# SENSITIVITY OF SYNCHRONIZED CHINESE HAMSTER CELLS TO ULTRAVIOLET LIGHT

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ABSTRACT The age response for lethality of Chinese hamster cells to ultraviolet light shows that they are resistant in $G_1$, sensitive as they move into and through the S phase and resistant again in $G_2$ and mitosis. Survival curves determined at different times in the cycle reveal that mitotic cells are the most resistant fraction, much more resistant than S cells, and more resistant than either $G_1$ or $G_2$ cells. The extent to which the age response is ilfluenced by nucleic acid and protein synthesis was investigated by using inhibitors of these processes. In the presence of inhibitors of DNA or protein synthesis added to $G_1$ cells before exposure, cell survival neither declines to the minimum survival of S cells nor rises subsequently to the resistance of $G_2$ cells. If, before exposure, DNA synthesis is arrested in the middle of S, when survival is at a minimum, the subsequent rise in survival during $G_2$ is not prevented. However when cycloheximide is added before exposure, during the middle of S, this rise is prevented. When actinomycin D, an inhibitor of RNA synthesis is added prior to exposure the age response is affected only slightly. Postirradiation treatment of $G_1$ and mid-S cells with inhibitors of DNA or protein synthesis maintains survival at a level characteristic of the age of the cells.

## INTRODUCTION

The response of mammalian cells to ultraviolet light (UVL) has been extensively studied recently in both synchronous and asynchronous cultures. The effects studied include cell survival (1, 2, 3, 4, 5), inhibition of DNA synthesis and induction of thymine dimers (6, 7, 8, 9, 10), chromosomal aberrations (11, 12) and the repair of UV-induced damage (13, 14, 15, 16). Photochemical events related to cell lethality are comparatively well known in bacteria (17) but not in mammalian cells.

The survival of synchronized mammalian cells exposed to UVL exhibits characteristic fluctuations as cells progress through the cycle (3, 4, 5). Studies of Chinese hamster cells by Sinclair and Morton (3) indicated a markedly different age response for survival after UVL from that of X-rays. Cells were more sensitive to UVL in S than in $G_1$ and $G_2$. These results have been confirmed by others (4, 5) in other

mammalian cells. The purpose of the investigations presented in this report is to establish the age response for lethality of Chinese hamster cells to UVL more precisely and to determine the extent to which this response is influenced by nucleic acid and protein synthesis.

## MATERIALS AND METHODS

The cells employed were two sublines derived from the V79 line of near diploid Chinese hamster cells. Both sublines (V79-S171 and V79-325) were grown on plastic petri dishes in EM-15 medium (similar to HU-15 (18) but without NCTC 109) 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 autoradiographically. Synchronous cells progress through the first cycle after synchronization a little more slowly (19). The V79-S171 subline had a short $G_1$ and it was desirable to determine the age response to UVL of another subline of Chinese hamster cells with a longer $G_1$, because it is known from X-ray studies (20) that additional structure in the age response usually is observed in long $G_1$ cell lines. Subline V79-325 was used for this purpose. The cells of this subline had an average generation time during these experiments of about 18 hr and progressed through $G_1$ in 5 hr, through S in 9.5 hours and through $G_2$ in 2 hr.

Synchronous populations at or near division were obtained by harvesting log-phase cells under controlled conditions using a combination of precooling and controlled shaking. The method of synchronization has been described in detail elsewhere (3, 19). After synchronization, aliquots of cells were inoculated into plastic petri dishes (85 mm in diameter) and followed throughout the subsequent generation cycle.

The response of mitotic cells cannot be effectively determined by this procedure, because at no time subsequent to synchronization and incubation were sufficient numbers of attached mitotic cells available, even at the end of the first cycle. Harvested cells were in mitosis but were not attached. Therefore, groups of log-phase plates were first exposed to UVL after removing the medium, and the plates were harvested for mitotic cells. Suitable aliquots of the population were plated, while others were used to determine cell concentration, multiplicity of cells in microcolonies and mitotic index (19).

In each experiment tests were performed to determine the degree of synchrony (3). The portion of the population synthesizing DNA was determined by pulse-labeling with tritiated thymidine (0.3 $\mu$c/ml, 3.0 c/mmole, for 15 min) at various imes after inoculation and scoring labeled cells by autoradiography.

A General Electric germicidal lamp (G15T8, 15 watt) was the source of ultraviolet light. The predominant wave length from this lamp was 254 nm. The source was mounted at a distance of 40 cm above the samples. Exposures were controlled by shutter and timer. The incident dose, at the exposure level, was 10.4 ergs/mm$^2$/sec, as determined by a Schwarz thermopile calibrated against a lamp standardized by NBS[1]. During these studies, the dose rate changed slightly and ultimately was 8.7 ergs/mm$^2$/sec. This change in the dose rate had no effect on the age response pattern. Exposures were conducted in room light, as no photoreactivation was observed in these cells under these conditions by Sinclair and Morton (3).

Cells attached to the dish were exposed to UVL after removal of the medium. Cells were rinsed with phosphate-buffered saline (PBS) before irradiation, except in experiments in

[1] National Bureau of Standards.

which this step was purposely omitted to establish its effect on the age response. The medium was replaced immediately after exposure to UVL.

The ability of single cells to form colonies was the criterion of the effect of UVL. Clones were scored after a postirradiation incubation period of 8 days for the V79-S171 subline and 11 days for the V79-325 subline. Stained colonies on the flat bottom of the plate only, were counted.

Inhibitors of different cellular processes were used in some experiments. The inhibitors were dissolved directly in EM-15 medium just prior to use. The final concentrations were: 1.0 mM hydroxyurea (HU), 7.5 mM thymidine (TdR), 5.0 μg/ml cycloheximide (CH), 0.01 μg/ml actinomycin D (AcD) and 0.5 μg/ml Colcemid. Their effect on DNA, RNA and protein synthesis was followed by labeling the cells for 15 min with tritiated thymidine, tritiated uridine (1.03 μc/ml, 29.0 c/mole) and tritiated leucine (6.6 μc/ml, 5.0 c/mmole), respectively, followed by autoradiography.

The synchronization method does not provide a suspension of single cells, but a population which is a mixture mainly of single and double cells. Therefore, it was necessary to correct all the survival data for cellular multiplicity, according to $S = 1 - (1 - f)^{1/\bar{N}}$, where $f$ is the observed colony survival, $S$ the desired single cell survival and $\bar{N}$ the average cellular multiplicity (3, 19). All the survival data (except for V79-325 cells, Fig. 2) are presented as single cell survival and in experiments with inhibitors, correction for toxicity was made.

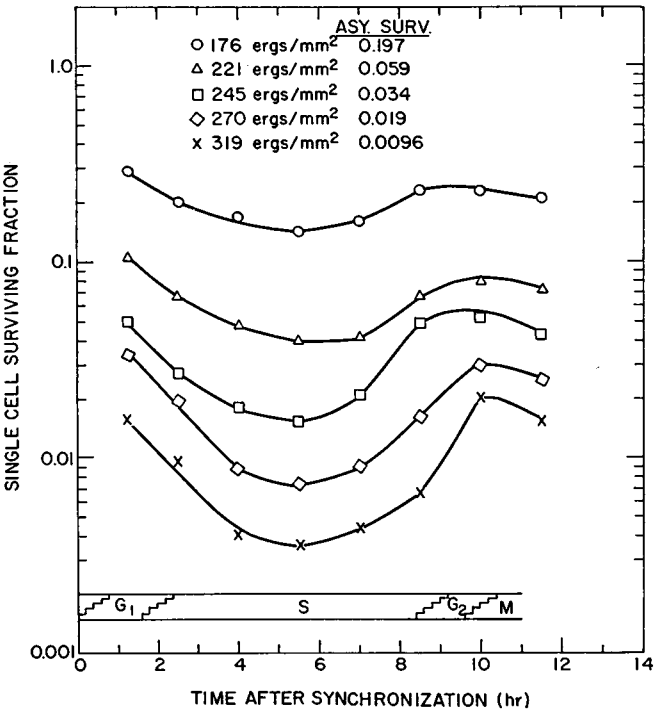


FIGURE 1 UVL age-response for colony formation of V79-S171 cells, for five different exposures. Average values of the survival for asynchronous cells are shown on the top of the figure.

