CHANGES IN REPAIR COMPETENCY AFTER 5-BROMODEOXYURIDINE PULSE LABELING AND NEAR-ULTRAVIOLET LIGHT

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ABSTRACT Synchronized V79 Chinese hamster cells, pulse-labeled with 5-bromodeoxyuridine (BrdUrd), show marked changes in the sensitivity to near-ultraviolet light during the cell cycle. Cells are least sensitive during the remainder of the S-phase after the BrdUrd pulse. They become maximally sensitive in the next cell cycle when the BrdUrd-labeled DNA presumably serves as the template for replication. This is followed by a return to relative insensitivity during the remainder of that S-phase. When BrdUrd is given both near the beginning and again near the end of the same S-phase, the increase in survival does not occur until DNA synthesis progresses beyond the time when the DNA made during the second pulse serves as a template. Furthermore, cells in the resistant phases of the cell cycle are sensitized by 1–2 mM caffeine. Survival curves are shown for the various cell ages of interest and are discussed in relation to the observed changes in functional repair capacity. The data support the hypothesis that lesions in the BrdUrd-containing DNA are effectively repaired after semiconservative replication. The data indicate that saturation of repair capacity and not target multiplicity is responsible for the appearance of a shoulder on these survival curves.

INTRODUCTION

This work examines the relationship in mammalian cells between the cell cycle phase and DNA damage under conditions that restrict that damage to a particular segment of the cell's genome. A preliminary account of this work has been presented earlier (Hagan and Elkind, 1977).

Ben-Hur and Elkind (1972*a*,*b*) found that moderate exposures to fluorescent light irradiation were lethal to V79 Chinese hamster cells only when the cells contained 5-bromodeoxyuridine (BrdUrd)-substituted DNA. Furthermore, cell killing was quantitatively related to the replacement of thymidine (dThd) by BrdUrd. In earlier studies, BrdUrd labeling for two or three generations was used. In the present work, short periods of BrdUrd labeling of synchronized V79 Chinese hamster cells were employed to sensitize a segment of the genome to near-ultraviolet light (NUV). This "pulse-labeling" technique allows the examination of the relationship between the cell-cycle position and cell killing and how the latter depends on functional repair processes.

A central problem in BrdUrd-mediated cell lethality has been the relative importance of bifilar vs. unifilar BrdUrd-labeling.[1] Djordjevic and Syzbalski (1960) reported a qualitative

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[1]The term bifilar refers to label in both strands of the DNA molecule whereas unifilar refers to single-stranded incorporation.

dependence of cell lethality upon the BrdUrd label configuration (bifilar vs. unifilar) for both ionizing and nonionizing irradiation of cells obtained from human sternal explants. However, evidence from bacterial survival measurements (Kaplan et al., 1962; Opara-Kubinska et al., 1961) and mammalian-cell cytogenetic data (Dewey and Humphrey, 1965) have not supported such a dependence. A recent report (Roufa, 1976) involving a study with Chinese hamster cells has also been interpreted as indicating a qualitative difference between the survival responses for bifilar and unifilar BrdUrd labeling. The use of BrdUrd pulse labeling in the present work permits the identification of sensitive or resistant cell-cycle phases that may account for the BrdUrd/NUV sensitivity differences in mammalian cells heretofore attributed to unifilar vs. bifilar labeling.

Cells containing BrdUrd-substituted DNA are killed by NUV light very likely because of DNA-associated lesions (Ben-Hur and Elkind, 1972*a*,*b*; Newman and Kubitschek, 1978; see also review by Hutchinson, 1973). The use of BrdUrd pulse-labeled synchronized cells not only restricts this lethal damage to the DNA, but also limits the damage to the segment of the DNA (and to a lesser extent its complementary strand) that is BrdUrd-substituted.

MATERIALS AND METHODS

Cell Culture

V79 Chinese hamster cells (Elkind and Sutton, 1960) were grown at 37°C in 2% CO_2 in Eagle's Minimum Essential Medium buffered with 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (Hepes) and one-third of the $NaHCO_3$ concentration normally used in Earle's saline. The medium was supplemented with 15% fetal calf serum and modified as described by Stanners et al. (1971) except that nucleosides were omitted. Dishes were routinely inoculated after cell synchronization, irradiated, and allowed to grow for 7–8 d for colony formation. The medium was not changed during incubation for colony formation. The cell inoculum was adjusted to produce 100–300 colonies per 100-mm Falcon tissue culture dish (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). Synchrony, the selective retention of cells in the late G_1-phase to early S-phase, was produced by the incubation of asynchronously growing cells for 3.5 h in growth medium containing 2 mM hydroxyurea (HU) (Sinclair, 1965), or by the selection of mitotic cells (as previously described by Sinclair and Morton, 1966) followed by their incubation in the HU medium for 3 h. After removal of the HU, the cells were rinsed with warm growth medium, and warm growth medium was added for further incubation.

