CELL KILLING BY ACTINOMYCIN D IN RELATION TO THE GROWTH CYCLE OF CHINESE HAMSTER CELLS

M. M. ELKIND, E. KANO, and H. SUTTON-GILBERT

From the Laboratory of Physiology, National Cancer Institute, Bethesda, Maryland 20014.
Dr. Elkind and Dr. Kano's present address is Biology Department, Brookhaven National Lab., Upton, Long Island 11973

ABSTRACT

Using Chinese hamster cells in culture, we have measured the effectiveness of actinomycin D to suppress division as a function of the position, or *age*, of a cell in its growth cycle. Cells were first exposed to millimolar concentrations of hydroxyurea in order to produce a synchronized population just before the onset of DNA synthesis. Thereafter, the survival response after 30 min exposures to actinomycin D was measured. Cells become resistant as they enter the S phase and then sensitive again in the latter part of S. When they reach G_2 (or G_2-mitosis) they are maximally resistant; at 1.0 μg/ml, for example, the survival in G_2 is 30-fold greater than it is in G_1. These results, plus measurements reported earlier on the interaction of damage in S cells due to actinomycin D and X-irradiation, suggest that the age-response pattern of the toxic effects of this drug probably reflects both the functional capacity of DNA-actinomycin complexes and the ability of this antibiotic to penetrate chromatin and bind to DNA.

INTRODUCTION

To study the biology of cell growth and division, an indirect approach often may be advantageous. Thus, inhibitors of growth processes may be used and, if they have fairly discrete effects and if their modes of action are fairly well understood, insights relative to the biology of normal states may be developed.

In connection with the growth and division of mammalian cells, we have examined the mechanism of division suppression by ionizing radiation (1), and more recently, by radiation plus certain drugs. Our results with radiation and actinomycin D have been particularly illuminating, first, because this antibiotic is known to bind preferentially to DNA in cells (2–5), and second, because we found that, at least in cells undergoing DNA synthesis, damage resulting from exposure to actinomycin D interacts with lethal (6) and sublethal radiation damage (7, 8). These studies support the conclusion that in mammalian cells a lesion at the level of DNA is mainly responsible for the loss of sustained proliferative ability (6, 7) even though cell metabolism resulting in overall cell growth continues for one to a few generations (see chap. 7 of ref. 9). The detailed nature of this damage involving DNA which leads to cell killing remains, however, unknown; therefore, an insight into the integrity of DNA consistent with function also remains unknown.

In view of the similar actions of actinomycin D and radiation (at least in cells undergoing DNA synthesis), we examined the dose-dependent kinetics of cell killing, due to drug action alone, and its variation through the growth cycle of Chinese hamster cells. (Similar studies relative to the variation of radiation-induced cell killing through the cycle of Chinese hamster cells have been reported [10].) We present here our results with this antibiotic and we will report elsewhere a detailed comparison between cycle-dependent

killing due to actinomycin D, radiation, and the bifunctional alkylating drug nitrogen mustard when these agents are used singly and together.

MATERIALS AND METHODS

General

Our results were obtained with a clonal line of Chinese hamster cells, designated *V79-753B*, which we originally derived from line *V*, a cell line from female lung tissue placed in culture by Ford and Yerganian (11). When growing while attached to glass or plastic, these cells double in number in 8–9 hr, have a fibroblast-like morphology, and produce tight, dense colonies ~2 mm in diameter in 8 days (untreated controls). The cells obtained from us by Robbins and Scharff (12), which have at most a very short period, G_1, after mitosis and prior to DNA synthesis, may or may not have been derived from *V79-753B*; still, the latter cells probably have a short G_1 period in view of their rapid doubling rate. Since colonies grew from single cells with close to 100% efficiency, the doubling time is close to the average generation time.

Our cell culture techniques have been previously described (13, 14) and are only briefly outlined here. Using a modified Eagle's medium (15) to which 15% fetal calf serum was added, we plated cells in appropriate numbers (e.g. from 2×10^2 to about 6×10^4) in 9 cm petri dishes and usually incubated them overnight (in a CO_2 incubator at 37°C) before an experiment was started. This was done to help insure that the distribution of cells throughout their cycle (i.e. the *age-density* distribution) was close to that corresponding to asynchronous, log-phase growth and hence was fairly reproducible from experiment to experiment. As a result of this overnight period, cell multiplicities increased from one to about three and, consequently, experiments were started with populations of microcolonies rather than single cells. After a given treatment sequence, cells were incubated long enough (up to 16 days) to obtain a maximum yield of colonies. The latter were stained with methylene blue (13) and counted.