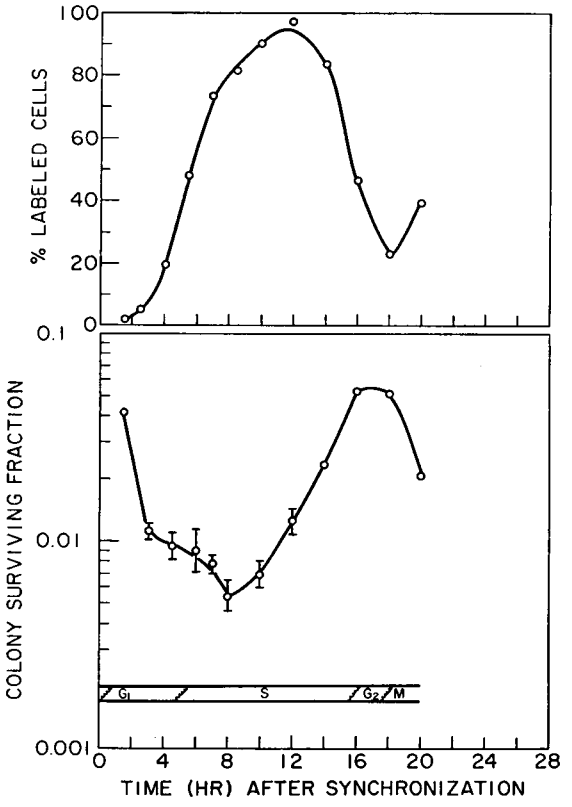


FIGURE 2 UVL age-response for colony formation of V79-325 cells after an exposure to 270 ergs/mm² (bottom). The limits shown by the error bars are for the standard deviation. Per cent of cells pulse-labeled with $H^3$-TdR against time after synchronization (top).

## RESULTS

### *Age Response for Lethality after UVL*

The age response of V79-S171 cells for five different exposures is shown in Fig. 1. The general pattern of these responses is the same at all levels. Cells are resistant in $G_1$, sensitive throughout S, and become resistant again in $G_2$. These curves show further that cells are most sensitive 5.5 hr after synchronization, which corresponds to the middle of the DNA synthetic phase. As the cells progress through the cycle the degree of synchrony gradually decreases due to random differences in the generation times among individual cells (20). At 10.0 hr, even though most cells are near the end of their first cycle, the population consists of a mixture of $G_2$, mitotic, and some S cells. The results at this time represent an average survival for this mixed population. It is probable, from these data alone, that cells at or near division are the most resistant.

In order to explore the survival throughout $G_1$, the V79-325 subline was used.

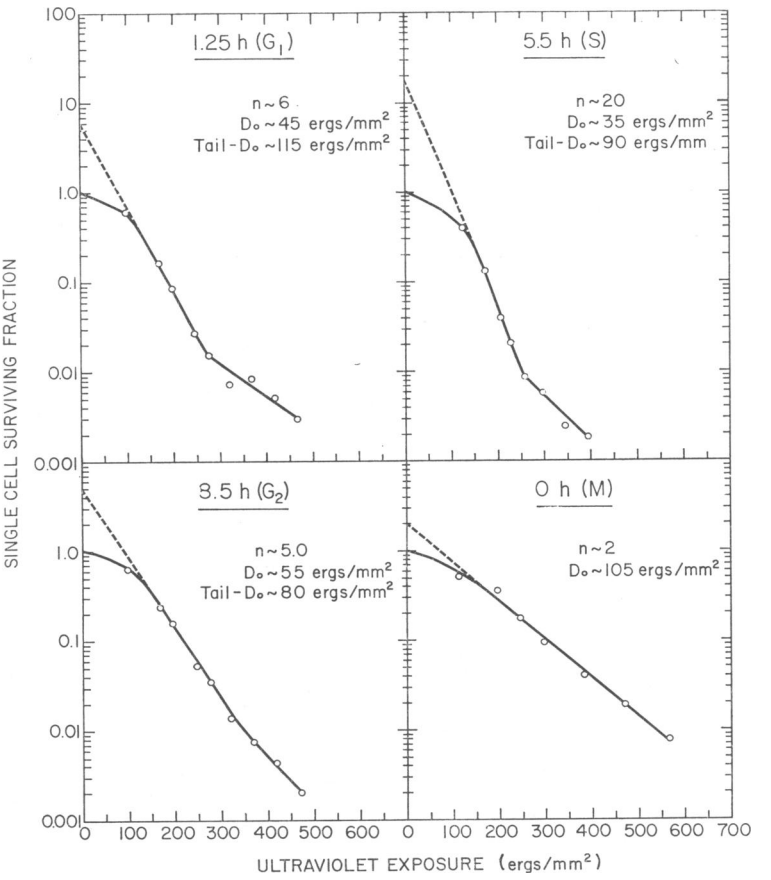


FIGURE 3 Survival curves of synchronized V79-S171 cells at different stages of the cell cycle.

Fig. 2 shows the age response of these cells. Some inflections in the response occurred in late $G_1$, but there was no significant departure from the pattern for V79-S171 cells except that possibly the "$G_2$" resistance developed earlier. In subsequent discussion we will refer to V79-S171 cells only.

### *Survival Curves at Different Cell Cycle Stages*

To obtain survival curves at selected cell stages, V79-S171 cells were irradiated with different doses of UVL at various times after synchronization. Results which are typical of several experiments are shown in Fig. 3. These curves were obtained in two sets of experiments, one set for interphase cells and the other for mitotic cells. Survival curves were determined at 1.25 ($G_1$) 5.5 (S), 8.5 ($G_2$) and 0 (mitosis) hr after synchronization. Mitotic cells are most resistant, much more resistant than S

TABLE I

PARAMETERS OF SURVIVAL CURVES AFTER UVL IRRADIATION

| Time after synchronization | Phase | Single cell extrapolation number | Shoulder width $D_q$ | $D_0$ | Tail $D_0$ |
|---|---|---|---|---|---|
| hr | | | ergs/mm$^2$ | ergs/mm$^2$ | ergs/mm$^2$ |
| 0 | M | 2 | 68 | 105 | - |
| 1.25 | $G_1$ | 6 | 82 | 45 | 115 |
| 5.5 | S | 20 | 100 | 35 | 90 |
| 8.5 | $G_2$ | 5 | 90 | 55 | 80 |
| V79-S171 asynchronous | - | 15 | 120 | 45 | |
| V79-325 asynchronous | - | 15 | 115 | 47 | |

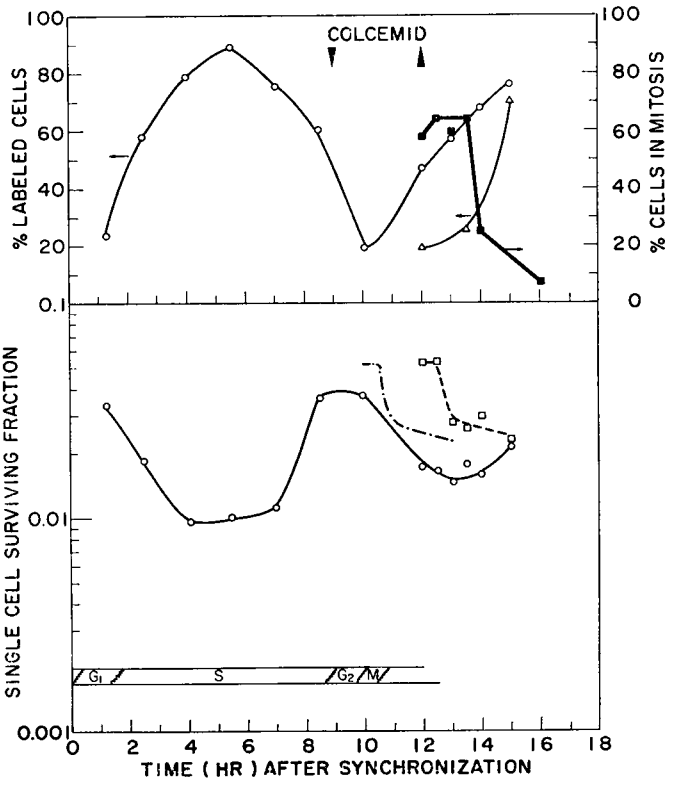


FIGURE 4 Changes in the sensitivity of V79-S171 cells in transition from mitosis to $G_1$, (270 ergs/mm$^2$). Top, per cent of cells pulse-labeled with $H^3$-TdR (open circles), pulse-labeling of Colcemid blocked cells (open triangles), mitotic index (closed squares). Bottom, age response for colony formation of non-blocked cells (open circles), age response of Colcemid blocked cells after removal of the drug (open squares). Curve without symbols, represents time correction for the length of Colcemid treatment for the M — $G_1$ transition.