Drugs

BrdUrd and 5-fluorodeoxyuridine (FdUrd) were purchased from the Calbiochem Corp. (San Diego, Calif.). Caffeine (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of either 1 or 2 mM in growth medium. After administration, caffeine remained in the medium during the incubation period for colony formation.

Mitotic Index

Cells, swollen by suspension in growth medium diluted 1:5 with glass-distilled water, were fixed on glass slides with Carnoy's solution. Slides were then stained with Giemsa, mounted, and scored. 500 cells were counted per data point.

NUV Light Irradiation

Cells attached in Falcon plastic dishes were exposed at 44 cm from a bank of 4 FS-20 Westinghouse tubular Sun Lamps (Westinghouse Electric Corp., Pittsburgh, Pa.). During irradiation, the dishes were rotated on a "lazy Susan" apparatus to expose the cells uniformly. The beam was filtered through the

cover of a 100-mm Falcon dish (0.975 mm of polystyrene). All exposures were at an exposure rate of 2.07 J $m^{-2}s^{-1}$ as determined with a Hilger-Watts thermopile (model FT 17.1, Hilger and Watts, Ltd., London, England) and a Keithly microammeter (model 150 B; Keithly Instruments, Cleveland, Ohio).

Relative DNA Synthesis

Cells were labeled overnight (16–17 h) with 0.1 μCi/ml of [^{14}C]dThd, 57 mCi/mmol (Schwarz/Mann Div., Becton, Dickinson & Co.). Mitotic cells were then selected (Sinclair and Morton, 1966) and inoculated onto coverslips in a 100-mm dish. The mitotic selection medium, containing 2 mM HU, was removed after 3.5 h and cells were returned to 37°C in fresh growth medium. At various times coverslips were removed, immersed in 37°C growth medium containing [^{3}H]dThd (20 μCi/ml, 6 Ci/mmol) for 15 min, and then transferred directly to a lysis solution. This solution consisted of 0.3 M NaOH, 0.01 M EDTA, 1.0 M NaCl, and Brij 58 (Honeywill-Atlas, Atlas Chemical Industries, Inc., Wilmington, Del.) added to a final concentration of 0.5%. After lysis at 4°C for 1 h, samples were neutralized with HCl, precipitated with trichloroacetic acid (TCA) onto glass fiber filters, and the radioactivity was counted in a toluene-based scintillation fluid. Relative rates of DNA synthesis were expressed as the ratio of counts per minute of ^{3}H to ^{14}C, as shown in Fig. 1.

DNA Extraction

Cells were first suspended in a buffer containing 0.1 M Tris, 0.01 M EDTA, and 0.2 M NaCl. Cells were then adjusted to a concentration of 1×10^6 cell/ml and lysed in a detergent mixture of 2% Sarkosyl (Geigg Chemical Corp., Ardsley, N. Y.) and 0.5% Brij 58 for 30 min at 37°C. The lysate was digested at 37°C for 30 min in 0.05 mg/ml RNAse (the RNAse had been preheated for 20 min at 80°C) and then digested for 60 min in pronase (self-digested) at 0.1 mg/ml. NaCl from a 5.0-M stock was added dropwise to produce a final concentration of 1.0 M. This solution was shaken for 1 h and then extracted with isoamyl alcohol:chloroform (1:24) for 30 min at room temperature. The mixture was centrifuged at 10,000 *g* to separate the phases. The aqueous layer was then removed and the extraction steps were repeated until no visible material was present at the interface. The final aqueous layer thus obtained was dialyzed for 60 h against repeated changes of 0.05 M phosphate buffer, pH 7.0.