Our results are expressed as the fraction of cells surviving a given treatment relative to the number of untreated control cells initially plated; standard errors in survival are shown where they are larger than the data points as plotted. In the analysis of the data, cell multiplicities greater than one are easily accounted for (see ref. 9), and for surviving fractions below ~0.2 they have the effect of simply increasing survival in proportion to multiplicity. Further details specific to particular experiments are noted in the legends.

Synchronization

Most of the results relate to responses at specific ages as a cell progresses through its growth cycle. To measure age-specific responses, we used cells synchronized by an hydroxyurea method. Hydroxyurea, a well-known inhibitor of DNA synthesis (e.g. ref. 16), was shown by Sinclair (17, 18) to be toxic to Chinese hamster cells when they are undergoing DNA synthesis (i.e. in their S phase). Further, the drug prevents cells in G_1, before DNA synthesis, from entering S without interfering with the division of those cells beyond S, in G_2 or mitosis. Accordingly, as we have shown (8), an initially asynchronous population is converted into one synchronized at the end of G_1 when cells are exposed to millimolar concentrations of hydroxyurea for two or more hr. After the drug is washed out of the plates, the cells progress through S, G_2, and M, increase in number by a factor of two, and continue to progress apparently in a normal way (7, 8).

In the experiments involving hydroxyurea treatment, zero hr is the end of the synchronization treatment and the time thereafter is a measure of the aging of cells starting with the S period.

RESULTS

Actinomycin D Binding to Dishes

Studies with actinomycin D are made difficult by two technical facts. The first, which is of biological importance, is that this antibiotic binds in cells. In earlier work we showed that the ^3H incorporated into Chinese hamster cells as ^3H-actinomycin D has an intracellular half residence time of 2.3 hr (8). In view of the sequestering properties that DNA has for this drug (2–5), this persistence of label probably represents the slow release of actinomycin D from chromatin. Some biological consequences of this will be noted later.

The second reason for difficulty in working with actinomycin D refers to its ability to bind to dishes. Because of this fact, which we demonstrate in Fig. 1 and later in a different way in Fig. 8, the use of a somewhat involved rinsing procedure is necessary to insure that effects of the drug are limited to the nominal exposure periods.

The uppermost curve in Fig. 1 (upper abscissa and right ordinate) shows the sensitivity of initially single cells to continuous exposure to actinomycin D. That is, from a suspension of single cells, dishes were inoculated containing the final concentrations of antibiotic shown, and they were incubated without medium change until colonies were formed. Toxicity does not set in until 0.002 μg/ml of drug is exceeded; essentially the same result is obtained whether glass or plastic dishes are used.

