ULTRAVIOLET LIGHT-INDUCED DIVISION DELAY IN SYNCHRONIZED CHINESE HAMSTER CELLS

ANTUN HAN, WARREN K. SINCLAIR, *and* C. K. YU

From the Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439. Dr. Han's present address is the Institut Ruder Boskŏvić, Zagreb, Yugoslavia.

ABSTRACT The age-dependent, ultraviolet light (UVL) (254 nm)–induced division delay of surviving and nonsurviving Chinese hamster cells was studied. The response was examined after UVL exposures adjusted to yield approximately the same survival levels at different stages of the cell cycle, 60% or 30% survival. Cells irradiated in the middle of S suffered the longest division delay, and cells exposed in mitosis or in G_1 had about the same smaller delay in division. Cells irradiated in G_2, however, were not delayed at either survival level. It was further established, after exposures that yielded about 30% survivors at various stages of the cycle, that surviving cells had shorter delays than nonsurvivors. This difference was not observed for cells in G_2 at the time of exposure; i.e., neither surviving nor nonsurviving G_2 cells were delayed in division. The examination of mitotic index vs. time revealed that most cells reach mitosis, but all of the increase in the number of cells in the population can be accounted for by the increase of the viable cell fraction. These observations suggest strongly that nonsurviving cells, although present during most of the experiment, are stopped at mitosis and do not divide. Cells in mitosis at the time of irradiation complete their division, and in the same length of time as unirradiated controls. Division and mitotic delays after UVL are relatively much larger than after X-ray doses that reduce survival to about the same level.

INTRODUCTION

Fluctuations in the lethal response of Chinese hamster cells to UVL as they age between one division and the next have been established (1, 2). In addition to lethality, the age-dependent effects of UVL upon division delay are required to describe the kinetics of cell populations irradiated with UVL.

Detailed descriptions of DNA synthesis and progression through the cell cycle have been published for various cell lines (3–6). The main effect of UVL appears to be delayed progression of the cells through the S phase. UVL induces age-dependent delays in division differing from those due to X-rays, the most notable

difference being the absence of a G_2 block after UVL (3, 6). Furthermore, division delay induced by UVL is much longer than that induced by X-radiation for exposures yielding comparable survival levels. No separate measurements of the division delays for surviving and nonsurviving cells have been made thus far, but the exposures used in the various published reports were such that the irradiated population consisted mainly of surviving cells (3, 4, 6).

The purpose of this investigation was to establish the age-dependent, UVL-induced division delay in surviving and in nonsurviving Chinese hamster cells. The effects were studied after two different levels of exposure, one of which yielded a majority of cells in the population as survivors and the other as nonsurvivors, and simultaneous measurements of the postirradiation growth kinetics of surviving and nonsurviving cells were made.

MATERIALS AND METHODS

The cells used in this study, V79-S171, were a subline derived from the V79 line of near-diploid Chinese hamster cells. The cells were grown on plastic Petri dishes in EM-15 medium (2) in a humid atmosphere of 2% CO_2 and air. The average generation time of the V79-S171 cells in log phase was about 10 hr, subdivided into a G_1 period of 1.5 hr, DNA synthetic period of 6.0 hr, G_2 period of 1.5 hr, and mitotic period of 0.5–1.0 hr, determined radioautographically (2).

Synchronous populations were obtained by harvesting log-phase cells under controlled conditions, using a combination of precooling and controlled shaking. The harvesting procedure for synchronized Chinese hamster cells has been described in detail elsewhere (1). Because division delay experiments require a large number of cells per sample to ensure a reasonable number of cell counts, the method of synchronization was slightly modified. Plates for harvest were incubated for 2 hr in Colcemid before synchronization to increase the yield of mitotic cells. The concentration of Colcemid used was 0.1 μg/ml, and its toxicity was negligible (3–5% at most). This procedure increased the yield about 2.5 times. The age response for lethality after UVL for Colcemid synchronized cells, was the same as that described previously for synchronous populations obtained without the drug (2).