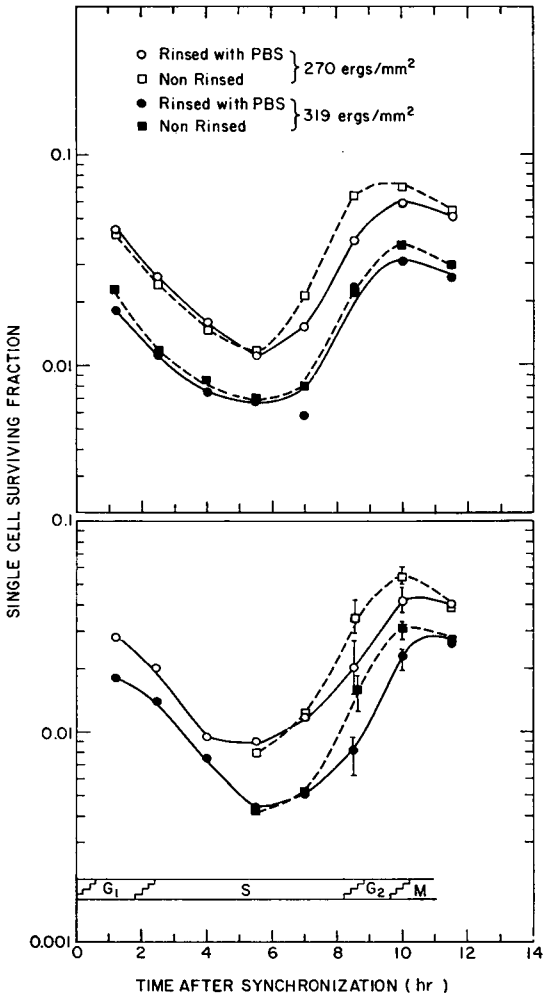


FIGURE 5 Age response for colony formation of rinsed (open and closed circles) and non-rinsed (open and closed squares) V79-S171 cells.

and more resistant than either $G_1$ or $G_2$ cells. The values for $D_o$ (the reciprocal slope of the survival curve) between the most sensitive (S), and most resistant (mitotic-M) cells, differ by a factor of about 3, while the extrapolation numbers differ inversely by a factor of about 10.

Survival curves for $G_1$ and $G_2$ cells indicate that their sensitivity to UVL is about the same. Evidently the elevation in survival at the end of the first cycle (see Fig. 1) is caused mainly by the presence of mitotic cells. Also, in survival experiments additional to those shown in Fig. 3 no significant difference in sensitivity between early (4 hr) and late S (7 hr) cells was found.

TABLE II

THE EFFECT OF RINSING ON UVL SURVIVAL*

| DOSE | TIME | $S_o$ | $S_r$ | $S_m$ | a | c | $S_t$ |
|---|---|---|---|---|---|---|---|
| ergs/mm$^2$ | hr | | | | | | |
| 270 | 8.5 | 0.063 | 0.038 | 0.224 | 0.585 | 0.134 | 0.086 |
| | 10.0 | 0.121 | 0.095 | | 0.265 | 0.202 | 0.135 |
| 319 | 8.5 | 0.03 | 0.0148 | 0.131 | 0.585 | 0.131 | 0.0297 |
| | 10.0 | 0.07 | 0.050 | | 0.265 | 0.247 | 0.0731 |

* Survival values given in this table are "colony survival" values not corrected to single cell. If single cell values are used instead, very similar values of 'c' are obtained.

The survival curve parameters ($n$ and $D_o$) are summarized in Table I, including data for asynchronous cells. Also included are numerical estimates of the shoulder width, $D_q$ (21).

At or near the 0.01 survival level, each interphase curve has a tail indicating the presence of a small fraction of the population that is more resistant to UVL. A similar small inflection has been observed in the survival curve of asynchronous cells. Because of the limitations imposed by the synchronization procedure, it is difficult to explore survival beyond the third decade (below 0.001 survival level), but a tail was observed in each experiment, except those for mitotic cells. It is likely that this tail is due to a small fraction of resistant mitotic cells for the following reasons: (*a*) 100% synchrony is never achieved, (*b*) inflections in survival curves generally indicate a mixture of at least two components (22), (*c*) mitotic cells are the most resistant component, and (*d*) the tail has a $D_o$ similar to that of mitotic cells.

In order to examine survival variations during the transition period between mitosis and $G_1$, cells were blocked in metaphase by the addition of Colcemid (0.05 μg/ml for 3.0 hr), and were irradiated at ½ hr intervals after its removal. The results, shown on Fig. 4, indicate that cells acquire the $G_1$ sensitivity no later than the completion of division. Thus, the observed low sensitivity of mitotic cells is characteristic of mitosis only.

### *Effect of Rinsing Prior to UVL*

It is generally considered desirable to rinse cells, after removal of medium, before UVL exposure, because of the high absorbance of 254 nm wave length UVL by the medium. In some experiments, however, this step is not desirable because it may result in the detachment of mitotic cells. It was important, therefore, to establish whether or not the rinsing is necessary. The age response for rinsed (with PBS) and nonrinsed cells therefore was determined (Fig. 5). Rinsing does not influence the

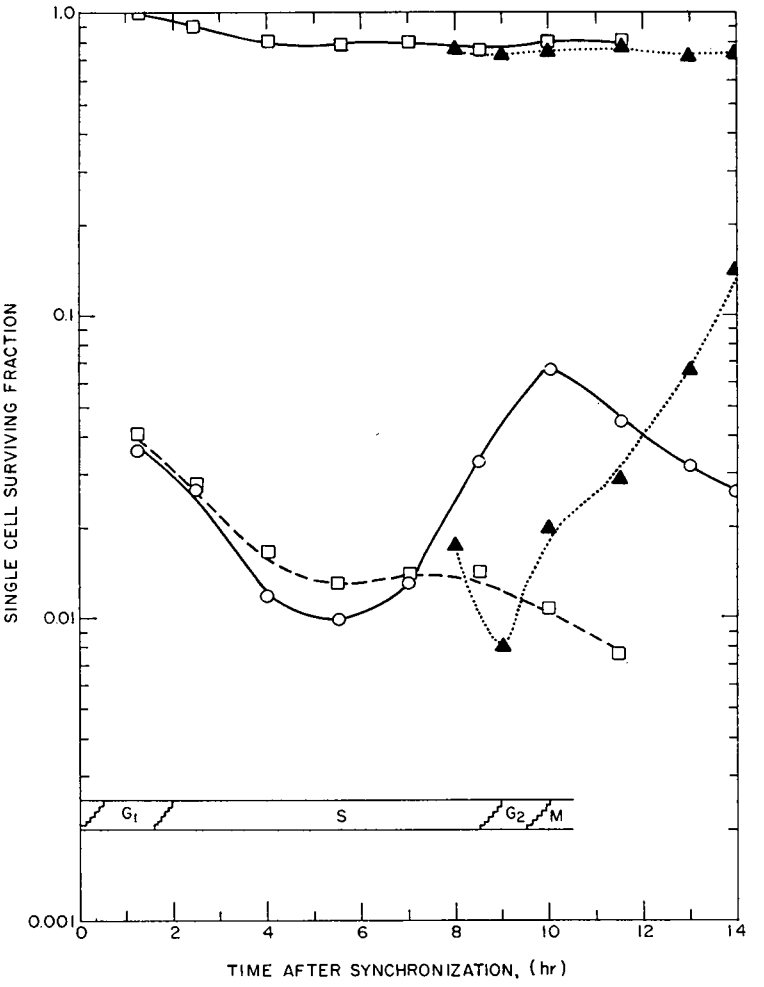


FIGURE 6 The effect of addition and removal of HU on the age response for colony formation of synchronous V79-S171 cells. Open circles, cells exposed to 270 ergs/mm². Age response of cells treated with 1.0 mM HU at 1.25 hr and exposed to 270 ergs/mm² at different times (open squares). Closed triangles, HU added at 1.25 hr and removed at 8.0 hr from cultures subsequently irradiated. Open squares and closed triangles at the top of the graph are the data for HU treatment only.