Cesium Chloride Gradients

DNA, extracted in phosphate buffer as above, was sheared by successive passages through a 22-gauge syringe needle. 1 ml of the DNA solution was then added to 3.80 g of CsCl (Beckman Instruments, Fullerton, Calif.). After agitation, the solution was poured into a 5-ml polyallomer tube and centrifuged in a SW 50.1 rotor (Beckman Instruments, L5-50 ultracentrifuge). Centrifugation at 33,000 rpm was performed at 20°C for 65 h. After centrifugation, gradients were fractionated by metered pumping from the bottom of the centrifuge tube through 20-gauge needles. Fractions of 100 μl each were collected on strips of Whatman 17 chromatographic paper (Whatman Inc., Clifton, N. J.), precipitated with 5% TCA, and washed twice in 95% ethanol. After drying, the paper strips were cut into 2-cm lengths, placed in scintillation vials, and the radioactivity was counted in a toluene-based scintillation solution.

RESULTS

For cells pulse-labeled with BrdUrd, the cell cycle dependency of NUV survival was determined in the following manner. Mitotic cells were accumulated at the G_1/S border by incubating them for 3.5 h in medium containing 2 mM HU. They were then incubated for 1 h in medium without HU. Following this, 1×10^{-7} M FdUrd was added to the growth medium for 15 min to inhibit *de novo* dThd production. BrdUrd was also added to a concentration of 1×10^{-4} M for 45 min. The latter medium was then removed, and after several rinses with warm growth medium the cultures were returned to 37°C in medium containing 1×10^{-5} M dThd.

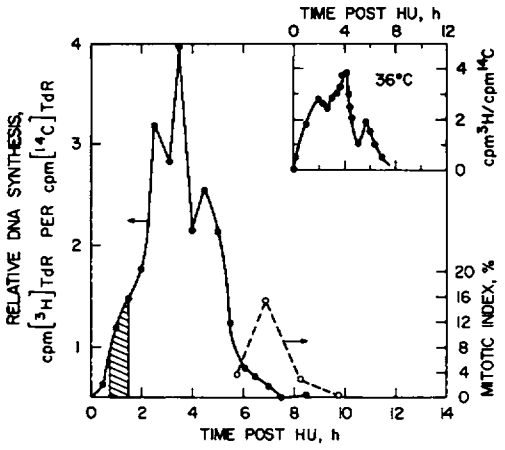

FIGURE 1

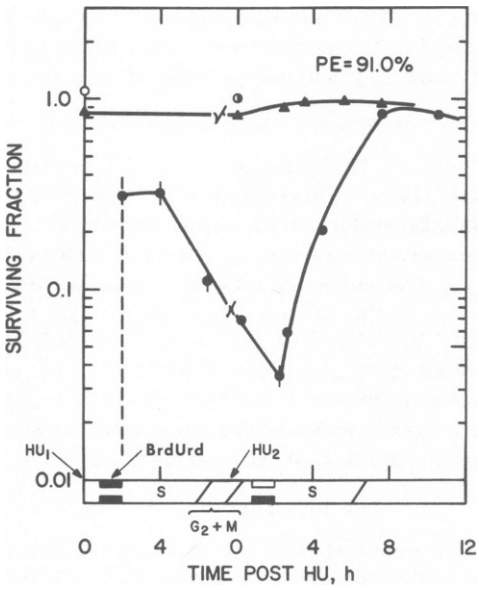

FIGURE 2

FIGURE 1 The mitotic index and relative DNA synthesis were measured in V79-Al885D cells as a function of time at 37°C after the removal of HU. Cells were mitotically selected before the HU exposure. To verify the pattern of relative DNA synthesis, an identically treated culture was incubated at 36°C to lengthen the S-phase (inset). The cross-hatched area represents the approximate time of BrdUrd labeling used in the experiments to follow.

FIGURE 2 The NUV age-response pattern of BrdUrd pulse-labeled V79-AL162 Chinese hamster cells. Cells, synchronized with HU_1, were BrdUrd labeled (●) or not (▲), as described in the text. Cells were irradiated with 1.25 kJ/m² of NUV light at various times after synchronization. The break in each curve represents the time of resynchronization by HU administration (HU_2) and the subsequent respreading of cells. Survival has been normalized to account for the toxicity of the first HU exposure. Other symbols shown are for BrdUrd labeling and no NUV (○) and BrdUrd plus resynchronization and no NUV (◑). The double closed bars indicate the period of BrdUrd labeling. The open bar indicates the corresponding time in the next cell cycle. Uncertainties, one standard error, are shown if larger than the data point. PE denotes plating efficiency.

