RADIOSENSITIVITY OF MAMMALIAN CELLS

IV. Effects of X-Irradiation on the DNA Synthetic Period in Synchronized Cells

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ABSTRACT The effect of X-irradiation on the timing of DNA synthesis in the Chinese hamster ovary cells has been investigated. Mitotically synchronized cells irradiated in mitosis or early G_1 exhibited a fixed, dose-independent (150–2000 rad) delay of 1.6 hr in entry into S, while the duration of S was unaffected. Cells irradiated during late G_1 or the first 0.8 hr of S were not affected either in time of initiation or duration of S. However, when cells 0.8 hr or more into S were irradiated, completion but not initiation of DNA synthesis was delayed, indicating a very precise separation of X-ray effects upon initiation and replication. There was no indication of a reordering of cells following irradiation and recovery, since cells in G_2 at the time of irradiation always divided before cells irradiated in S. The results suggest that two separate functions required for initiation and continued replication of DNA may be differentially sensitive to X-irradiation.

INTRODUCTION

The effect of X-irradiation on timing of DNA synthesis in mammalian cells varies greatly with cell type (Sinclair, 1967: Little, 1968). Since DNA synthesis has been implicated in the variation of survival and division delay with position in the life cycle (Sinclair, 1968), the effects of X-irradiation on the DNA synthetic period (S) of the Chinese hamster ovary cell, which exhibits a constant delay in division when irradiated at any time throughout the life cycle (Walters and Petersen, 1968 *a*), were investigated.

EXPERIMENTAL METHODS

Propagation in suspension of Chinese hamster ovary cells in F-10 medium and synchronization of cell growth with excess thymidine or by selectively detaching mitotic cells from monolayer cultures (600 ml, 0.9 mitotic fraction, 0.7–1.0 $\times$ 10^5 cells/ml) have been described elsewhere (Walters and Petersen, 1968 *a*). The cell doubling time ranged from 16 to 18 hr.

Mitotically synchronized cells were X-irradiated (250 kvp, 30 ma, 200 rad/min, 2.6 mm Cu equivalent HVL) in suspension at various times after synchronization, and aliquots (5 ml) were pulse-labeled with thymidine-^{3}H (15 min, 1 μCi/ml, 6 Ci/mmole) at regular intervals for autoradiography (Walters and Petersen, 1968 *b*). Each family of curves (Figs. 1–3) represents data from a single experiment in which all cells were taken from a single synchronized population. Slight variations in time of initiation of the DNA synthetic period (S) among experiments can be sufficient to obscure the very precise nature of the measured effects: therefore, each experiment contained its own internal control.

RESULTS

Fig. 1 A presents an example of the data obtained when early G_1 cells are irradiated. Mitotically synchronized cells were irradiated 1.5 hr after synchronization (1 hr after division) with doses ranging from 500 to 1200 rad. Entry into S was dose independently delayed for 1.6 hr for all cultures, although the duration of S was unaffected. Similar results were obtained with cells irradiated in mitosis with doses ranging from 150 to 2000 rad. Thus, cells irradiated in mitosis and early G_1 exhibit a fixed, dose-independent delay of entry into S while the duration of S is unaffected. These results are similar to those seen with L cells (Mak and Till, 1963) but are different from the results in another line of Chinese hamster cells (Sinclair, 1967) and of HeLa cells (Terasima and Tolmach, 1963 *a* and *b*; Brent, Butler, and Crathorn, 1966).

When cells were irradiated with 500 rad at 2.5, 3.5, and 4.5 hr after synchronization (Fig. 1 B), neither the time of initiation nor the duration of S was affected. However, when cells were irradiated at 7, 8, 9, and 10 hr after synchronization (Fig. 2 A), a very unexpected result was obtained. Cells irradiated at 7 and 8 hr were not delayed in entry or exit from S, while those irradiated at 9 and 10 hr were not delayed in entry into S but were retained in S for 2 additional hr. Thus, over the period of only 1 hr the response of irradiated S cells changed drastically. When cells were irradiated from 9 to 12 hr after synchronization, a fixed increase above control in the maximum number of cells in S was always noted. The rate of exit from S, although delayed, was not significantly different from the control. Fig. 2 B shows the effect of irradiating cells during exit from S. Although the uncertainty of the data increases at longer times after synchronization, the retention of cells in S does not appear to be significantly different from that of cells irradiated earlier in S. The dose dependence of retention of cells in S is shown in Fig. 3 A, where thymidine-synchronized cells (single blockade) were irradiated in early S. The synchronized populations, irradiated with 200, 400, and 600 rad, were retained in S for 0.8, 1.4, and 1.9 hr, respectively, although initiation of the second round of replication was delayed by the same time as division (0.012 hr/rad [Walters and Petersen, 1968 *a*]). The data presented above are different from those obtained with other systems. The absence of any effect on timing (initiation and duration) of S for cells, for example, irradiated from 2.5 to 8.0 hr after synchronization is in contrast to an apparent stimulation reported in other cell lines (Sinclair, 1967). Although the dose-dependent retention in S of

cells actively synthesizing DNA is a commonly noted characteristic of irradiated mammalian cells, the very precise separation of the X-ray effect on initiation (entry into S) from replication between 8 and 9 hr after synchronization has not been re-