As was pointed out previously (7) the response of mitotic cells to agents such as X-rays or UVL cannot be determined adequately by the standard synchronization procedure followed by exposure, because at no time subsequent to synchronization were sufficient numbers of attached mitotic cells available, even at the end of the first cycle. Therefore, the Colcemid-blocked cells were first exposed to UVL (after removal of medium), the plates were then harvested for mitotic cells, and suitable aliquots of the population were plated.

In each experiment tests were performed to determine the degree of synchrony (1). The portion of the population synthesizing DNA was determined by pulse labeling with tritiated thymidine (0.128 μCi/ml, 14 Ci/mmole) at various times after inoculation, and by scoring labeled cells radioautographically.

The source of UVL was a General Electric germicidal lamp (G15T8, 15 w) (General Electric Co., Instrument Div., West Lynn, Mass.), the predominant wavelength from which was 254 nm. Cultures were placed 40 cm from the UVL source, and the incident exposure at this level, as determined using a Schwarz thermopile (Schwarz/Mann, Orangeburg, N.Y.), was 8.4 ergs/mm^2 per sec. Details of the UVL irradiation and the dose rate measurements were described previously (2).

Cells attached to the dish were exposed to UVL after removal of the medium without

rinsing because it had previously been shown that rinsing can remove mitotic cells (2). The induced division delays were examined after exposures which yielded about 60% or 30% survival, the UVL exposures being adjusted to yield approximately the same survival levels at different stages of the cycle.

As a function of time after irradiation, single cell suspensions obtained by trypsinization were counted electronically to determine the growth of the total population. These suspensions were also diluted as needed and plated for colony formation to determine the viable fraction of the total population and its growth. The procedure for the determination of growth of controls was identical, except for the absence of exposure. The pooled contents of two plates generally constituted a sample. From each sample three dishes were inoculated for a determination of the viable cells present using colony formation as the end point. Cells in the suspension were counted electronically by passing them through a 100 μ aperture of a Coulter counter (Coulter Electronics, Industrial Div., Hialeah, Fla.). The signals were fed through a 400-channel analyzer and scaler (Nuclear-Chicago, RIDL Div., Des Plaines, Ill.) and the volume distributions read out by means of an X-Y plotter (8).

The average mitotic delay (as distinct from division delay resulting from assessment of cell number) was determined by scoring mitotic index as a function of time after synchronization, and by comparing control and irradiated cells. This method has been employed previously with X-rays (9).

RESULTS

The lengths of the division delay, after exposures adjusted to reduce survival to about 60% at different stages of the cell cycle, are shown in Fig. 1. To display the killing effect of UVL, the total number of cells in the population is normalized to 1.0, and the increase in the number of surviving cells is shown relative to the total number of cells in the population at the time of exposure.

The control population starts to divide at 9.0–9.5 hr, and the growth data in control cells obtained from both total cell counts and colony counts (Fig. 1, panel A), are in good agreement for the first two cycles. Differences occur after the second division, i.e. in the third cycle, where the increase in the number of cells is larger when determined by colony counts.

The results of cell number and surviving cells, after exposures which yielded about 60% survival, show that cells irradiated in the middle of S exhibited the longest division delay (8–9 hr). Cells irradiated in G_1 or mitosis have about the same smaller delay in division ($\sim$5 hr) and cells exposed in G_2 were not delayed. The lengths of mitotic delays, as determined by scoring mitotic index as a function of time, are shown in panel F of Fig. 1. These results yield delays similar to those shown in the other panels. Thus mitotic delay and division delay do not differ significantly.

The growth data presented in Fig. 1 indicate that division in the total population and in the surviving cells was delayed by essentially the same amount. After exposures which yielded about 60% survival the viable fraction predominates and the growth curves obtained are essentially those of surviving cells. In order to examine possible differences or similarities in division delay between surviving and