age response in the first half of the generation cycle but appears to affect it during the later part of the cycle (top half, Fig. 5). Therefore, another experiment was carried out with additional plates per point in the second half of the cycle for a more precise determination of this effect. The results of this experiment are presented on the bottom part of Fig. 5. Differences in survival between rinsed and unrinsed cells at 8.5 and 10.0 hr were statistically significant, while at 5.5 and 7.0 hr no significant differences were observed.

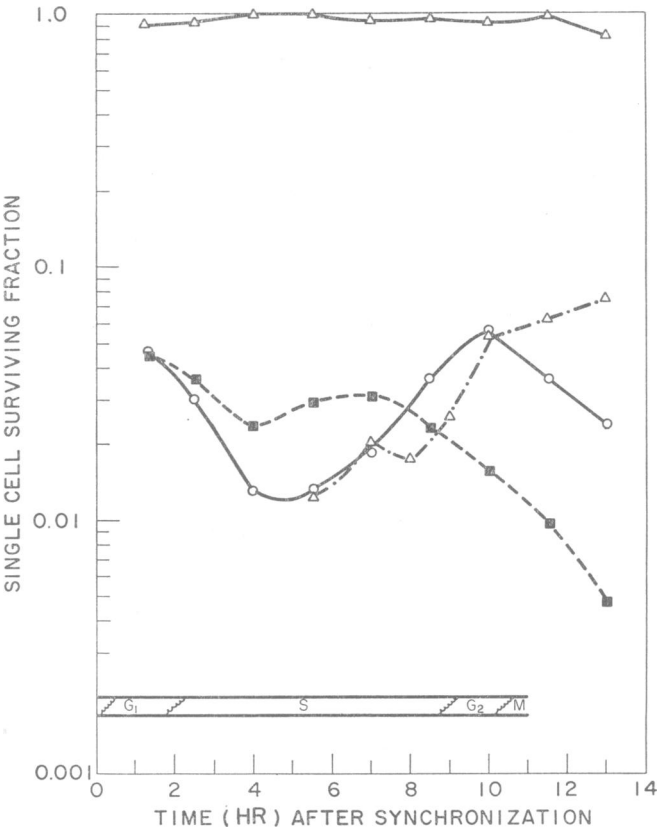


FIGURE 7 The effect of excess TdR on the age response for colony formation of V79-S171 cells. Open circles, age response of V79-S171 cells exposed to 270 ergs/mm²; closed squares, 7.5 mM TdR added to $G_1$ cells (1.25 hr), and 270 ergs/mm² at different times. The drug was removed immediately after exposure. Open triangles, 7.5 mM TdR added to mid-S cells (5.5 hr), and 270 ergs/mm² at different times, the inhibitor removed immediately after exposure. Open triangles at the top of the graph are the data for excess TdR treatment only.

The fact that differences in survival occur at times (8.5 and 10 hr) when some or most of the cells are dividing suggests that these differences may be due to the removal of mitotic cells by rinsing. This suggestion can be tested by simple algebraic considerations.

Cells at time $t$ (in $G_2$) consist of three main components, a fraction '$a$' of cells still in S, a fraction '$b$' of cells at or about age $t$ and a fraction '$c$' of cells already in mitosis. If the survival of these three components after a given dose of UVL is $S_s$, $S_t$ and $S_m$, respectively, the survival of nonrinsed cells in the mixture, $S_o$ is given by

$$S_o = aS_s + bS_t + cS_m \cdots \quad (1)$$

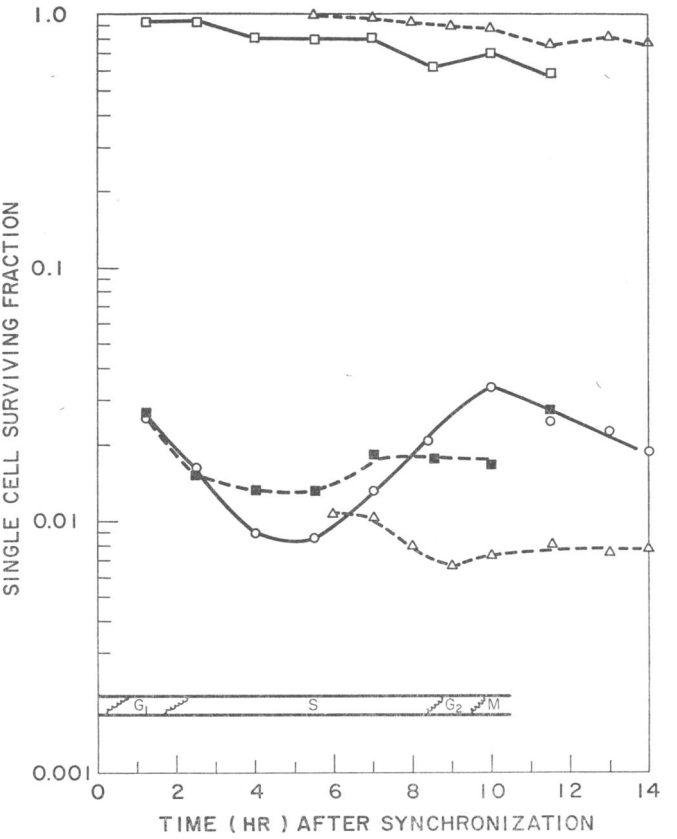


FIGURE 8 The effect of cycloheximide on the age response for colony formation of V79-S171 cells. Open circles, cells exposed to 270 ergs/mm² at various times after synchronization; closed squares, 5.0 μg/ml of CH to $G_1$ cells (1.25 hr) and 270 ergs/mm² at different times, the inhibitor removed immediately after exposure; open triangles, the same treatment as before, except that CH was added to mid-S cells (6.0 h). Open squares and triangles at the top of the graph are the data for CH treatment only.

furthermore, if the mitotic cell component is removed by rinsing, the survival of rinsed cells

$$S_r = \frac{a}{1-c} S_s + \frac{b}{1-c} S_t \cdots \quad (2)$$

and thus

$$c = \frac{S_o - S_r}{S_m - S_r} \cdots \quad (3)$$

The results of some calculations, applying these expressions to the survival data