The remaining two curves in Fig. 1 (lower

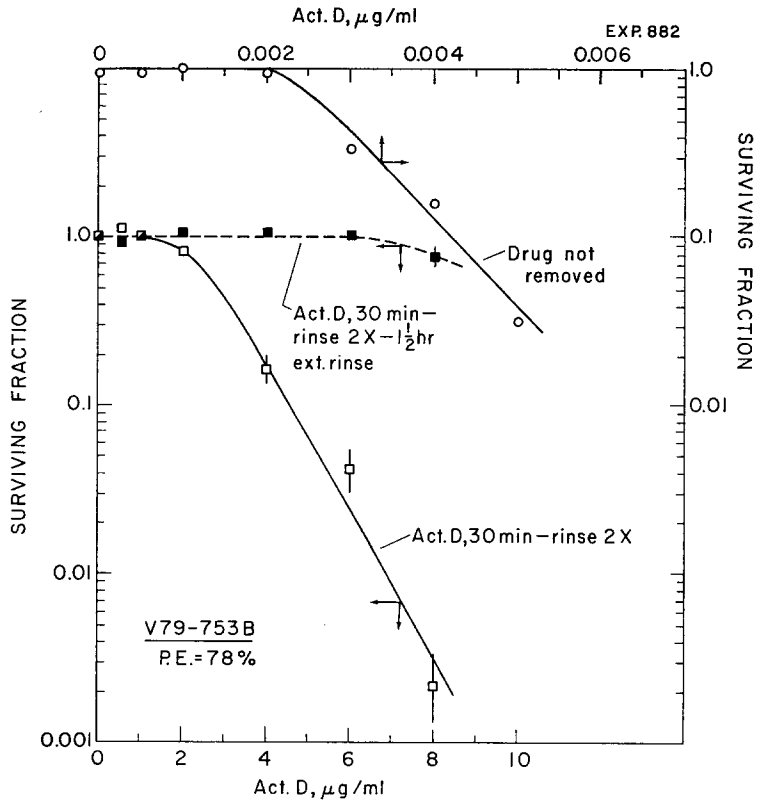

FIGURE 1 The effect of the treatment of 9-cm plastic petri dishes (Falcon Plastics, Division of B-D Laboratories, Inc. Los Angeles, Calif.) with actinomycin D *before* the addition of cells. The open circles trace the effect on colony formation when cells are inoculated into medium containing drug, with no change of medium thereafter. The squares show the results of pretreatment of dishes with varying concentrations of drug and two different rinsing procedures. (*P.E.* = plating efficiency: the per cent of cells in the starting population able to form colonies.)

abscissa and left ordinate) show the effect of binding of actinomycin D to plastic dishes (open squares) and how the effect of this binding can be accounted for (closed squares). *Before* the inoculation of cells, medium containing the concentrations of drug shown was added. After incubation for 30 min (at 37°C in a CO_2 incubator), this medium was sucked out, the dishes were rinsed twice (~14 ml/rinse), and normal growth medium plus cells was added. Even though this procedure diluted the residual actinomycin D concentration by at least a factor 2×10^4, significant cell killing resulted (open squares). However, if after the two prompt rinses, a third rinse was introduced 1½ hr later, and before fresh medium and cells were added, essentially no cell killing resulted. Thus, in 1½ hr, under normal conditions of medium, temperature, and pH, enough actinomycin D comes off the plastic surfaces to kill large fractions of cells. For example, after the treatment of dishes with 4 μg/ml followed by two prompt rinses, enough actinomycin D is released to yield about 0.004 μg/ml (in 10 ml), as judged from the results traced by the uppermost curve.

A result similar to that traced by the closed squares in Fig. 1 was obtained when glass dishes were used and two prompt rinses *only* followed exposure to actinomycin D. From measurements with ^{3}H-actinomycin D in 20 ml glass vials (the size used for scintillation counting), we know that the drug binds to glass and that more than half of it is released in 1½ hr. Hence, it appears that plastic binds more actinomycin D, releases more in 1½ hr, or both.

To avoid problems due to drug binding to surfaces, in the following experiments two prompt

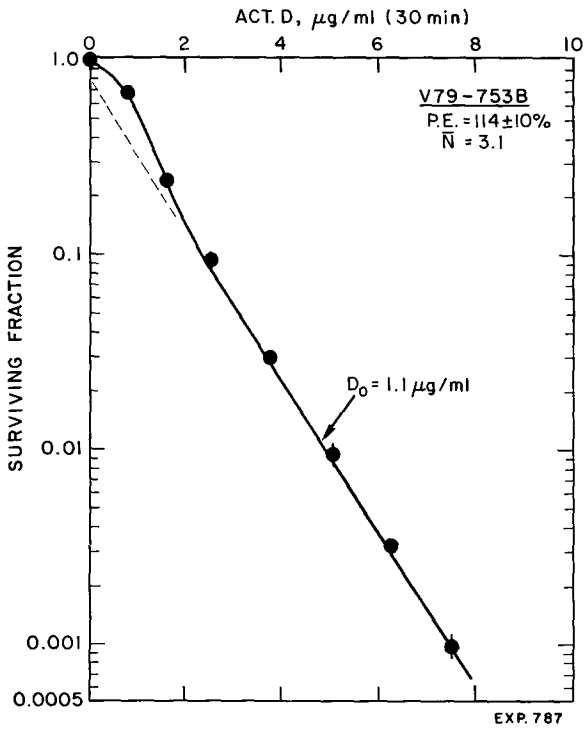

FIGURE 2 The survival curve of asynchronous Chinese hamster cells exposed to increasing concentrations of actinomycin D for 30 min (37°C). D_0 is the dose of actinomycin which reduces survival by 1/e (= 0.37) along the straight line portion of the curve. (*P.E.* = plating efficiency; $\bar{N}$ = cell multiplicity at the start of the experiment.)

rinses plus a 1½ hr "extended rinse" were used (except as noted) even when glass dishes were employed.