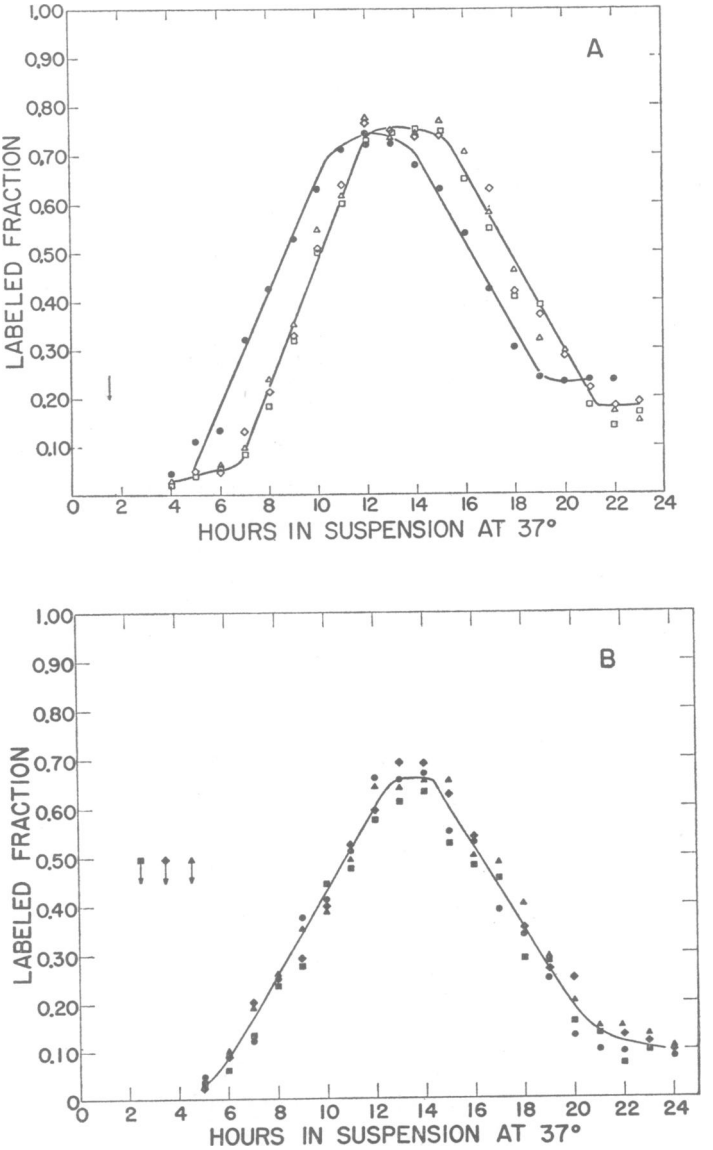

FIGURE 1 Effect of X-irradiation on the DNA synthetic period. (A) Synchronized cells were irradiated 1.5 hr after mitosis with (□) 500, (◇) 800, or (△) 1200 rad. Closed circles denote control. (B) Synchronized cells were irradiated with 500 rad at the time after mitosis indicated by the arrows. Circles represent the control. Each data point is that fraction of cells labeled after a 15 min pulse with thymidine-^{3}H.

ported. The data demonstrate very clearly that there exists a time early in S during which one cannot affect the timing of DNA synthesis. The constant percentage increase in maximum number of cells in S when irradiated from 9 to 12 hr and the observation that apparently all the cells after 9 hr were retained in S suggests that this time is 0.8 hr into S. Before this time (from 2.5 to 8.0 hr) the DNA synthetic period is