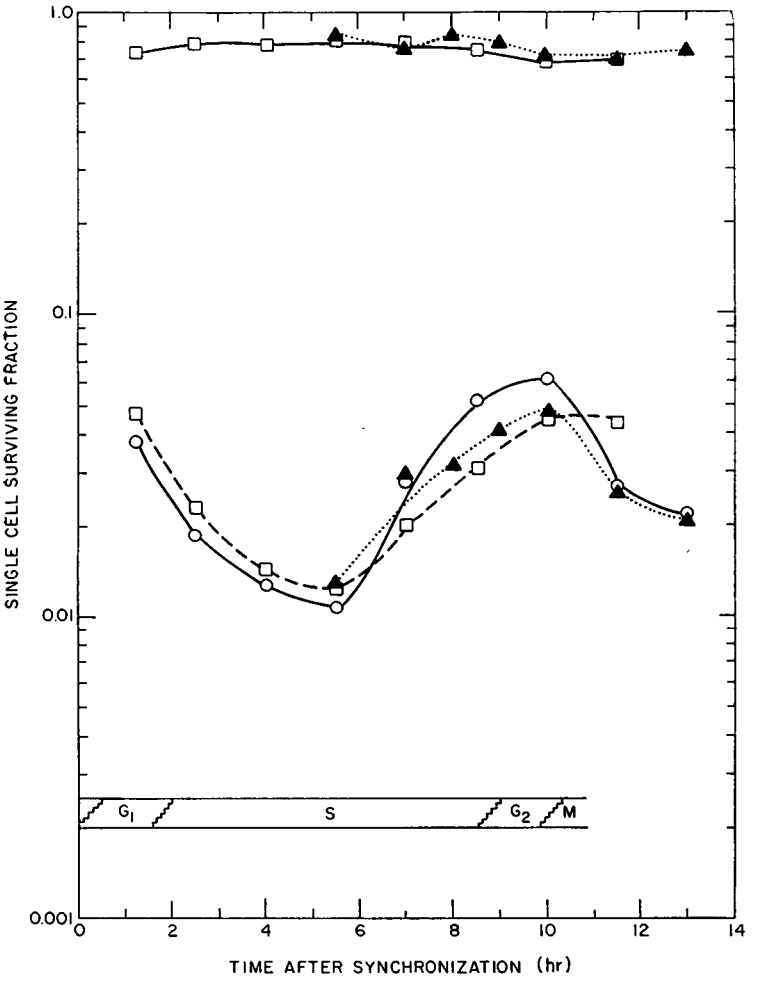


FIGURE 9 The effect of actinomycin D on the age response for colony formation of V79-S171 cells. Open circles, cells exposed to 270 ergs/mm² at various times after synchronization; open squares, 0.01 μg/ml of AcD added to $G_1$ cells (1.25 hr) and 270 ergs/mm² at different times the drug removed immediately after exposure; closed triangles, the same as preceding, but AcD added to mid-S cells (5.5 hr). Open squares and closed triangles at the top of the graph are the data for AcD treatment only.

at 8.5 and 10 hr in order to find '*c*', are indicated in Table II. A value for mitotic index of 20–25% is often obtained experimentally for '*c*' at 10 hr, and 13% at 8.5 hr compares reasonably with a value of 11% sometimes obtained at this time. [Note that rinsing unirradiated plates results in similar differences for '*c*' but many plates are required for adequate statistics.]

Thus, the observed differences in the survival of nonrinsed and rinsed cells can be accounted for by the removal of mitotic cells by rinsing.

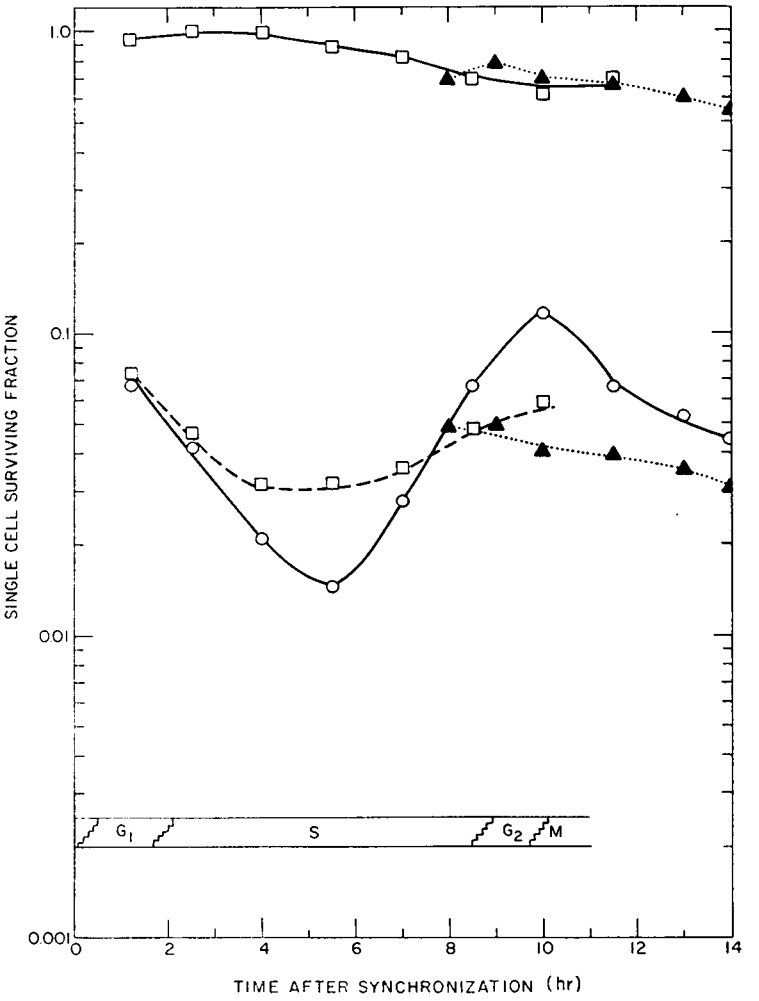


FIGURE 10 The effect of addition and removal of cycloheximide at 8.0 hr on the age response for colony formation of V79-S171 cells. Open circles, cells exposed to 270 ergs/mm² at various times after synchronization; open squares, 5.0 μg/ml of CH added to $G_1$ cells (1.25 hr) and the drug removed immediately after exposure; closed triangles, CH added to $G_1$ cells (1.25 hr), removed at 8.0 hr, and 270 ergs/mm² as a function of time after removal of the inhibitor. Open squares and closed triangles at the top of the graph, are the data for CH treatment only.

### *Effect of Inhibitors Added Prior to UVL*

Age response studies have established that the peak sensitivity of Chinese hamster cells to UVL is in the middle of the S phase. This suggests a role for DNA synthesis in controlling the sensitivity of the cell to UVL. Since there may be additional controlling factors, experiments were undertaken to evaluate the sensitivity of cells to

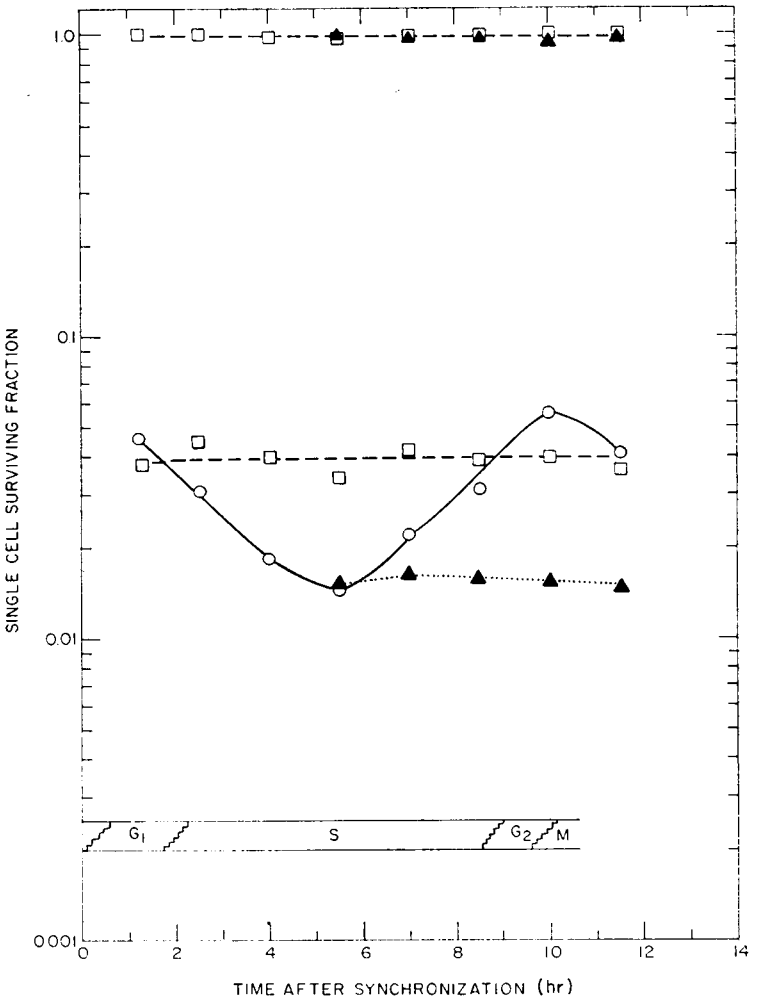


FIGURE 11 The effect of postirradiation treatment of $G_1$ and S cells with excess TdR. Open circles, cells exposed to 270 ergs/mm² at different times after synchronization; open squares, 270 ergs/mm² at 1.25 hr ($G_1$), 7.5 mM TdR added immediately after exposure, and removed as a function of time; closed triangles, the same as before, but 270 ergs/mm² delivered to mid-S cells (5.5 hr). Open squares and closed triangles at the top of the graph are the data for TdR treatment only.