Survival of Asynchronous Cells

Using cells grown overnight and the rinsing procedure outlined, we measured the dose-dependence of the loss of colony formation of asynchronous cells. Fig. 2 shows the survival curve of cells treated with actinomycin D for 30 min at 37°C. 30 min is short enough to approximate acute treatment and yet long enough to be manageable. 60 min exposures yield essentially the same curve if each concentration is halved.

Two features are prominent in Fig. 2. The first is that if there are cells which are completely resistant to actinomycin D, for example, because of lack of penetration into the cell (3), the proportion of these is less than 1 per 1000. The second feature is that the curve has an inflection point indicative of at least two moieties whose survival curves differ appreciably (see chap. 2, ref. 9). For this not to be the case, it would be required that cells become more resistant as the concentration of the drug is increased, a possibility which is highly unlikely in the case of radiation-induced cell killing (4) but which cannot be ruled out here on purely logical grounds. However, it will be clear presently that a mixed population effect is responsible for the inflection in Fig. 2

Single-Dose Age-Responses of Actinomycin D Relative to X-Irradiation

To analyze the basis for the inflection in the curve in Fig. 2, as well as to find out how the response to actinomycin D varies with position of a cell, or its *age*, in its growth cycle, synchronized cultures were used. In Fig. 3, the variation in survival response after exposure to a constant dose of drug is shown and, in addition, the age-response for a fixed X-ray dose as measured in the same experiment. The latter curve serves not only to permit a comparison of the age-response functions of these two agents, but also as an age marker since we know that the maximum in X-ray survival occurs in the latter half of the S phase (10). Further, we know that zero hr in Fig. 3 is the G_1-S border and that, from earlier work, division occurs at about 7 hr (7).

With the foregoing in mind, Fig. 3 shows that *V79-753B* cells are resistant to actinomycin D at the same age as they are resistant to X-rays (i.e.

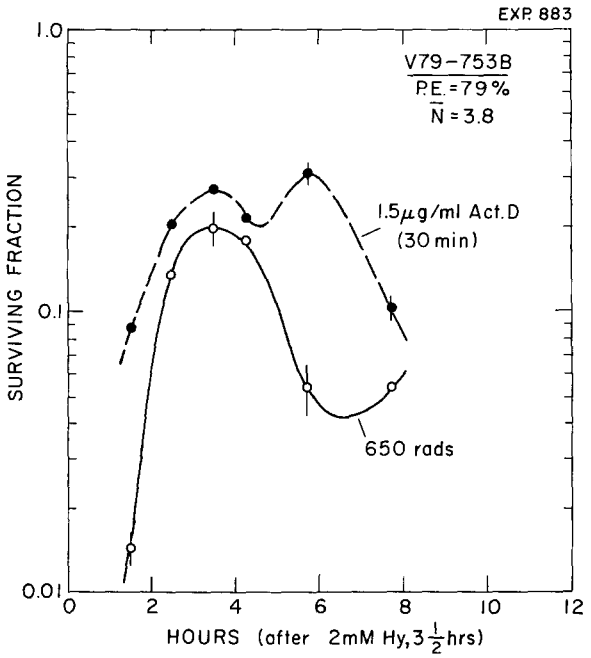

FIGURE 3 Age-response functions after actinomycin D and X-ray treatment of Chinese hamster cells synchronized with hydroxyurea (*Hy*). (X-irradiation: 55 kv, 722 rads/min.)