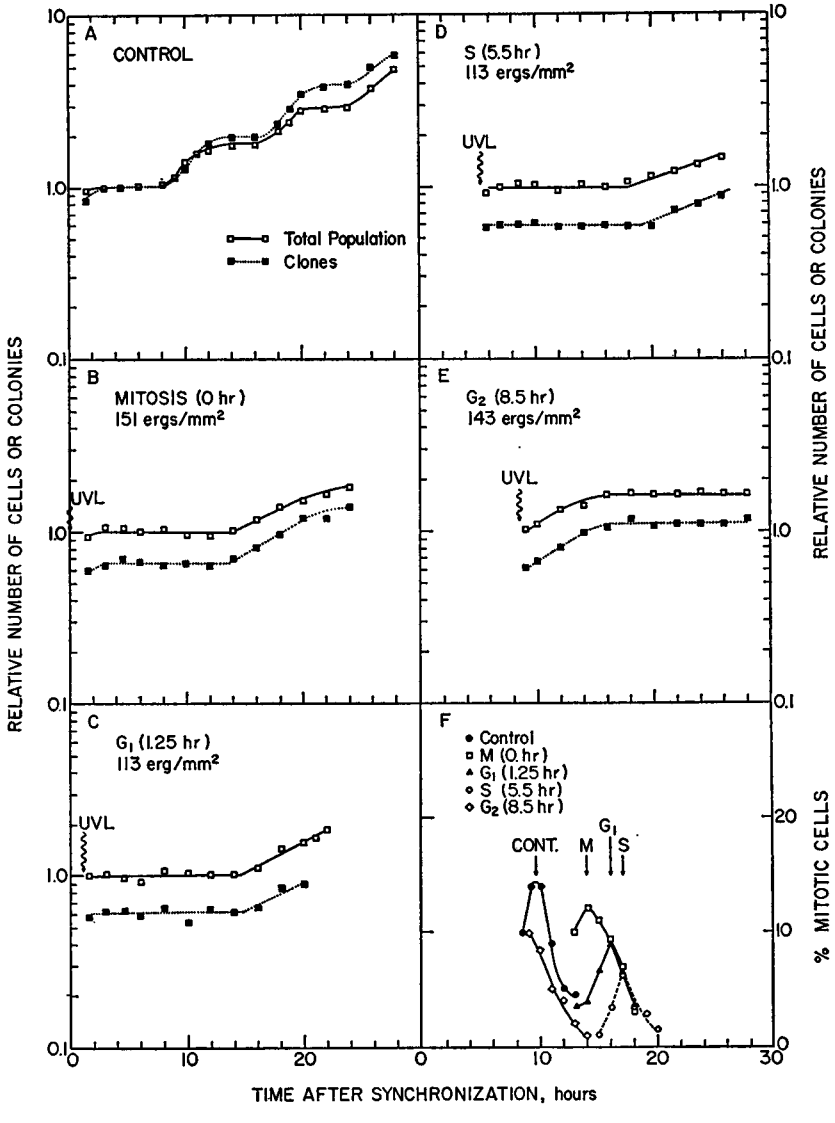

FIGURE 1 Ultraviolet light-induced division delay in Chinese hamster cells after exposures adjusted to reduce survival to about 60%. Exposures applied and the age of cells at the time of irradiation are shown on each panel (B–E). Open and closed squares trace the growth of total population and surviving cells, respectively (panels A–E). Mitotic index data are shown on panel F, and the symbols are explained on the panel.

nonsurviving cells a further set of experiments was undertaken in which the exposures were increased to reduce the survival to about 30% at each stage of the cycle. The results are shown on Fig. 2.