Neutral isopycnic sedimentation of the DNA from G_2-phase cells, BrdUrd-labeled in the above manner, demonstrated that the BrdUrd was limited to a small fraction of the genome. For these experiments, cells were labeled overnight with [^{14}C]dThd (0.1 μCi/ml, 57 mCi/mmol), synchronized, and BrdUrd-labeled as above. Although 3–5% of the genome was shifted to a density of 1.758 g/cm³, the density of >90% of the genome remained unchanged (i.e., a density of 1.700 g/cm³). Under the same conditions, but with [^{3}H]BrdUrd used as the density label, >80% of the tritium label appeared in a separate peak with a density of 1.758 g/cm³. The data of Luk and Bick (1977) for the density shift of essentially totally BrdUrd-substituted DNA from Chinese hamster cells suggests that the BrdUrd replacement of the hybrid material was >90% when semiconservative DNA synthesis is assumed. When 1×10^{-4} M BrdUrd and 1×10^{-7} M FdUrd remained in the growth medium for 7 h, >95% of the DNA was shifted to the hybrid density. The remaining 5% of the DNA carried no density label.

The NUV survival of the BrdUrd pulse-labeled cells was then addressed. Fig. 2 shows the variation in survival, the age-response pattern, in the remainder of the first cycle, and the second cycle for cells labeled with BrdUrd and then exposed to a fixed dose of NUV at various times thereafter. The phases of the cycle were located from the results shown in Fig. 1 and earlier data for this cell line (Sinclair and Morton, 1966). Survival remained constant for part of the first S-phase and then decreased through the G_2- and M-phases to a minimum value in the next cell cycle. To improve the synchrony in the second cell cycle, cells were held near the G_1/S border by a second treatment with HU (see legend, Fig. 2). A minimum in survival occurred 2 h after release from this block, a time equivalent to 10.5 h after cells were released from the first G_1/S block. An increase in survival began at this point and continued through the remainder of this S-phase.

Subsequent to the experiment in Fig. 2, the cell line was recloned in an effort to develop a population with improved synchronization properties. The recloned cells, designated V79-AL885D, were treated in the manner already described except that a second HU block was omitted, and to obtain the same degree of BrdUrd substitution, the FdUrd concentration had to be increased to 2×10^{-6}M. The age-response pattern of V79-AL885D cells (Fig. 3) is qualitatively the same as that described for V79-AL162 cells (Fig. 2). However, with the second G_1/S block omitted, it was possible to detect survival changes in G_1. Furthermore, fine

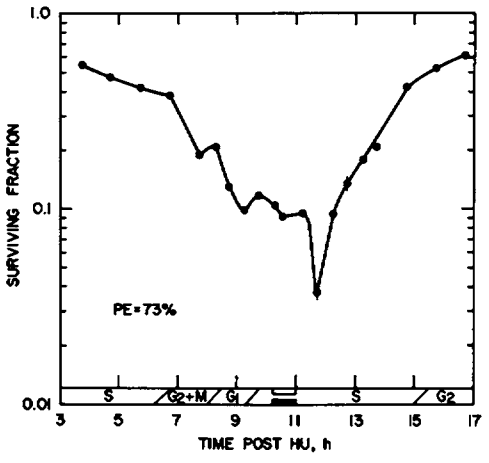

FIGURE 3

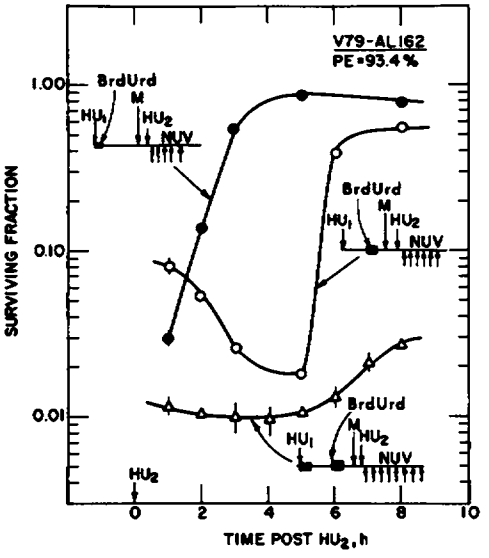

FIGURE 4

FIGURE 3 The NUV age-response pattern of BrdUrd pulse-labeled V79-AL885D Chinese hamster cells. Cells, synchronized by mitotic selection into medium containing HU, were BrdUrd-labeled as described in the text. As a function of the incubation time they were irradiated with 1.0 kJ/m^2 of NUV light. Other details are as for Fig. 2. PE denotes plating efficiency.