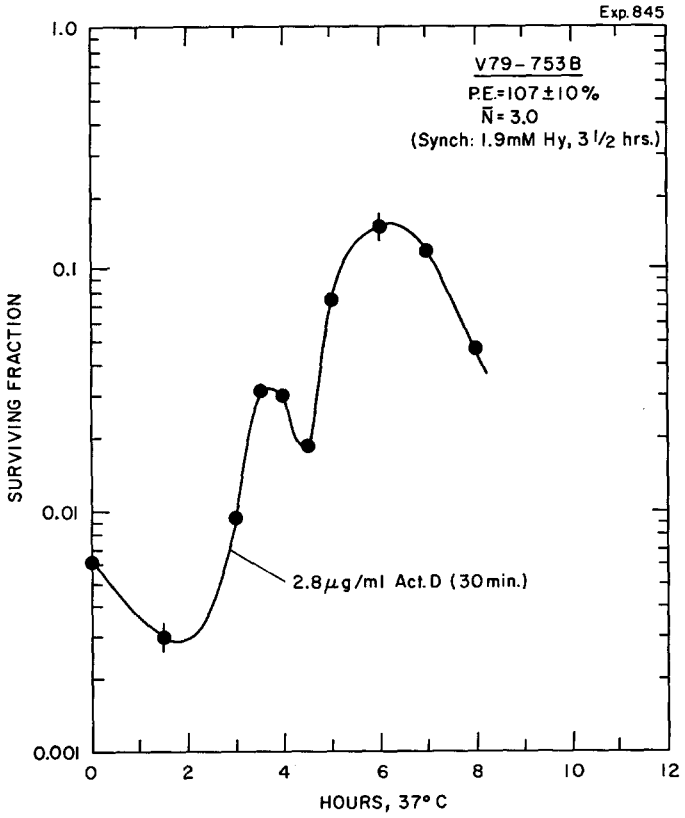

FIGURE 4 Age-response function of Chinese hamster cells after treatment with a higher concentration of actinomycin D than in Fig. 3. Other details as in Fig. 3 and earlier figures.

the latter part of the S period) but that, in addition, they are resistant in G_2 or G_2-mitosis. This latter point is even clearer when a higher concentration of actinomycin D is used as in Fig. 4. In addition to a general downward displacement of the whole age-response curve after 2.8 μg/ml (30 min), in Fig. 4 it is also clear that late S cells and G_2 cells respond differently.

G_2 Contamination after Synchronization

In Fig. 4, there are three prominent features. There are the two maxima already noted but, in addition, the minimum at 1½ to 2 hr suggests that cells are most sensitive to actinomycin D during the beginning of the S period. We explore this latter point first.

Because synchronizing techniques are not perfect, and because of the prominent resistance of G_2 (or G_2-mitosis) cells to actinomycin D treatment, it seemed likely to us that the first minimum in Fig. 4 was due to imperfect synchronization. That this is very likely the case is shown in the next two figures.

Fig. 5 shows the effect of two different periods of hydroxyurea treatment on the initial portions of the single-dose age-response patterns for actinomycin D and X-irradiation. The squares are for a 2 hr synchronizing period and the circles are for a 5 hr synchronizing period. Both the radiation data (open symbols) and the actinomycin D data (closed symbols) show a lowering of the initial portion of the age-response patterns with increasing synchronization time. Indeed, 5 hr of exposure to 2 mM hydroxyurea eliminates completely the minimum in the X-ray response, a result consistent with the greater relative sensitivity of G_2 cells to radiation compared to actinomycin D. These data suggest, therefore, that with increasing synchronization time, the proportion of G_2 (or G_2-mitosis) cells decreases and that the first minimum in Fig. 4 is a result of small G_2 contamination of the population at the end of the synchronization period.

G_1 and Early S Survival Curves

To make clear that the preceding is essentially correct, and to determine as well a more complete picture for the age-dependent structure in Figs. 3 and 4, survival curves were measured immediately after synchronization and 2 hr later with the same

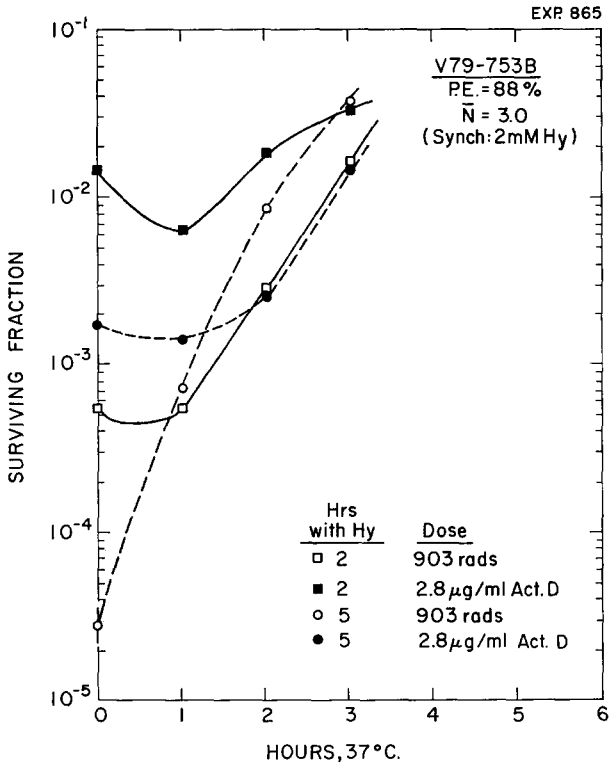

FIGURE 5 The effect of increasing periods of synchronization with hydroxyurea (*Hy*) on the initial portions of X-ray (open symbols) and actinomycin D (closed symbols) age-response patterns. (Details as in Fig. 3.)