UVL when various cellular processes were inhibited. Hydroxyurea (HU) and excess thymidine (TdR) were used as inhibitors of DNA synthesis, cycloheximide (CH) as an inhibitor of protein synthesis and actinomycin D (AcD), as an inhibitor of RNA synthesis. Suitable tests with labeled precursors were provided in each experiment to ensure that the drugs in the concentrations used produced inhibitory effects in the cells.

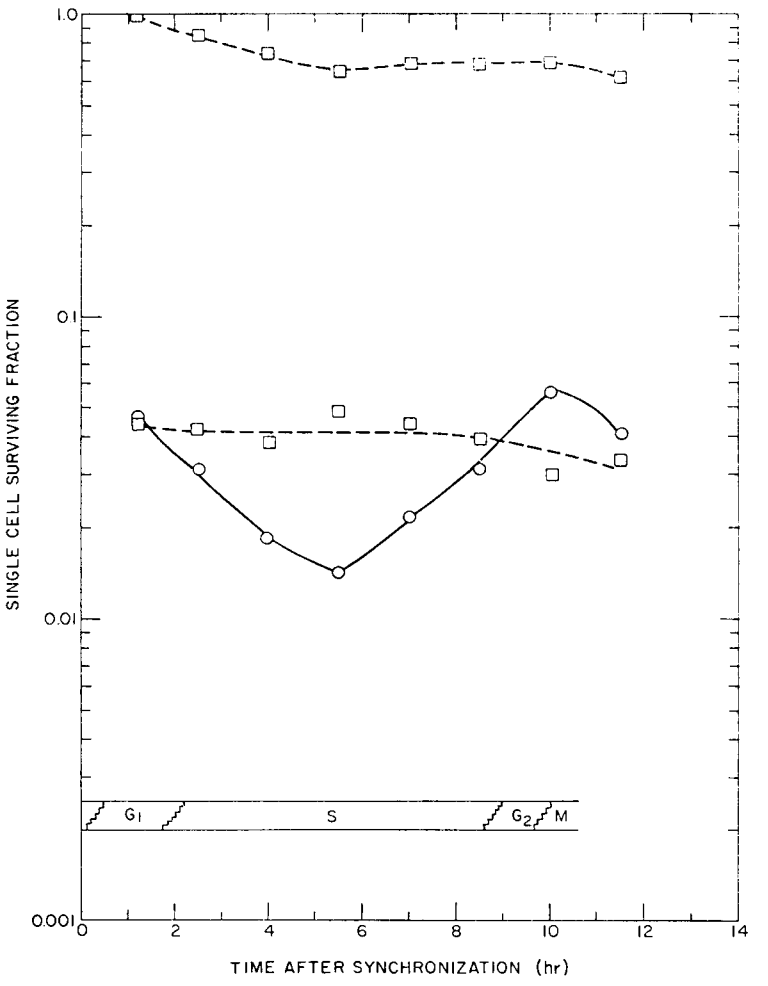


FIGURE 12 The effect of postirradiation treatment of $G_1$ cells with 1.0 mM HU. The same as Fig. 12, except that 1.0 mM HU was added instead, after $G_1$ cells (1.25 hr) were exposed to 270 ergs/mm$^2$.

Fig. 6 shows the results of an experiment in which 1.0 mM HU was added to the cells at 1.25 hr, i.e. when most of cells were in $G_1$. The few S cells present at this time were killed by the drug (23), and the rest were prevented from entering S phase, as demonstrated by failure of these cells to incorporate $H^3$-TdR. After the inhibitor was added, cells were irradiated with 270 ergs/mm$^2$ at different times, and the drug was removed immediately after exposure. The survival of HU-treated cells declined initially as did that of the untreated controls, leveled off for a period of 2–3 hr, and thereafter continued to decline. There was no subsequent rise. If the drug was removed from the cultures at 8.0 hr, DNA synthesis started rapidly, and survival, after an initial dip, increased significantly, even exceeding the survival level normally

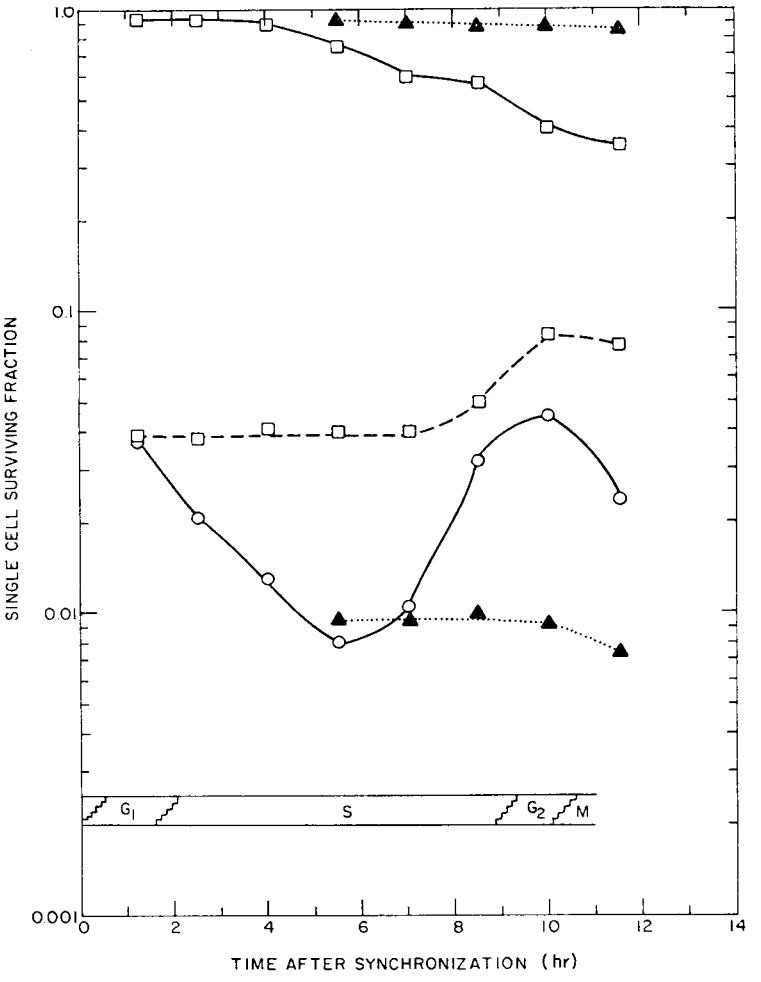


FIGURE 13 The effect of postirradiation treatment of $G_1$ and S cells with cycloheximide. The same as Fig. 12, but 5.0 $\mu$g/ml of CH was added after $G_1$ (1.25 hr) and mid-S (5.5 hr) cells were exposed to 270 ergs/mm$^2$.

seen for uninhibited cells. This higher maximum survival mainly is due to improved synchrony resulting from the drug treatment, and is also observed in experiments in which HU was used to improve the synchrony. Thus, after removal of HU, cells progress through the cycle in an almost normal fashion, delayed only for the length of time they were inhibited by HU.

To determine whether this response is the result of the inhibition of DNA synthesis or an effect specific to HU, and also to arrest DNA synthesis during S without killing cells, DNA synthesis was inhibited by a different agent, excess TdR. This

drug, when added at 7.5 mM to synchronized cultures, is not toxic and does not kill S cells. The absence of DNA synthesis in the presence of excess TdR is more difficult to establish, because $H^3$-TdR incorporation can not be applied. The method used here was the same as that already described elsewhere (24). The results showed that cells treated with excess TdR (7.5 mM), passed through DNA synthesis with a delay which corresponded to the length of the treatment.