FIGURE 4 The effect of the time of the BrdUrd pulse on the age-response pattern. Cells were labeled with BrdUrd as illustrated schematically in the figure (see text). After resynchronization with hydroxyurea (HU_2) and respreading, cells were irradiated with 1.5 kJ/m^2 of NUV light at the times indicated. M indicates the approximate time of mitosis. Other details are as shown in Fig. 2. PE denotes plating efficiency.

structure, such as the approximately twofold survival increase at mitosis, was apparent that was not detected with V79-AL162 cells, probably due to the improved synchrony.

An examination of the results in Figs. 2 and 3 permits the identification of the following cycle-specific characteristics. Both sets of data show that immediately after BrdUrd labeling, cells exhibited only a modest degree of sensitivity to NUV with little or no change during the first S-phase. For irradiation toward the end of this latter period, survival started to decrease and continued to fall until G_1 of the next cycle. The small increase in survival at the G_1/S border was consistently observed in several experiments. Although, as shown in Fig. 3, a sharp minimum was occasionally observed just after the time when the BrdUrd pulse would be copied, this was observed in only two of six experiments. In all six experiments, however, the lowest survival was observed immediately after DNA containing the BrdUrd pulse would be expected to be serving as a template. Subsequent to this minimum, survival increased progressively during the remainder of the second S-phase.

To examine the inferred connection between the copying of the BrdUrd-containing DNA and minimum survival, the position of the pulse in the S-phase was shifted. As the inserts in Fig. 4 indicate, three parallel cultures were synchronized with HU (HU_1), pulse-labeled with BrdUrd as shown, synchronized a second time at the next G_1/S border (HU_2), respread to yield single cells, and then irradiated with a fixed dose of NUV at the times shown. Hence, the age-response patterns shown extend from the point of resynchronization through the S-phase of the first complete cell cycle after BrdUrd labeling. Two cultures received single BrdUrd pulses at either early or late S-phase and the third culture was labeled at both times. Both single-pulse curves demonstrate that the increase in survival occurs immediately after synthesis upon the BrdUrd-substituted DNA template. In the case of the two-pulse protocol (triangles), survival did not start to increase until after the growing point would have been expected to pass the second pulse.

The single-dose survival variations described in Figs. 2 and 3 were examined more completely by measuring survival curves at selected times. Fig. 5 shows survival curves at three times after synchronization. The curve at 4.5 h corresponds to the relatively high single-dose survival observed in the S-phase after BrdUrd labeling. At 9.0 h, the survival curve corresponds to the G_1 period of the second cycle, where the shoulder is lost. The curve at 12 h corresponds to the ascending single-dose survival in the second S-phase after the DNA growing point has passed the template containing BrdUrd. The "tails" on the survival curves show sensitivities about the same as those of unlabeled cells and hence most likely represent small fractions of cells not adequately labeled with BrdUrd, if at all.

Because the tails in Fig. 5 make uncertain the estimation of survival curve parameters, additional survival curve data were obtained with cells synchronized by the combined application of mitotic selection plus HU. As shown in Fig. 6, at 4.5 h after the removal of the HU, the survival curve parameters of both sublines are essentially the same (see legend). The remaining two curves in Fig. 6 are for V79-AL885D cells exposed either at 60 or 80 min after a second HU synchronization, 8 h after the first. Clearly, the single-dose sensitivity of these cells in Fig. 3 reflects a survival curve with a small shoulder and a steep final slope. Curves for 60 and 80 min after a second synchronization should correspond to just before and during the copying of the DNA template containing BrdUrd. Hence, little or no change occurs during this period, although the tail on these curves suggests that the synchrony was not perfect. The implications of these data are discussed later.