The patterns observed are generally similar to those shown in Fig. 1, and again

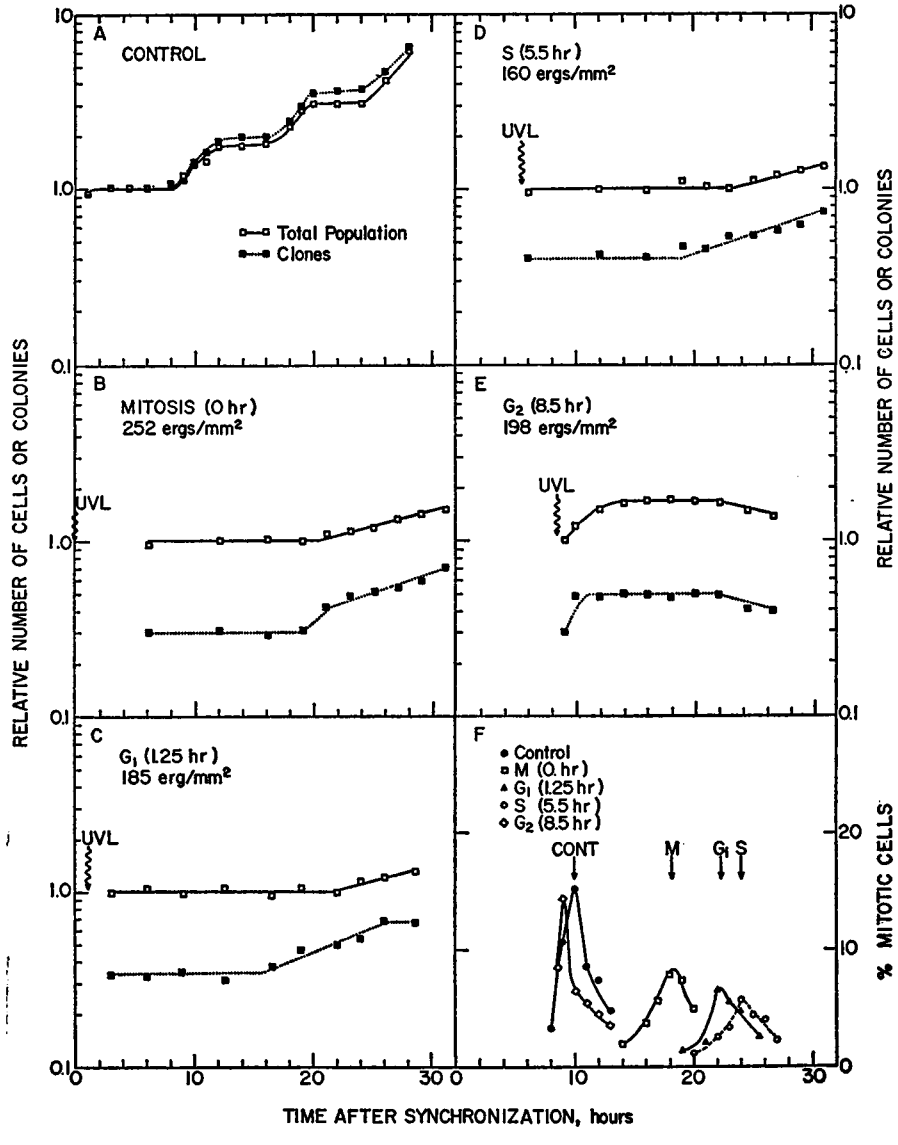

FIGURE 2 Ultraviolet light-induced division delay in Chinese hamster cells after exposures adjusted to reduce survival to about 30%. Exposures applied and the age of cells at the time of irradiation are shown on each panel (B–E). Open and closed squares trace the growth of total population and surviving cells, respectively (panels A–E). Mitotic index data are shown on panel F, and the symbols are explained on the panel.

no delay is observed in G_2. The magnitude of the delays at other stages was increased and now the differences between surviving and nonsurviving cells are clearly evident. The delay in the total population is longer than for viable cells only. The results for mitotic index (Fig. 2, panel F) agree best with those for the total