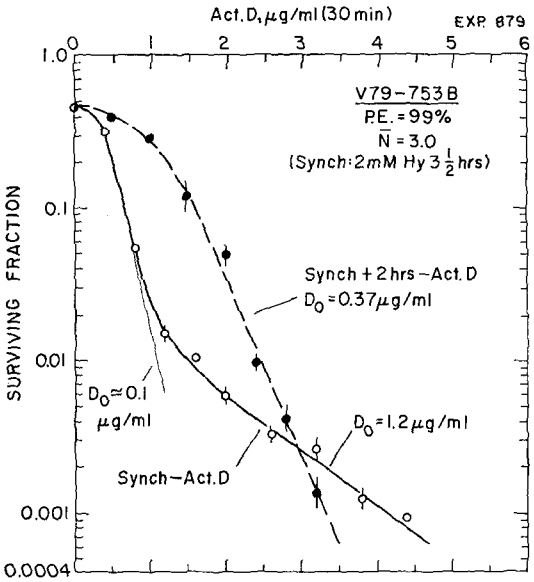

FIGURE 6 Actinomycin D survival curves of Chinese hamster cells immediately after synchronization (open circles) and two hours later. The drop in survival from 1.0 corresponding to zero actinomycin D concentration resulted from the killing of S cells during the hydroxyurea (*Hy*) synchronization. (Other details as in earlier figures.)

conditions of synchronization as in Figs. 3 and 4.

In Fig. 6, "Synch-Act. D" means treatment with the antibiotic immediately after the washing out of the hydroxyurea. Before discussing these curves, we note first that the surviving fraction corresponding to zero drug concentration is 0.48. As compared to untreated controls, after synchronization treatment only the surviving fraction is reduced because of the toxic effect that hydroxyurea has for S cells which is reflected in this initial decrease. The drop in surviving fraction is less than that corresponding to the proportion of the population in S after overnight growth (60–70%), because some of the microcolonies at that time, which contain one or more S cells, also contain one or more cells not in S.

In Fig. 6, the curve obtained immediately after synchronization is reminiscent of the one in Fig. 2, in that it has an inflection; in addition, the D_0 values of the terminal portions of both curves are essentially equal. This suggests that in both instances the terminal portions of the curves are due to cells in the same age interval. 2 hr after synchronization the curve appears to represent an homogeneous population. No inflection is present. It is also evident, however, that even if the tail of the curve traced by the open circles is not considered, in the first 2 hr after synchronization there is a considerable change in curve shape. Thus, in Fig. 6 a contamination with a small percentage of G_2 cells (or less) immediately after synchronization is suggested by the tail (see also Fig. 7). And since small absolute changes in the proportion of G_2 cells would lead to large relative fluctuations in survival, we should get, as we do, large variations in survival at zero hr after synchronization in different experiments (e.g. compare data in Figs. 4–6 at 2.8 μg/ml).

In addition to a D_0 dose, a survival curve can be characterized by a shoulder width D_q or an extrapolation number n (see chapters 2 and 3, ref. 9). D_q is the dose intersected by a back extrapolate of the straight line portion of a curve at a surviving fraction corresponding to no treatment (e.g. 0.48 in Fig. 6), and n is the ordinate intersected by the same back extrapolate relative to that for no treatment. (These parameters are related: $D_q = D_0 \ln_e n$.) Mindful that most of the initial shoulder of the open circle curve might be due to multiplicity (multiplicity after synchronization is about the same as it is at the start of synchronization (6), and if we attribute the tail of this curve to a G_2 contamination, we see that G_1-S cells are very sensitive, first, because their survival curve has a narrow threshold, but also because the curve is steep. The D_0 indicated in Fig. 6, 0.1 μg/ml, is probably an overestimate because of the influence of the tail portion on the initial portion of the curve. 2 hr after synchronization, not only has the D_0 value increased by about 4-fold, but the shoulder has become clearly appreciable. Since the incorporation kinetics of ^{3}H-actinomycin D is linear with time and concentration at least with asynchronous cells (8), it seems unlikely that the survival curve shoulder reflects nonlinear drug incorporation. Rather, the shoulder suggests a capacity for sublethal drug damage in analogy with the implications of a shoulder in radiation survival curves (see chap. 6, ref. 9).