The excess TdR treatment was applied to $G_1$ cells (1.25 hr) and to cells in the middle of S (5.5 hr). The purpose of the latter was to determine whether cells can still acquire the resistance normally seen in $G_2$ if DNA synthesis is arrested during S. The results are shown in Fig. 7. When the agent was added to $G_1$ cells (1.25 hr), the initial decline in sensitivity was not as great as with HU, but the eventual decline and overall pattern of response is very similar. No rise is seen at later stages of the "cycle". A small increase in survival, between 4 and 7 hr, may be due to the presence of a few S cells which were not completely inhibited.

The addition of TdR in the middle of S did not prevent the development of the resistance normally seen in uninhibited, irradiated cultures, although the development was somewhat slower.

In order to determine whether other changes would occur if other cellular processes were arrested in addition to DNA synthesis, cycloheximide (CH), an inhibitor of protein synthesis, was added to $G_1$ (1.25 hr) and S cells (6.0 hr). This agent, at a concentration of 5.0 $\mu$g/ml, reduces the rate of protein and DNA synthesis to a negligible value very rapidly, but RNA synthesis is reduced more slowly (24). Treated and control populations were exposed, subsequently, to 270 ergs/mm$^2$ at various times. As shown in Fig. 8, the response of $G_1$ cells during the first six hr was essentially the same as in the presence of excess TdR. However, later survival did not decrease, but either remained at the same level or showed a tendency to increase. When added in $G_1$, CH suppressed the peak in resistance normally seen at the end of the cycle. If the agent was added at 6.0 hours it decreased survival slightly, initially, and then survival leveled off.

In a similar experiment, actinomycin D (AcD), an inhibitor of RNA synthesis, was added at 1.25 or 5.5 hr. Cultures were treated with AcD, and subsequently received 270 ergs/mm$^2$ at various times. The results, presented on Fig. 9, show that AcD, added to either $G_1$ or middle S cells, affected the age response to only a small extent. The differences observed may be due solely to the toxicity of this agent.

In an additional experiment, CH was added at 1.25 hr, and the cultures were incubated until 8.0 hr, when the agent was removed. As a function of time after the removal of inhibitor, cells were exposed to 270 ergs/mm$^2$, and these results are shown in Fig. 10. Survival of temporarily arrested cells did not increase, but instead decreased slightly. Thus, while the effects of HU inhibition can be completely and rapidly reversed, the effects of CH inhibition were not reversed within the time period of this experiment.

*Effect of Inhibitors Added after UVL*

In order to determine whether any changes occur in survival, if different cellular processes are arrested immediately after irradiation, cultures were exposed to 270 ergs/mm$^2$, either at 1.25 or at 5.5 hr. Inhibitors of DNA synthesis (HU or excess TdR), or protein synthesis (CH), were added immediately after exposure, and removed as a function of time thereafter. Fig. 11, shows the results of the experiment, where excess TdR was given to cells immediately after exposure at 1.25 or 5.5 hr. In both cases, the survival did not change after exposure, but remained at the same level as that of the control for corresponding age. Essentially the same result was obtained if HU was applied at 1.25 hr (Fig. 12), or CH at 1.25 or 5.5 (Fig. 13). When CH was added to $G_1$ cells (1.25 hr), and kept for more than seven hr, survival started to increase. This rise coincides with the rise normally seen in untreated cultures at this time and might have been caused by a small number of cells which were not completely inhibited by the agent.

## DISCUSSION

The results for the UV sensitivity of synchronized Chinese hamster cells reported here are in good agreement with those of Sinclair and Morton (3) for the same cell line. There are, however, new and more complete data in the present report which establish the sensitivity of different phases of the cycle more precisely.

The following summarizes the UVL age response for lethality in Chinese hamster cells: cells are least sensitive in mitosis, more sensitive after division is completed and most sensitive in the middle of the DNA synthetic phase; sensitivity then decreases toward the end of the cell generation cycle. Cell sensitivity immediately after and just before division does not differ significantly. The rise in sensitivity after mitosis may occur as early as late telophase. (This is indicated from the data of Fig. 4, in which cell survival starts to decline somewhat earlier than the mitotic index.) It was not possible to determine if survival rises as sharply at the $G_2$ — M transition as it declines at the M — $G_1$ transition.

It was concluded from earlier work (3), that survival increases some time after DNA synthesis begins. The same observation has been made by others with other cell lines (2, 4). The results presented here show more precisely the timing of survival changes in Chinese hamster cells. Survival decreases until the middle of the S phase, and only begins to rise in the latter part of S, achieving a maximum at the end of the cycle.

The question arises—are survival changes gradual throughout $G_1$, S and $G_2$ (as they appear) or are there characteristic sensitivities associated with each of these stages (in which case the smooth age response results only from inadequate synchrony at each transition point)?

During the first half of the cycle the cell population consists of $G_1$ and S cells only and their proportion is available to us as a function of time from pulse labeling

with $H^3TdR$. This information plus simple algebra of the type presented earlier (equation 1 for two components only) indicates that the observed survivals after 270 ergs/mm$^2$ are consistent with a single value of survival for $G_1$ cells and another single value for S cells. The view that $G_1$ has a single characteristic sensitivity is also supported by the data of Djordjevic and Tolmach (5) in Hela cells, in which, during a long $G_1$ period of almost 12 hr, only minor changes in survival occurred. Later in the cycle of V79-S171 cells, mitotic cells become important and eventually the main contributor to the observed survival. Since we do not have measurements of mitotic index for cultures for which survival was assayed, we are unable to make reliable estimates of the sensitivity of $G_2$ cells at different times. The data of Table II indicate that the survival of $G_2$ cells increases with age, although this observation may result from the incomplete removal of mitotic cells. Thus, from these data it is not possible to determine whether $G_2$ has a characteristic sensitivity or whether there is a gradual increase in survival of V79-S171 cells from late S through $G_2$ to mitosis.

The age response data obtained here are in good agreement with those reported for Hela S3 cells by Djordjevic and Tolmach (5). In both cases, the results were obtained in cells attached to bottom of the dish during exposures, and by similar synchronization methods, i.e., collection of loosely attached mitotic cells. However, higher UVL exposures are needed to produce the same fluctuations in Chinese hamster cells that are observed in Hela cells. For example, to achieve the same difference in survival between $G_1$ and mid-S as observed in Hela S3 cells after 70 ergs/mm$^2$, a dose of 270 ergs/mm$^2$ has to be delivered to Chinese hamster cells. Hela S3 cells are apparently more sensitive to UVL than Chinese hamster cells, as is also the case for X-rays (20). Nevertheless, the age response patterns for these two cell lines are in good agreement.

The age responses for cell lethality after UVL, reported earlier for D98/AG cells (2) and for L cells (4), differ in some respects from that reported here for Chinese hamster cells. However, in the case of D98/AG cells, the difference may be more apparent than real. Inspection of the growth curve of D98/AG cells and the survival curves made at different times after FUdR reversal shows that cells are most resistant 8 hr after this reversal, which corresponds to the time when cells begin to divide. No specific measurements of the sensitivity of mitotic cells to UVL have been reported prior to this study, and as we have shown here, mitotic cells are very resistant to UVL. The decrease in sensitivity observed in D98/AG cells at the end of the cycle is probably due mainly to mitotic cells. Whether the mitotic cells of the other commonly used established cell lines display the same low UVL sensitivity must be demonstrated before a general conclusion can be reached. The UVL age responses for different cell lines reported so far, do not seem to be qualitatively different especially, with some allowance for difficulties in determining the duration of cell stages. The small differences observed may be explained by differences in experi-

mental conditions (e.g., cells in suspension vs. attached cells, methods of synchronization, etc.).