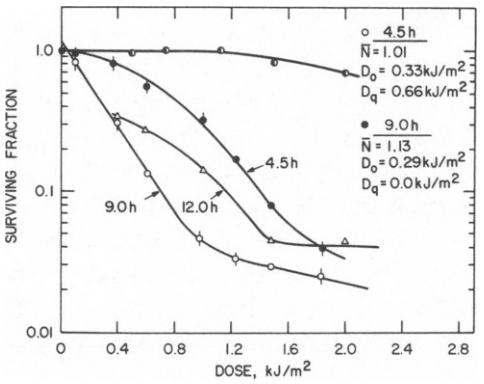

FIGURE 5

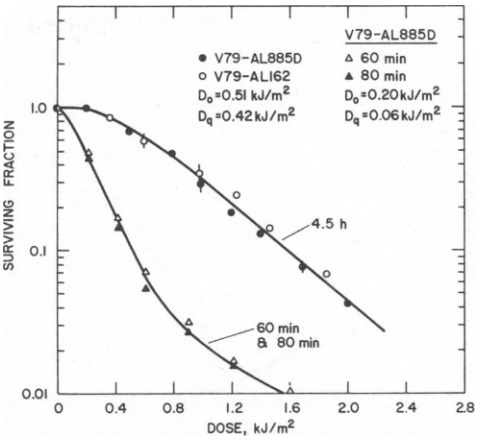

FIGURE 6

FIGURE 5 Cell survival curves for the BrdUrd pulse-labeled V79-AL162 Chinese hamster cells exposed to NUV at: (●) 4.5 h after synchrony; (○) 9.0 h after synchrony; (△) 12.0 h after synchrony. These cultures were administered BrdUrd as described for Fig. 2. Also shown is the NUV survival curve for an asynchronous population of cells receiving no BrdUrd (○). D_0 is the dose required to reduce the survival by a factor of 1/e on the exponential portion of the survival curve. D_q, the quasi-threshold dose, represents the extrapolated dose at which the exponential portion of the survival curve intercepts unity survival. This value is then corrected for cell multiplicity as previously described (Elkind and Whitmore, 1967). $\bar{N}$ is the cell multiplicity at the time of irradiation.

FIGURE 6 NUV survival curves of BrdUrd pulse-labeled V79 Chinese hamster cells synchronized by mitotic selection into HU. Circles represent the survival of the two V79 sublines irradiated at 4.5 h after HU removal. The triangles represent survival curves at 60 and 80 min after a second HU synchronization. The target multiplicity, exp (D_q/D_0), corresponding to the data at 4.5 h is 2.3; at 60–80 min after the second synchronization, the target multiplicity is 1.3. Other details are as for Fig. 5.

The cells irradiated at 12.0 h after the removal of HU, shown in Fig. 5, comprised two subpopulations (in addition to the tail). The initial portion of the curve appears to follow that at 9.0 h and thereafter a shoulder region is evident. Since these cells were not resynchronized, the cell age of 12.0 h corresponds to 3–4 h after the second HU synchronization in Fig. 2. Hence, in Fig. 2 the increase in cell survival from 3 to 8 h after resynchronization results from the development of a moiety whose survival curve has a shoulder region approximately equivalent to that of the 4.5-h survival curve.

Thus, there are two cell-age intervals, after BrdUrd labeling, in which the cells exhibit relative resistance to NUV exposure. In both instances, the DNA replication fork would have passed the BrdUrd region. This suggests that the shoulders of the survival curves in question represent the effect of a repair process occurring after DNA replication.

Accordingly, we examined the influence of posttreatment with caffeine on survival (for a review of caffeine studies, see Timson, 1977). As shown in Figs. 7 and 8, the presence of caffeine does enhance cell killing. However, the NUV age-response pattern retains the same general shape as that observed in the absence of caffeine. Additionally, when 2 mM caffeine was added, the survival minimum appeared to have been delayed for $\simeq$ 2 h. The caffeine effect on the survival curve at 4.5 h postsynchronization, shown in Fig. 8, illustrates the specific survival curve parameters affected by caffeine. Whereas caffeine did decrease the dose required to reduce the survival by a factor of 1/e on the exponential portion of the