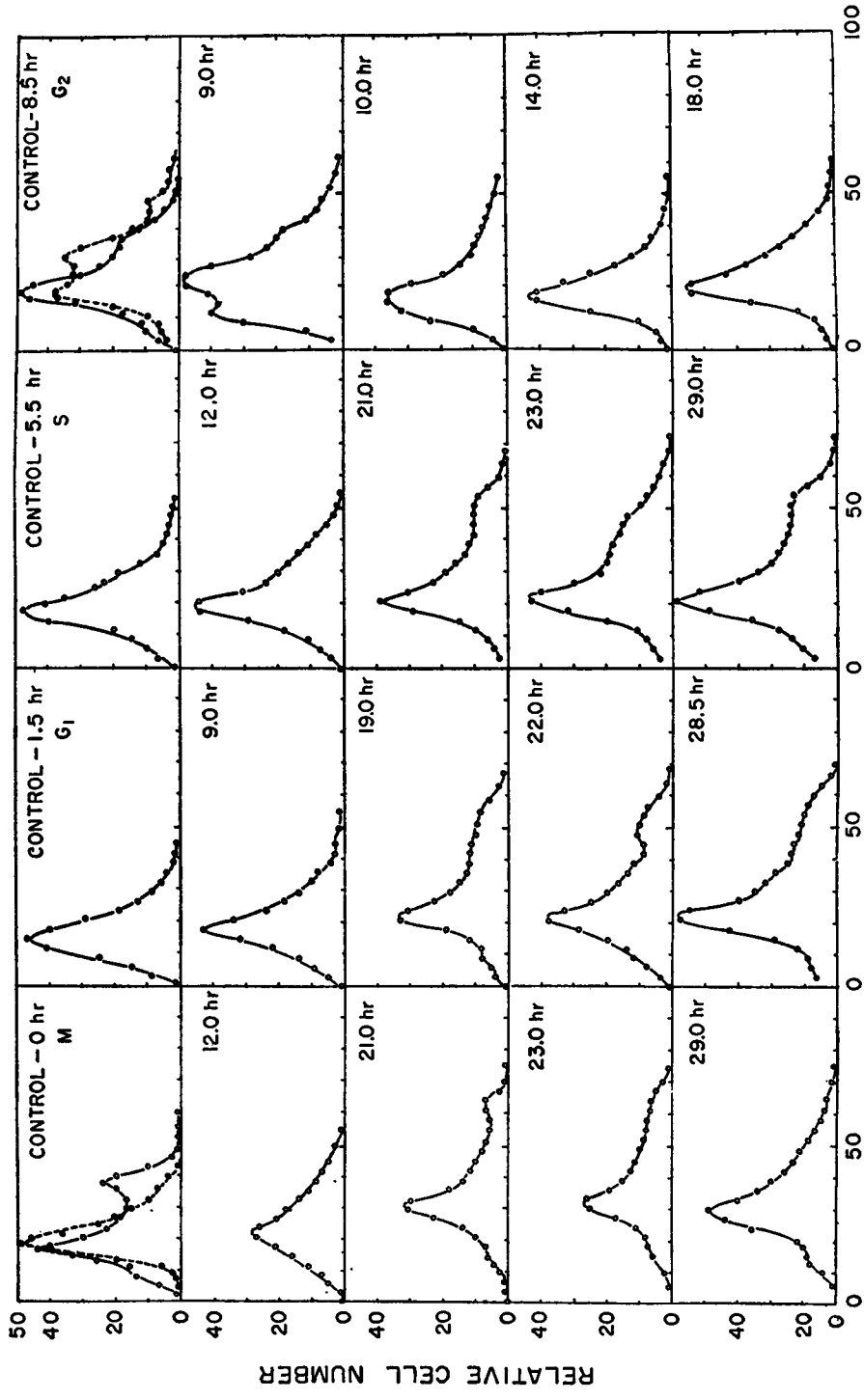

FIGURE 3 Cell size distributions of Chinese hamster cells at various stages of the cell cycle. Top row represents the distributions of unirradiated cells. Closed circles on the first and last panels trace the size distributions of an asynchronous population and that at 10.0 hr after synchronization, respectively. The cell size changes as a function of time, after exposure (to 0.3 survival level, see Table I) at various stages of the cycle are shown in the perpendicular columns.

TABLE I

DIVISION DELAY AFTER EXPOSURE OF UVL DESIGNED TO REDUCE SURVIVAL TO ABOUT 0.6 OR 0.3 SURVIVAL, RESPECTIVELY

Time of exposure after synchrony	Stage of the cycle	UVL dose	Survival	Time of division after exposure 1 hr			Division delay		
				Total pop.	Clones	M.I.*	Total pop.	Clones	M.I.*
hr		*ergs/mm²*			*hr*			*hr*	
Control				9.0	9.5	9.7	—	—	—
0	Mitosis	151	0.65	14.0	13.7	14.0	5.0	4.2	4.3
		252	0.30	21.0	19.0	18.5	12.0	9.5	8.8
1.25	G_1	113	0.62	14.8	15.0	16.0	5.8	5.5	6.3
		185	0.34	22.0	16.0	22.4	13.0	6.5	12.7
5.5	S	113	0.60	18.4	19.2	17.0	9.4	9.7	7.3
		160	0.41	23.0	19.0	24.0	14.0	9.5	14.3
8.5	G_2	143	0.62	9.0	9.0	9.0	0	0	0
		198	0.30	9.0	8.5	9.0	0	0	0