Since we used the drug hydroxyurea to synchronize and hold cells at the G_1-S border, it is a possibility that at least some of the enhanced cell killing by actinomycin D just after synchronization reflects the combined effects of both drugs (Fig. 6). However, in experiments like those in Figs. 3 and 4, in which the age-response pattern of synchro-

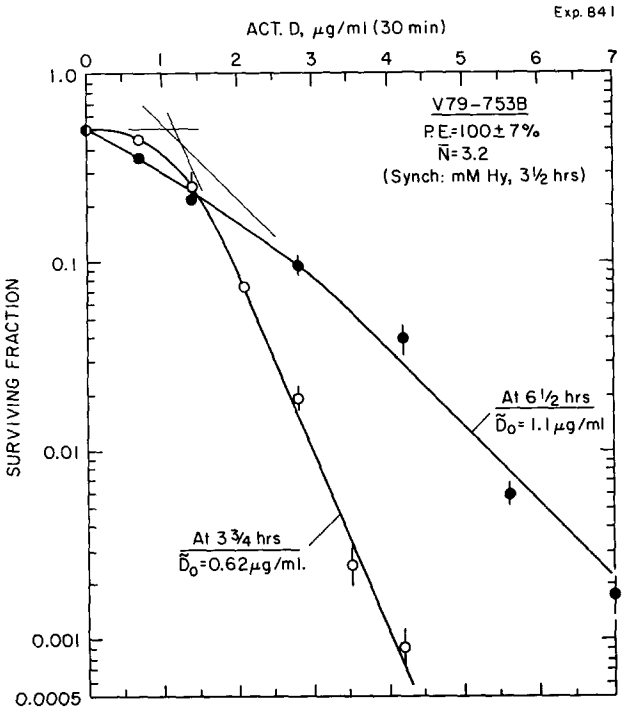

FIGURE 7 Actinomycin D survival curves at the times of the maxima in Fig. 4. (Other details as in earlier figures.)

nized cells was followed into the second interdivisional period, a clear minimum was observed at a time corresponding to the second G_1-S region. Therefore, this latter age appears to be the most sensitive, suggesting, perhaps, that the entire G_1 period might be relatively sensitive at least in a cell line having a short G_1 period.

Late S and G_2 (-Mitosis) Survival Curves

Continuing with the survival properties of cells as they progress in their growth cycle, we now consider the survival curves at the times of the two maxima in Figs. 3 and 4. This is important to do, first, because we are not always able to resolve the minimum between 4 and 5 hr (a not unreasonable lack of resolution in view of the narrowness of the minimum and our use of 30 min exposures), and second, because we must consider the possibility that the maximum in late S represents only a contamination with cells whose rate of aging is sufficiently rapid so that they are in fact in G_2 while most of the population is still in late S.

Fig. 7 shows two survival curves at, or close to, the two maxima in Fig. 4. Compared to that of early S cells (Fig. 6), the survival curve of late S cells (3¾ hr) is about half as steep. Still, the curve for late S cells has an appreciable shoulder, like that for early S cells. By 6½ hr, the terminal slope of the survival curve has decreased by another factor of 2, but now it is not clear that the curve still has a shoulder larger than that due to multiplicity (9). However, by the time cells reach a nominal age of G_2 (or G_2-mitosis), we would expect the age dispersion in the population to have increased, due to varying growth rates, beyond that present immediately after synchronization. Hence, it seems reasonable to us that the survival curve for a narrow age interval of cells at the second maximum in Fig. 4 could have an appreciable shoulder, indicative of a capacity for sublethal damage, which is masked in Fig. 7 at 6½ hr due to the presence of less resistant cells.

Finally, with respect to the inflections in the curves in Fig. 2 and Fig. 6 (open circles), we note that the D_o's involved are essentially the same as that for 6½-hr cells in Fig. 7. This supports our view that the terminal portion of the survival curve of asynchronous cells and the tail of the curve immediately after synchronization are due to G_2 cells. Also, since there is no evidence of a resistant tail in the survival curve at 3¾ hr in Fig. 7, we conclude that the maximum at this age in Figs. 3 and 4 is not due to a G_2 contamination