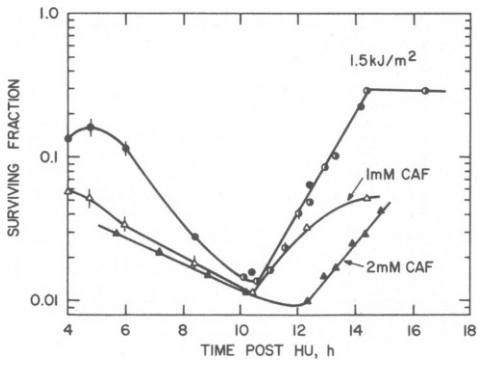

FIGURE 7

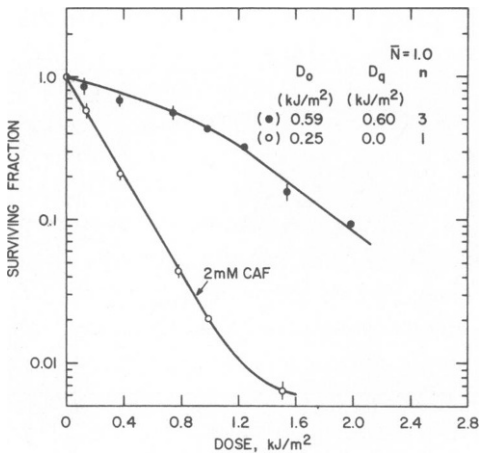

FIGURE 8

FIGURE 7 The effect of caffeine (CAF) on the NUV age-response pattern of V79-AL162 Chinese hamster cells pulse-labeled with BrdUrd. Cells labeled as in Fig. 2 and a repeat of the same experiment (●, ◑). Cells treated as above except that 1 (△) or 2 mM (▲) caffeine was added immediately after irradiation.

FIGURE 8 Caffeine effects upon the S-phase survival curve of BrdUrd pulse-labeled V79-AL162 Chinese hamster cells. Cell were synchronized and labeled as in Fig. 4 and irradiated in S-phase (4.5 h after HU removal). Control cells (●); cells treated with 2 mM caffeine after irradiation (○). The symbol $\bar{n}$ is the target multiplicity. All other symbols are as indicated in the legend of Fig. 5.

survival curve (D_o) from 0.59 to 0.25 kJ/m^2, the quasi-threshold dose (D_q) decreased from 0.6 kJ/m^2 to $\simeq$ 0. From these curves and several repeats of the experiment, we may state that the D_o value in the presence of caffeine (1–2 mM) is 0.27 ± 0.02 (SE) kJ/m^2 and that the D_o measured in the absence of caffeine is 0.55 ± 0.06 (SE) kJ/m^2. However, the principal effect of caffeine is the large reduction in the shoulder of the curve.

DISCUSSION

Cells, BrdUrd-labeled over only a portion of their genome, experience large variations in NUV sensitivity as a function of cell age. These cells, unifilarly substituted, are minimally sensitive to NUV immediately after the incorporation of the BrdUrd. Their ability to survive a constant dose of NUV, however, may decrease by 30–50-fold when the irradiation is delayed until the time of semiconservative synthesis upon the BrdUrd-substituted template. The likelihood that the foregoing is correct was supported by the observation that when the BrdUrd label was shifted to late S-phase, there was a corresponding shift in the period of NUV sensitivity. Additionally, the cell cycle-dependent changes in NUV sensitivity were largely eliminated when BrdUrd pulses were administered both early and late in S-phase. The two-pulse exposure reduced the cell-cycle dependency in the subsequent cycle by maintaining a high sensitivity through the portion of S-phase between the two pulses. The residual small degree of cycle-dependent cell killing observed in the two-pulse experiment is consistent with the almost complete lack of a cycle dependency for continuous, uniform BrdUrd labeling (Ben-Hur and Elkind, 1972*a*).

From these observations it is possible to account for apparent qualitative differences in sensitivity attributed to strand-symmetry of the BrdUrd label (i.e., unifilar vs. bifilar labeling). In the earlier work unifilar labeling was followed immediately, or after a short period of incubation, by the UV (Djordjevic and Szybalski, 1960) or NUV exposure (Roufa, 1976). For an asynchronous culture, this protocol produces two qualitatively distinct cell populations. One component of the population would consist of S-phase cells, which, having just incorporated BrdUrd, would be relatively photo-resistant. The second component would consist of cells that had aged into G_2-, M-, G_1-phases during the labeling and subsequent chase periods. From the data shown in Figs. 2 and 3, we would expect these latter cells to become progressively photo-sensitive. The corresponding survival curves would be of the biphasic type, e.g., as traced by the open triangles in Fig. 5. The latter curve is similar in shape to the survival curves reported by Djordjevic and Szybalski (1960) and by Roufa (1976). Hence, what has been attributed by these authors to qualitative differences in survival due to unifilar vs. bifilar BrdUrd labeling might in fact reflect only a cell age-dependent variation in sensitivity. As already noted, cells in which essentially the entire genome is labeled with BrdUrd (i.e., bifilarly labeled DNA) yield a survival curve lacking a shoulder (Ben-Hur and Elkind, 1972*a*), primarily because some part of the DNA containing BrdUrd in S-phase cells is in the vicinity of a growing fork. From the data shown in Figs. 3 and 4, cells in the other cycle phases would be inherently quite photo-sensitive after uniform BrdUrd labeling.

