division after return of the irradiated cells to 37°C after 2.4 hr at 4°C indicates that no recovery occurred during the time that the cells were held at 4°C. The initial increase in cell number after return of the irradiated cells to 37°C represents division of cells nearer than 0.9 hr to division at the time of irradiation and cooling.

The implications of the existence of a specific segment of the cell population that is apparently insensitive to radiation has been discussed at length (Walters and

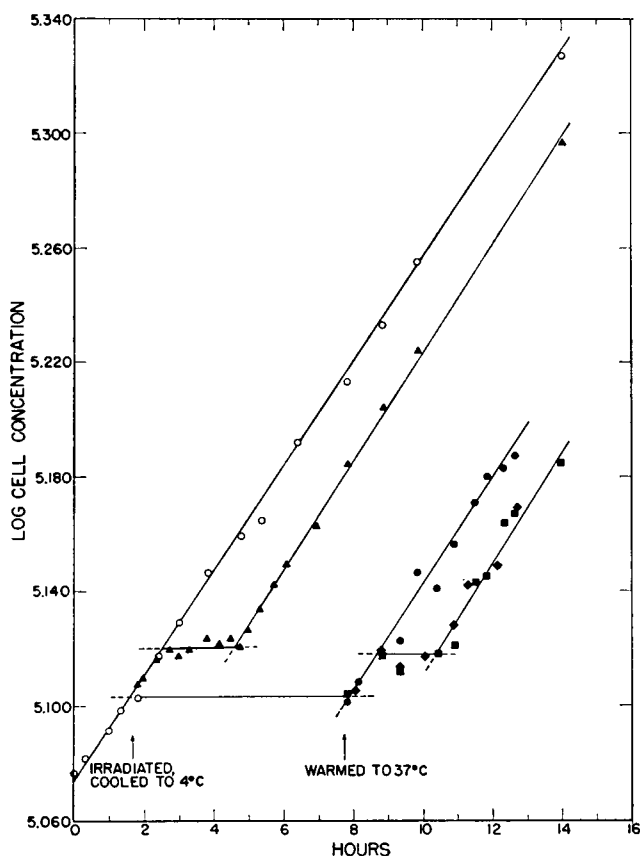


FIGURE 2 Effect of variation of dose rate on the growth of asynchronous cells exposed to 200 rads of  $^{60}\text{Co}$  gamma radiation during incubation at  $4^{\circ}\text{C}$ . The times of respective treatments are shown by the arrows. Each culture was treated as follows: (O) untreated control; (▲) cells that were acutely irradiated (60 rads/min) but not cooled; (●) unirradiated cells maintained at  $4^{\circ}\text{C}$  for 6.2 hr, then warmed; and (■ and ◆) effect on cells of chronic irradiation (32 rads/hr) during the 6.2 hr period at  $4^{\circ}\text{C}$ .

Petersen, 1968 *a*), and it is clear that cells in this segment of the life cycle, having completed all biochemical preparations sensitive to radiation, will divide despite exposure to increasingly larger doses of radiation but will exhibit quantitative delay in the next generation. As expected, however, these radiation-insensitive cells were prevented from dividing by cooling. The initial increase in cell number, due to cells beyond the sensitive point, was followed by a period of 2.0 hr during which no division occurred. After resumption of division, the rate of increase in cell number paralleled that of the controls, both irradiated and unirradiated. The yield of cells was again quantitative, indicating that simultaneous irradiation and cooling did not affect short-term viability.

Similar results were obtained when asynchronous cells were cooled at various

times during the delay period after irradiation. In every case the total division delay time, exclusive of incubation time at 4°C, was experimentally indistinguishable from the irradiated control which was not cooled. Thus, cells cooled increasingly later in the recovery period resumed division in proportionately less time after being returned to 37°C, indicating that again no recovery occurred during the time that cells were held at 4°C. When the recovery process is interrupted by incubation at sub-optimal growth temperatures, it will resume after return to 37°C from the point attained at the time the temperature was lowered.

Since recovery apparently does not occur at 4°C, it should be possible to accumulate damage leading to division delay by irradiating cells at 4°C with a very low dose rate. After the return of cells to 37°C, the induced delay period should be equivalent to that of cells acutely receiving the same total dose. The data in Fig. 2 illustrate the results of an experiment that uses asynchronous cells and that compares the effect on division of 200 rads delivered acutely at 37°C (60 rads/min) with the effect from the same radiation dose delivered over a 6.2 hr period to cells at 4°C (32 rads/hr). The response of cells irradiated at the ~100-fold slower rate was similar in every respect to that of cells acutely irradiated. There was an increase in cell number for 0.9 hr after the return to 37°C, followed by a period of 1.7 hr during which no division occurred. The division rate after recovery was the same as that of the control. In comparison the duration of the division delay period of acutely irradiated cells was 1.8 hr. It is quite clear that cells exposed at a relatively low dose rate while maintained at 4°C responded as though acutely irradiated once the temperature was returned to 37°C.

## DISCUSSION

It is becoming increasingly apparent that very real differences exist between radiation-induced damage leading to division delay and that ultimately leading to cell death. In both cases, however, repair does occur; division spontaneously resumes in irradiated cells, while survival is enhanced by dose fractionation (Elkind and Sutton, 1960). Nevertheless, the conditions which alter repair are different for division delay and survival, suggesting that the repair mechanisms may also differ. Various inhibitors of DNA and RNA synthesis have been found to reduce the survival of irradiated mammalian cells (Elkind et al., 1967 *a*; Weiss and Tolmach, 1967; Sinclair, 1968). Inhibitors of protein synthesis (Phillips and Tolmach, 1966; Elkind et al., 1967 *b*), oxidative phosphorylation (Dalrymple et al., 1967), and suboptimal growth temperatures (Elkind et al., 1965; Whitmore and Gulyas, 1967) apparently do not inhibit repair processes leading to an increased survival, although conflicting reports do exist in the literature (Phillips and Tolmach, 1966). However, recovery of the ability of irradiated cells to divide is prevented by the absence of protein synthesis or by incubation at 4°C and is unaffected by concentrations of inhibitors

that are adequate to completely prevent normal DNA and RNA synthesis (Walters and Petersen, 1968 b)—conditions differing from those influencing survival.

Results reported here demonstrating the absence of recovery of the ability to divide when cells are incubated at 4°C, are consistent with the differences between the two types of repair discussed above and suggest that recovery of the ability to divide involves enzymatic synthesis. The repair of potentially lethal damage may include a nonenzymatic component, as suggested by Elkind and his coworkers (Elkind et al., 1967 a; 1967 b), but it is difficult to envision a model for repair in a biological system devoid of enzymatic reactions. It seems more likely that, as suggested by Phillips and Tolmach (1966) and Whitmore and Gulyas (1967), a situation exists analogous to that seen in bacteria (Witkin, 1966) wherein particular agents enhance survival by preventing the cell from expressing a defect and allowing additional time for repair of the lesion. However, this does not appear to be the case for damage leading to division delay in Chinese hamster cells. Either treatment with cycloheximide or incubation at 4°C, instituted at any time during the delay period and subsequently reversed, can interrupt repair without affecting the total dose-dependent recovery interval. Thus, in contrast to the beneficial effect of low temperature on survival, no immediate repair which appreciably shortens division delay occurs with protein synthesis inhibition or in the cold.

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