^{125}I-UdR INDUCED DIVISION DELAY

MARTIN H. SCHNEIDERMAN, BRUCE F. KIMLER, ALAN S. KIRSCHNER, *and* DENNIS B. LEEPER, *the Department of Radiation Therapy and Nuclear Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania 19107*
KURT G. HOFER, *Institute of Molecular Biology, Florida State University, Tallahassee, Florida 32306*

ABSTRACT Mitotic selection for cell cycle analysis was used to investigate the effects of ^{3}H and ^{125}I, incorporated into DNA, on the cell cycle progression of Chinese hamster ovary (CHO) cells. The results indicate that S-phase cells were delayed and G_2 cells were not.

Radioactive precursors of DNA have been used extensively to measure and/or mark cellular DNA. The use of these precursors presupposes that the decay of the radioisotopic moiety will not interfere with the normal functions of the organism being studied. However, as long ago as 1958 Painter et al. (1) indicated that decay of incorporated radioactive precursors interfered with growth in HeLa cells. Since the study of Painter et al., increased evidence of the effects of incorporated radioactive DNA precursors on cell survival, cell progression, and the integrity of DNA have been reported (2–15). Most of the studies required freezing or suboptimal growth temperatures to allow a measurable accumulation of damage due to radioisotopic decay. Recently, Ehmann et al. (5) used flow microfluorometry to show an effect of a 20-min pulse of [^{3}H]TdR (20 μCi/ml, 15 Ci/mM) on cell cycle progression. The cell cycle distribution 4 h after treatment indicated parasynchronization as the cells accumulated in late S or in the G_2 + M phases. The technique of mitotic selection for cell cycle analysis, described by Schneiderman et al. (16), is an extremely sensitive measure of the perturbation of progression of late S, G_2, and M cells (16–19)[1,2] and was used in this investigation to study the effect of radioactive precursors incorporated into DNA.

By using the mitotic selection technique to determine the rate at which Chinese hamster ovary (CHO) cells progress into a small selection window in mitosis, $\simeq$4–22 min before division (16), we were able to observe the early effects of 10-min pulses of [^{3}H]TdR (5 μCi/ml, 50 Ci/mM) and ^{125}I-UdR (0.25–4.0 μCi/ml, 1.86–2.2 $\times$ 10^{6} Ci/M) on cell progression. The results illustrated in Fig. 1, panels A, B, and C demonstrate a decrease in mitotic cell yield (i.e., a decrease in the rate at which cells progress into the selection window) starting at about 70–80 min after treatment with 5 μCi/ml [^{3}H]TdR or varying concentrations of ^{125}I-UdR (0.25–4.0 μCi/ml). The nadirs of

[1]Schneiderman, M. H., L. A. Braby, and W. C. Roesch. 1977. Division delay after low X-ray doses and the effect of cycloheximide. *Radiat. Res.* In press.
[2]Schneiderman, M. H. Analysis of mammalian tissue culture cells: mitotic index, labeling index, and liquid scintillation counting using a new Gelman filter. In preparation.