* M.I. = mitotic index.

population, whereas the delay for viable cells is shorter than these, suggesting that the majority of cells reach mitosis. Furthermore, all of the increase in cell number for total population can be accounted for by the increase in the viable cell fraction. Thus, the nonsurviving cells presumably are present during most of the experiment, but apparently are stopped at mitosis and do not divide. It is also possible that some nonsurviving cells do not reach mitosis at all. This is indicated by cell size distribution data presented in Fig. 3.

The set of curves along the top of Fig. 3 represent the distributions recorded at various times after synchronization (open circles). Closed circles on the first and last panel show the cell size distribution of an asynchronous population and that observed at 10 hr after synchronization (i.e., when most cells divide), respectively. The distribution obtained immediately after harvesting, that is, 0 hr, as well as any other recorded at the time of cell division, is characterized by two peaks. One peak is in the region of smaller sizes, showing cells which just have completed their division (very early G_1 cells), and the other peak is for large mitotic cells about to divide. The rest of the curves, recorded at various stages of the cycle, show that cells grow larger as they age in their interdivisional cycle, as was demonstrated previously by Sinclair and Ross (8).

Although not shown, the size distributions immediately after exposure (to 0.3 survival level, see Table I) at any time in the cycle, were the same as for controls. In Fig. 3 the perpendicular columns illustrate the cell size changes after exposures at selected stages of the cycle. The essential features of these curves are: (*a*) for cells irradiated in mitosis, G_1, and S, average cell size does not change for about 9–12 hr after irradiation; (*b*) a few hours before an increase in the cell number occurs the

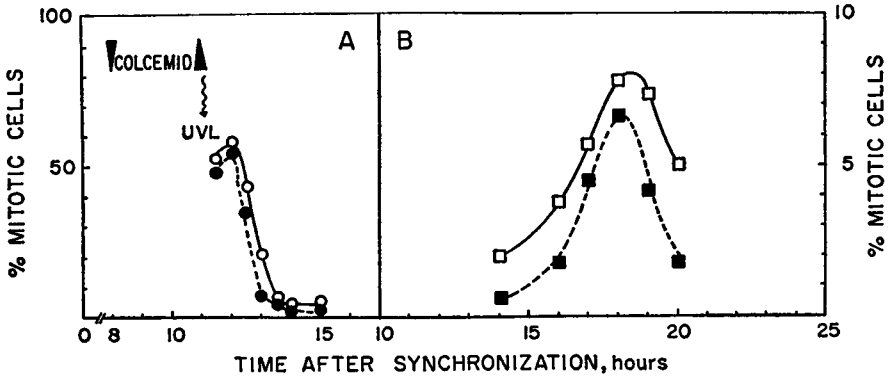

FIGURE 4 The effect of ultraviolet light on duration of mitosis in Chinese hamster cells and the frequency of abnormal mitosis. Panel A: open circles trace the per cent of mitotic figures in the control population, and the closed circles in cells exposed to UVL 252 ergs/mm², immediately after removal of Colcemid. Arrows indicate the length of Colcemid block, and the time of exposure. Panel B: open squares show the mitotic index data at the end of the division delay for cells exposed to 252 ergs/mm² in mitosis, and closed squares trace the percentage of mitotic figures exhibiting demolished chromosomes ("chromatin mass"). The data shown by open squares include both normal and abnormal mitotic figures.

distributions indicate the presence of some cells with larger volumes, presumably those which have reached mitosis, and the second peak is visible at the end of division delay period; and (*c*) there is a fraction of cells in the population which did not undergo size changes during the delay period. Apparently in these cells metabolic activity was severely limited by irradiation, and they will probably not divide at all. Consequently, this peak may indicate the nonsurviving cells, whose further progression through the cycle was prevented by irradiation. The curves presented (as well as those not presented here) confirm the absence of giant cell formation after UVL, as was suggested by others using a different method of observation (10, 11).