In microorganisms, there is strong evidence that DNA is a principal target for UVL. The mechanism of cell killing is comparatively well-known for these cells and in many instances their survival can be correlated with the existence of repair mechanisms (17, 25, 26, 27). There are also a number of indications that the DNA molecule could be the principal target for UVL in mammalian cells, e.g., survival pattern through the cycle (3, 4, 5), the effect of UVL on DNA synthesis (6, 7, 8, 9, 10, 14), action spectra for cell survival (28) and for chromosomal aberrations (12). Photochemical events leading to lethality in mammalian cells remain largely unknown, in spite of numerous investigations on the effects of UVL in these cells. The studies on the induction of thymine dimers, for example, indicate that this kind of damage is not as important in the survival of mammalian cells as in some bacteria. After a dose of several hundred ergs/mm$^2$, many dimers are produced (8, 9, 10), yet these cells survive. Excision of thymine dimers has been found in only one of the commonly used established cell lines (14). For many others, attempts to demonstrate the existence of an excision process have been unsuccessful (8, 10, 15). In bacterial cells, thymine dimers interfere with normal DNA synthesis, and this could be the mechanism of UVL-induced mutations (31). Lack of excision of thymine dimers in some cell lines may mean that mammalian cells in vitro, being diploid and polyploid, have many of their genes inactive and thus damage produced in some genes would not affect cell survival.

The results of the postirradiation application of inhibitors to $G_1$ and mid-S cells, indicate that postirradiation inhibition of either DNA or DNA and protein synthesis, does not affect cell survival but maintains it at a level characteristic of the age of the cells. These results are consistent with observations by Arlett in asynchronous Chinese hamster cells (32). Therefore, it would seem that either V79 cells do not have an enzymatic repair mechanism for UVL-induced damage or that these inhibitors do not prevent unscheduled DNA synthesis (33) from providing the necessary repair.

The dependence of UVL survival upon macromolecular synthesis, as shown here, is evidently complex. The acquisition of the S-period sensitivity by $G_1$ cells is not prevented by HU (as was also found in Hela cells (5) using FudR) or by AcD, but is prevented, at least partially, by TdR or CH. This suggests that the S-period sensitivity is not determined directly by DNA synthesis but rather by a process concomitant with it which cannot proceed in the presence of excess TdR or CH. However, the development of the $G_2$ resistance after S, was prevented by HU or TdR, partially by CH, and not at all by AcD, when these agents were added in $G_1$. Furthermore, this resistance was not prevented by TdR or AcD added in mid-S, but was prevented by CH added in mid-S. We can conclude, therefore, (1) that arresting RNA synthesis has no direct effect on UVL survival, (2) that arresting the onset of DNA synthesis may prevent the development of sensitivity in S (depending upon the method of

interrupting DNA synthesis) but always prevents the development of $G_2$ resistance, (3) that arresting DNA synthesis during S does not prevent resistance developing in $G_2$ but that arresting protein synthesis during S does.

These facts are consistent with the following: first, that it is not DNA synthesis but some process concomitant with it that is responsible for the sensitivity of S cells and, second, that $G_2$ resistance develops only if DNA synthesis has proceeded for at least part of S but this resistance can be prevented by a protein inhibitor added at the middle of S. The exact timing of events during S which control the development of $G_2$ resistance should be the subject of further investigation. Since both the development of S sensitivity and $G_2$ resistance can be prevented by a protein inhibitor, it is tempting to think of the processes involved being the formation of specific proteins.

Again, not inconsistent with this view, is the very low UVL sensitivity of mitotic cells, which may be related to their low metabolic activity. In addition to DNA, RNA synthesis ceases during mitosis (29, 30), and the rate of protein synthesis is slowed down (34) and thus biological damage may not be as readily expressed. Further evidence that proteins are involved in the UVL response is provided by the action spectra for mitotic spindle destruction and anaphase delay in microbeam irradiated cytoplasm. These spectra show that wavelengths considerably shorter and longer than 254 nm are more effective. Such wavelengths may be characteristic of specific proteins (31). In addition, Mitchison has recently reported[2] that, in phases other than S, the action spectrum for yeast inactivation has a peak of 280 nm rather than 254 nm, indicative of molecular involvement other than DNA. The foregoing is not necessarily inconsistent with DNA being the principal target molecule. In mammalian cells, at least, particular proteins may be concerned with the expression of damage to DNA.

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## REFERENCES

1. LEE, H. H., and T. T. PUCK. 1960 *Radiat. Res.* **12**:340.
2. ERIKSON, R. L., and W. SZYBALSKI. 1963. *Radiat. Res.* **18**:200.
3. SINCLAIR, W. K., and R. A. MORTON. 1965. *Biophys. J.* **5**:1.
4. RAUTH, A. M., and G. F. WHITMORE. 1966. *Radiat. Res.* **28**:84.
5. DJORDJEVIC, B., and L. J. TOLMACH. 1967. *Radiat. Res.* **32**:327.
6. CLEAVER, J. E. 1967. *Radiat. Res.* **30**:795.

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[2] Mitchison, J. M. 1968. Oral communication at the "Symposium on Control of Molecular Events During the Cell Cycle." Argonne National Laboratory, Argonne, Ill.

7. DOMON, M., and A. M. RAUTH. 1968. *Radiat. Res.* **35**:350.
8. TROSKO, J. E., E. H. Y. CHU, and W. L. CARRIER. 1965. *Radiat. Res.* **24**:667.
9. STEWARD, D. L., and R. M. HUMPHREY. 1966. *Nature.* **212**:299.
10. KLIMEK, M., and M. VLASINOVA. 1966. *Int. J. Radiat. Biol.* **11**:329.
11. HUMPHREY, R. M., W. C. DEWEY, and A. CORK. 1963. *Radiat. Res.* **19**:247.
12. CHU, E. H. Y. 1965. *Mutat. Res.* **2**:75.
13. RAUTH, A. M. 1967. *Radiat. Res.* **31**:121.
14. REGAN, J. D., J. E. TROSKO, and W. L. CARRIER. 1968. *Biophys. J.* **8**:319.
15. KLIMEK, M. 1965. *Neoplasma* **12**:459.
16. HAN, A., B. MILETIC, and D. PETROVIĆ. 1964. *Int. J. Radiat. Biol.* **8**:187.
17. HANAWALT, P. C. 1968. *In* Current Topics in Photophysiology. A. C. Giese, editor. Academic Press, Inc., New York. **4**:204–245.
18. ELKIND, M. M., and H. SUTTON. 1960. *Radiat. Res.* **13**:556.
19. SINCLAIR, W. K., and R. A. MORTON. 1966. *Radiat. Res.* **29**:450.
20. SINCLAIR, W. K. 1968. *Radiat. Res.* **33**:620.
21. ALPER, T., J. F. FOWLER, R. L. MORGAN, D. D. VONBERG, F. ELLIS, and R. Oliver. 1962. *Brit. J. Radiol.* **35**:722.
22. ELKIND, M. M., and W. K. SINCLAIR. 1965. *In* Current Topics in Radiation Research. M. Ebert and A. Howard, editors. North-Holland Publishing Company, Amsterdam. **1**:167–218.
23. SINCLAIR, W. K. 1965. *Science.* **150**:1729.
24. SINCLAIR, W. K. 1967. *Proc. Nat. Acad. Sci. U.S.A.* **58**:115.
25. SETLOW, R. B., and W. L. CARRIER. 1964. *Proc. Nat. Acad. Sci. U.S.A.* **51**:226.
26. BOYCE, R. P. 1964. *Proc. Nat. Acad. Sci. U.S.A.* **51**:293.
27. BOLLUM, F. J., and R. B. SETLOW. 1963. *Biochim. Biophys. Acta.* **68**:599.
28. TODD, P., T. P. COOHILL, and J. A. MAHONEY. 1968. *Radiat. Res.* **35**:390.
29. PRESCOTT, D. M., and M. A BENDER. 1962. *Exp. Cell Res.* **26**:260.
30. TAYLOR, J. H. 1960. *Ann. N.Y. Acad. Sci.* **90**:409.
31. BROWN, D. Q., and R. E. ZIRKLE. 1967. *Photochem. Photobiol.* **6**:817.
32. ARLETT, C. F. 1967. *Int. J. Radiat. Biol.* **13**:369.
33. RASMUSSEN, R. E., and R. B. PAINTER. 1966. *J. Cell Biol.* **29**:11.
34. SALB, J. M. and P. I. MARCUS. 1965. *Proc. Nat. Acad. Sci. U.S.A.* **54**:1353.