The appearance and disappearance of the shoulder on the NUV survival curve of BrdUrd pulse-labeled cells also has implications for the molecular basis of the sensitivity variation through the cell cycle. In qualitative terms, a shoulder-type curve could mean that sublethal damage must be accumulated for cell killing because of a multiplicity of target sites in each cell (Elkind and Whitmore, 1967). In these experiments these sites would presumably correspond to the BrdUrd-labeled DNA segments. Alternatively, a threshold-type curve could result from the repair of lesions that are potentially lethal (for a review see Alper, 1977). In this case, as the amount of substrate (i.e., the number of lesions) increases with dose, the repair system becomes progressively saturated. As a result, cell killing starts and approaches a limiting rate with increasing dose. This saturating dose is set by the enzymatic activities of the repair system (constitutive and induced). In consideration of these models, the first change in target multiplicity occurs at the mitosis after label incorporation. Here, the BrdUrd sites should segregate symmetrically (chromatid exchanges notwithstanding), and thus the sites should decrease by a factor of two. Because of this, one might expect the shoulder width of the survival curve to change appreciably (i.e., twofold) at mitosis. Although the data in Fig. 6 show that the apparent target multiplicity decreases by approximately this factor during the cell cycle (see legend), this decrease occurs not at mitosis but gradually from late S-phase in the cycle of the BrdUrd administration to the S-phase of the following cycle. In addition, the difference in final slopes of the curves in Fig. 6 indicates that the cyclic change in response involves more than simply a change in target multiplicity. The hypothesis that target multiplicity does not account for the observed changes in survival is strengthened by the cell survival values observed during synthesis beyond the BrdUrd region of the template. Here the number of BrdUrd sites per cell remains essentially unchanged. The amount of BrdUrd per genome, however, is roughly halved. Notwithstanding, the shoulder returns to the survival curve. This behavior is not predicted by target theory considerations.

Saturation of a repair system offers a more plausible explanation of the survival curve changes observed. In this case the loss of the shoulder at the time of DNA synthesis would reflect a diminished repair capacity. This lower repair capacity could result from the production of a lesion whose toxicity is specifically related to the proximity of the growing fork. The specificity of BrdUrd and light effects for DNA replication has been previously shown both by increased sister chromatid exchanges (Kato, 1974) and by aberrant DNA initiation (Povirk and Painter, 1976). Conversely, the return of the shoulder upon synthesis beyond the last BrdUrd site would result from the passage of the growing fork.

The hypothesis of a DNA repair mechanism operating at the four-stranded stage is supported by the caffeine data. Both the age-response pattern (Fig. 7) and the survival curves (Fig. 8) demonstrate the ability of caffeine to enhance cell killing during the periods of NUV resistance. Additionally, the survival curve shoulder, suggestive of a repair capacity as noted above, is eliminated by caffeine added to the postirradiation incubation medium. (Clearly, target multiplicity changes cannot account for the caffeine effect.) Furthermore, because caffeine is effective at times greater than 4 h after passage of the growing fork, an inhibition of repair is indicated. This inhibition of repair is most likely indirect (Goth and Cleaver, 1976).

Because the resistance to BrdUrd/NUV was observed after replication past the BrdUrd site, it seems reasonable to hypothesize that repair of the BrdUrd/NUV lesion is facilitated by a four-stranded substrate. If valid, this hypothesis can only offer a partial explanation of the age-response pattern. Both the sharp increase in NUV sensitivity observed in late S-phase (Fig. 5) and the small increase in survival observed in the G_1 or early S-phase (Fig. 5) are consistently observed from experiment to experiment and are yet to be explained.

Because the exposure of BrdUrd-substituted DNA to NUV irradiation produces a number of distinct molecular lesions (reviewed by Hutchinson, 1973), it is not possible to relate our survival measurements to a specific lesion. The cell cycle dependency of the repair of specific molecular lesions will be addressed, however, in a separate work.

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