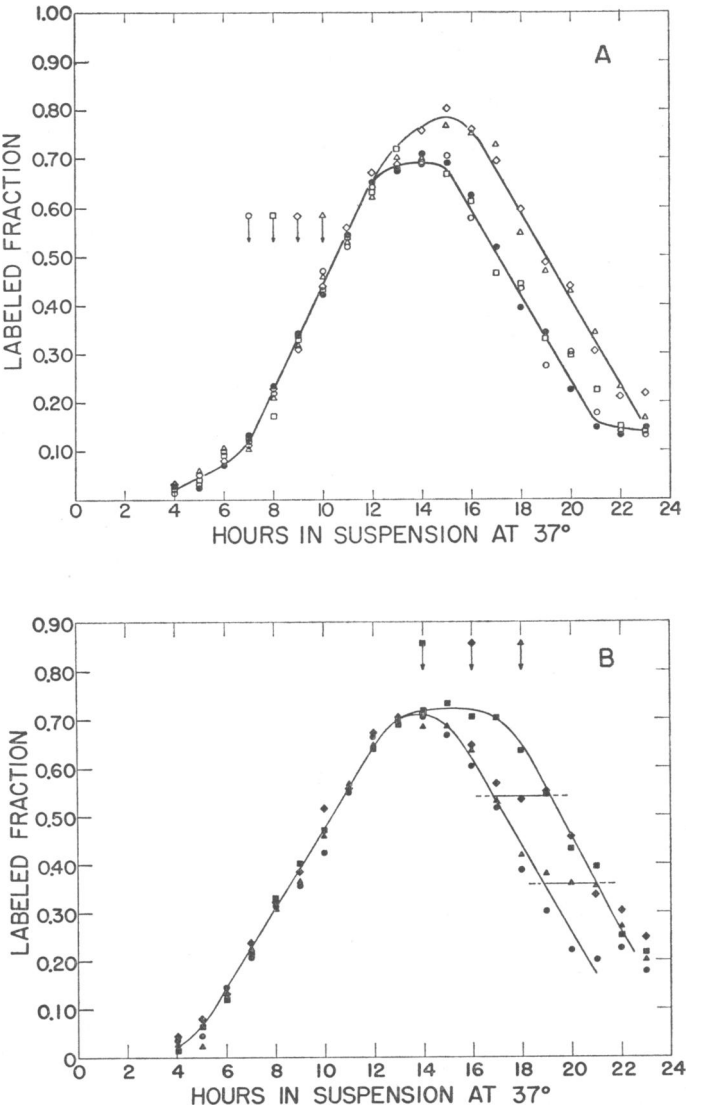

FIGURE 2 Effect of X-irradiation on initiation and completion of DNA synthesis. Mitotically synchronized cells were irradiated with 500 rad at the times indicated by the arrows. Closed circles denote the control in both (A) and (B). Each data point is that fraction of cells labeled after a 15 min pulse with thymidine-^{3}H.

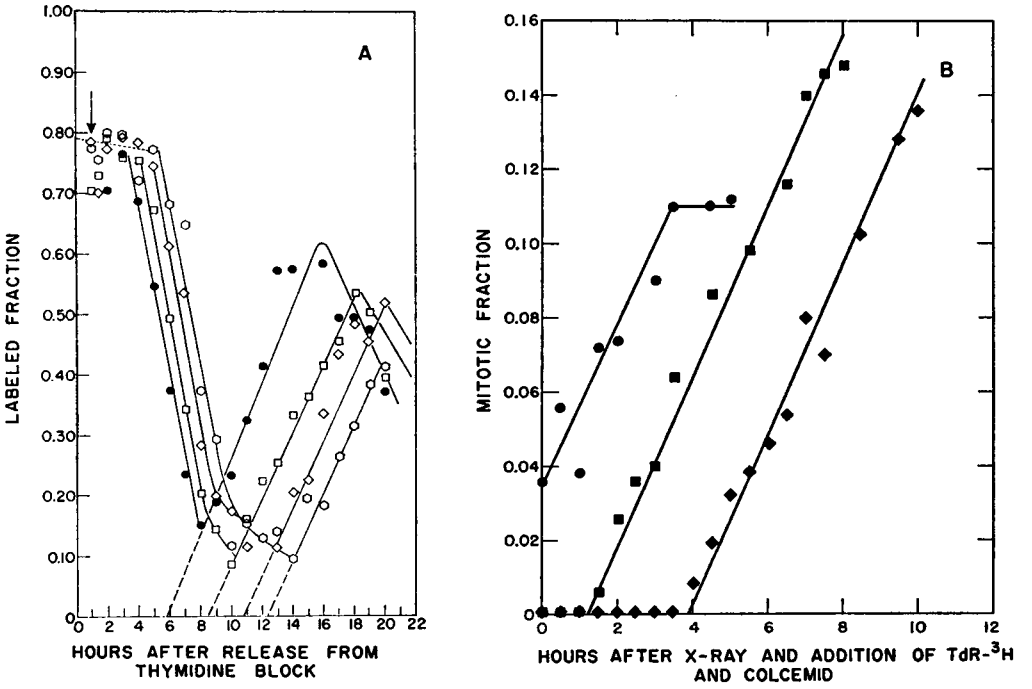

FIGURE 3 Dose-dependent retention and temporal distribution of irradiated S cells. (A) Thymidine-synchronized cells (single blockade) were irradiated 1 hr after release with (□) 200, (◇) 400, and (○) 600 rad, respectively, and pulse-labeled with thymidine-^{3}H for 15 min. Closed circles represent the control. (B) Randomly growing cells were irradiated with 200 rad and simultaneously treated with thymidine-^{3}H (Tdr-^{3}H) and colcemid. Circles and squares represent accumulation of unlabeled and labeled mitoses, respectively, in the control, while the diamonds represent accumulation of labeled mitoses in the irradiated population.

unaffected, while at later times initiation occurs normally but replication is apparently prolonged.