In order to determine whether UVL affects the length of cell division, (since X-irradiation does not increase the *duration* of division [12–14]), cells were blocked in metaphase by addition of Colcemid (0.1 μg/ml) at the end of the first cycle. After 3 hr of incubation, the drug was removed, cells exposed to UVL, 252 ergs/mm², and mitotic index scored at ½-hr intervals. The results shown on Fig. 4 (panel A), indicate that cells in mitosis at the time of irradiation complete their division in the same length of time as unirradiated controls. These cells, however, show in their subsequent division, a very large percentage of mitotic figures with demolished chromosomes ("chromatin mass") (panel B, Fig. 4), the phenomenon previously described by Chu (15). This phenomenon is also present when cells are irradiated at the other ages, but to a much lesser extent.

The lengths of division delays after exposures designed to reduce survival to 60% or 30% are summarized in Table I.

DISCUSSION

The following summarizes the results of this study of the UVL age-dependent response for induced division delay; cells irradiated in the middle of S suffered the longest division delay, cells exposed in G_1 or mitosis have about the same delay in division, and cells irradiated in G_2 were not delayed. All of the cell increase at the time of the first postirradiation division can be accounted for by the increase of the viable cell fraction. Thus, the nonsurviving cells are presumably stopped at mitosis and do not divide. This is indicated from the data for mitotic index vs. time, at 30% survival.

Previous studies (1, 2) dealing with the age-dependent UVL lethality have established that these Chinese hamster cells are least sensitive in mitosis, become more sensitive during G_1, and are most sensitive in the middle of the DNA synthetic phase; sensitivity then decreases at the end of the cycle. The sensitivity of G_1 and G_2 cells was found to be about the same. For both lethality and division delay, the most sensitive phase in the division cycle is the S phase. This is also true for the production of thymine dimers (16), and chromosome damage (17).

The delays observed for cells exposed in mitosis or G_1 may be the consequence of characteristic sensitivities of these two stages, or they may be the result of difficulties in passing through the DNA synthetic phase. Progression through the S phase depends upon the rate of DNA synthesis, and UVL inhibits this process severely (18–20). Studies on cell progression following UVL irradiation with various cell lines have established that cells exposed in G_1 move to S without any delay; that is, no G_1–S block was observed (3, 4, 6). This information plus our own observations[1] indicate that division delays observed for cells irradiated in mitosis or G_1 are the consequence of the slowed progression through S, caused apparently by damaged DNA synthesis and replication. This view is also supported by the data of Thompson and Humphrey (21) on L-P59 cells, which showed that the length of division delay in the first cycle was inversely related to the age of cells at the time of irradiation, but that delay in the second division after exposure was a direct function of the age at the time of irradiation. The foregoing also explains the absence of delay for cells in G_2 (in the dose range examined). These cells advance at the normal rate, and are not delayed in their first postirradiation division, but they exhibit considerable delay in the subsequent cycle (see panel E in Figs. 1 and 2). Therefore the UVL damage to the DNA seems not to interfere with the advancement of G_2 cells to mitosis, but apparently affects the progression of these cells through their subsequent cycle.

The age response data for division delay presented here are in good agreement with those reported for HeLa (3), T, and DON cells (6). Specific measurements of the induced division delay for surviving and nonsurviving cells have not been re-

[1] Data presented in 1968 at 16th Annual Meeting of American Radiation Research Society, Houston, Texas. Unpublished.

ported before this study, as previous reports (3, 6) have dealt with a majority of surviving cells only. We have shown here that a difference exists in the length of division delay between surviving and nonsurviving cells. The observed difference indicates that all of the increase in cell number is due to the viable cell fraction only. Thus the nonsurviving cells, although present during most of the experiment, apparently do not divide (22). Both surviving and nonsurviving cells irradiated in G_2 completed their division without delay, as is evident from the measurements of cell number and mitotic index (panels E and F, Fig. 2). It is probable, however, that nonsurviving G_2 cells would be stopped at the next division.

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