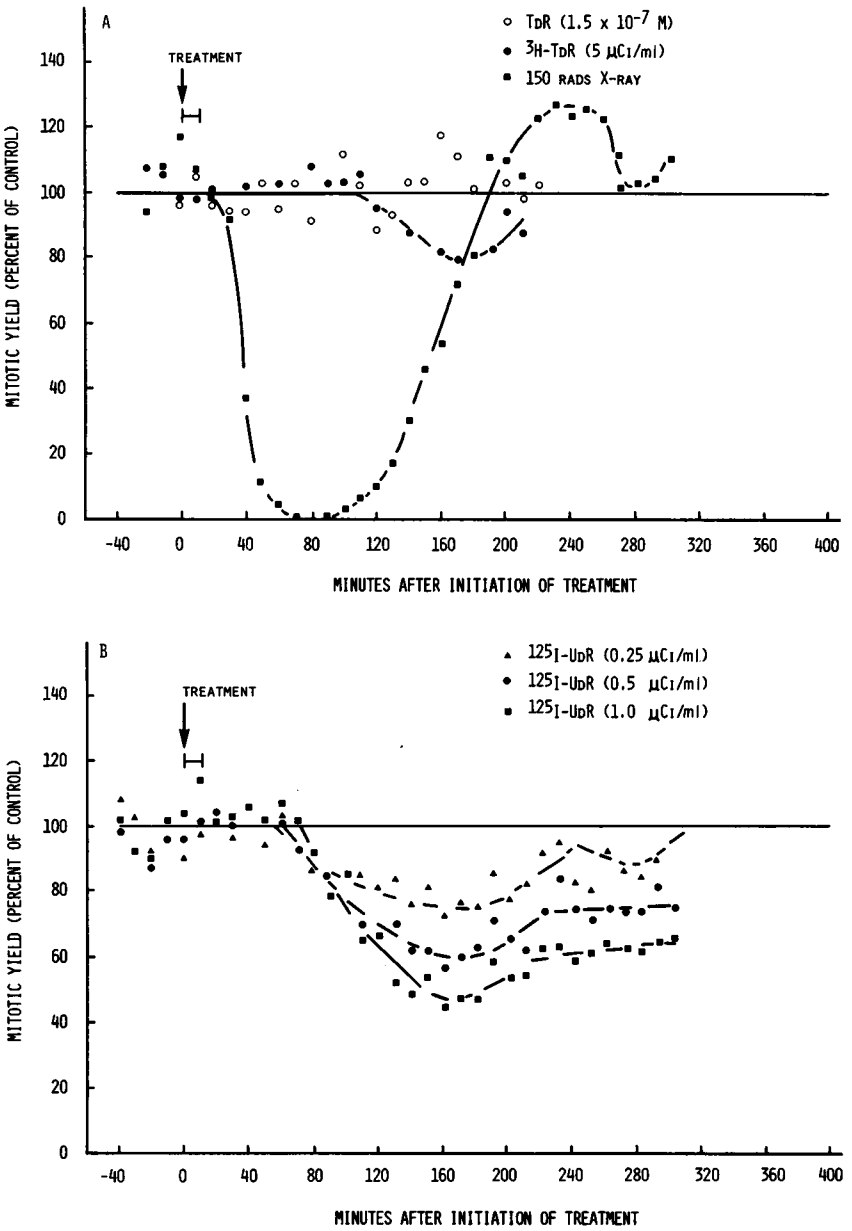

FIGURE 1 The mitotic yield after 10-min treatments with labeled and unlabeled DNA precursors or X-rays. (A) Mitotic yield after TdR (1.5×10^{-7} M), [^{3}H]TdR (5 μCi/ml), (1.5×10^{-7} M), or X-rays (150 rads). (B) Mitotic yield after various concentrations of ^{125}I-UdR. (C) Mitotic yield after ^{125}I-UdR or I-UdR.

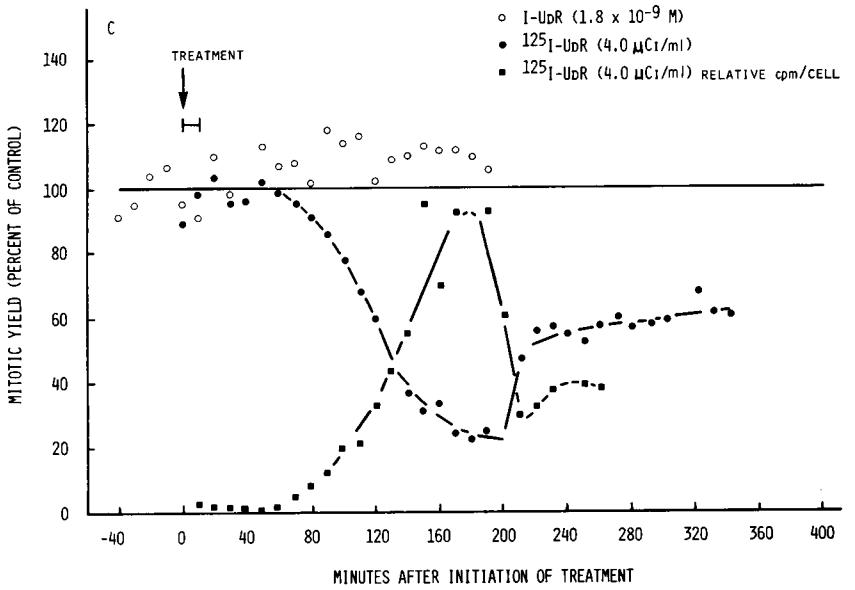

FIGURE 1 *(continued)*

the ^{125}I-UdR curves decrease as the concentration (or dose) of ^{125}I-UdR was increased. These results indicate that the first cells to be affected by the incorporated radioactive DNA precursors were 86–96 min before division (70–80 plus 16 min for the average time of selection before division, i.e., the S/G_2 boundary) for the fastest-moving cells. In addition, when the incorporated radioactivity of the selected cells (exposed for 10 min to 4 μCi/ml ^{125}I-UdR) was measured by crystal scintillation counting, no activity was measured until 70–80 min after treatment (Fig. 1 C). Moreover, the inverse relationship between cell count and cellular radioactivity after exposure to 4 μCi/ml ^{125}I-UdR indicates that the ability of cells to progress into mitosis was related to the amount of incorporated ^{125}I-UdR. Progression of S-phase cells (which had incorporated ^{125}I-UdR) was affected while G_2 cells (which had not incorporated ^{125}I-UdR) was not.

A distinctive feature of the 4 μCi/ml ^{125}I-UdR curve is the change in cell yield between shakes 36 and 37 or 210 min after treatment. Since the mitotic index was determined for each shake,[2] there is no doubt that the jump in cell count is due to a change in the mitotic yield. In addition, the 0.5 and 1.0 μCi/ml ^{125}I-UdR curves show a similar response at approximately the same time (210 min after treatment). These results imply that cells, distributed in the last 130 min of S-phase and exposed to ^{125}I-UdR, are more sensitive to radiation-induced progression delay than the cells distributed earlier in the S-phase.

Although these results clearly show an immediate effect of ^{125}I-UdR incorporation on cell progression, we can only speculate as to its mechanism. The ^{125}I-UdR data presented by Warters and Hofer (14) indicate that the ultimate "elementary target locus" for radiation-induced division delay was not the entire nucleus but some smaller substructure within it. The data presented here suggest that the most "sensitive target"

for radiation-induced delay is either in the DNA synthesized during the last 130 min of S-phase (heterochromatin [20]) or the various cellular structures, such as histones and membranes, which may be closely associated with this DNA. However, in conclusion, although decay of ^{125}I incorporated into DNA results in division delay, this by no means suggests that radiation damage to this link in the long chain of events leading to mitosis and division is the only target for radiation-induced delay.[1]

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