Since irradiated (up to 800 rad) Chinese hamster ovary cells grow exponentially at the control rate after regaining the ability to divide, the distribution of irradiated S and G_1 cells was determined by irradiating and simultaneously adding thymidine-^{3}H (0.2 μCi/ml) and colcemid (Puck, Sanders, and Petersen, 1964). Accumulated mitoses were assayed autoradiographically, and the results are shown in Fig. 3 B. It will be noted that labeled mitoses from the irradiated population trailed the control labeled population by 2.5 hr (the division delay period for 200 rad), although exit from S at this dose was delayed by only 0.8 hr (Fig. 3 A) and time of entry into S for most G_1 cells was unaffected by the radiation. As expected from experiments employing cell counting techniques (Walters and Petersen, 1968 *a*, 1968 *b*), the rate of accumulation of labeled mitoses was the same for both control and irradiated populations, showing that the temporal distribution of irradiated S and G_1 cells paralleled that of the control.

DISCUSSION

From previous studies (Walters and Petersen, 1968 *b*) and from the results shown in Fig. 3 B, we know that cells which were in G_2 at time of irradiation subsequently divided before cells in S at irradiation and that cells located prior to the time marking the end of RNA synthesis essential for division cannot complete division-related RNA synthesis (i.e. move closer in time to division) in the immediate postirradiation period. More precisely, irradiated cells retain the same temporal distribution in traversing two defined points in the life cycle (namely, the time marking the end of essential RNA synthesis for division and division itself) as the unirradiated controls. However, as demonstrated above, cells irradiated in S complete DNA replication much more quickly than they regain the ability to divide. Results from the two studies appear to be incompatible unless one considers the possibility that, although capable of initiating and completing DNA synthesis, such irradiated cells do not proceed with further biochemical preparations for division. That is, completion of DNA synthesis in the irradiated cell would not imply an obligatory completion of any of the remaining biochemical requirements for division.

In view of the dose-independent delay of entry into S of cells irradiated in M and early G_1 (Fig. 1 A) and yet the previous observation of dose-dependent division delay (Walters and Petersen, 1968 *a*), it seems likely that the effects of irradiation on timing of DNA synthesis are related not to the temporal position of the cell in the life cycle but to the state of the cell with respect to the components (e.g. enzymes) involved in DNA synthesis. Established line cells have high levels throughout their entire life cycle of many of the enzymes required for initiation and replication of DNA synthesis (Gold and Helleiner, 1964; Turner, Abrams, and Lieberman, 1968), which may account for varying effects of irradiation on DNA synthesis. On the other hand, the dose-dependent, radiation-induced delay of initiation of DNA synthesis is the predominant response of primary tissue explants (Lieberman, Abrams, Hunt, and Ove, 1963: Little, 1968) and regenerating liver (Bollum, Anderegg, McElya, and Potter, 1963) which must synthesize most, if not all, of the enzymes required for DNA synthesis during the transition to the active biosynthetic state. From our earlier studies it appears that irradiation can affect the synthesis of functional proteins in Chinese hamster cells having completed RNA but not associated protein synthesis essential for division (Walters and Petersen, 1968 *b*). Furthermore, we have shown that irradiation of this cell can induce marked changes in the protein composition of the chromatin and that this change is specific for the arginine-rich histone fraction f3 (Gurley, Hardin, and Walters, 1970). Thus, there is reason to believe that irradiation can alter the state of the cell with respect to particular protein species. There is no reason to suspect the same would not be true of specific enzymes.

If irradiation can influence the synthesis and/or localization of specific proteins, then the variable effects on DNA synthesis seen with different mammalian cells might be expected, as would the precise timing of radiation effects on initiation and

replication reported here. Accordingly, it is tempting to speculate that two separate enzyme functions are involved here, each with a unique sensitivity (Crathorn and Shooter, 1963). The nature of the effect also suggests very precise timing of the DNA synthetic period itself. Indeed, the rate of entry into and exit from S is the same for populations irradiated in M or G_1, indicating that the duration of S is constant. A correlation between the point 0.8 hr into S, where irradiated Chinese hamster cells are retained in S, with the rate of DNA synthesis will be the subject of another communication (in preparation).

The authors wish to acknowledge the technical assistance of P. C. Sanders and S. G. Carpenter.

This work was performed under the auspices of the U. S. Atomic Energy Commission.

Received for publication 16 January 1970 and in revised form 11 March 1970.

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