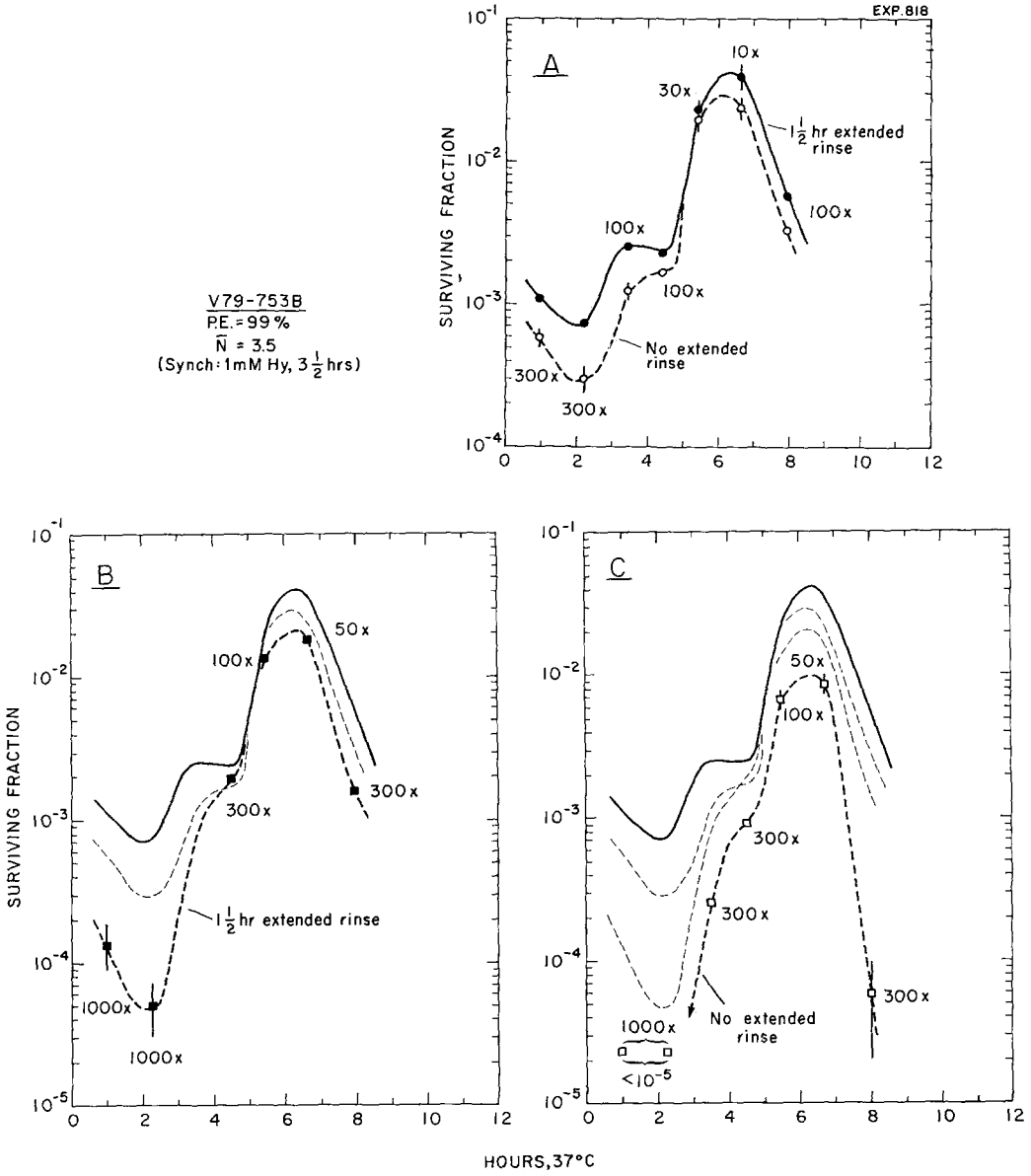

FIGURE 8 Actinomycin D age-response curves for an exposure of 3.8 μg/ml for 30 min at the times shown after synchronization with hydroxyurea (*Hy*). "10X" stands for a cell inoculum of 2×10^3 cells per 9 cm dish, "100X" stands for 2×10^4 cells, and so on. In panel *A* for equal inocula at the times shown, the effect is shown of an extended rinse after two prompt rinses. In panel *B*, the upper two curves are from panel *A* and the squares trace the effect of higher cell inocula at the times shown with optimal rinsing. The upper three curves in panel *C* are from panel *B*. The open squares show the result of inadequate rinsing plus higher cell inocula. Other details as in earlier figures. (Courtesy of Elkind, et al., ref. 8.) Dr. Elkind and Dr. Kano's present address is Biology Department, Brookhaven National Lab., Upton, Long Island 11973

but represents, indeed, a characteristic of late S cells.

Cell-to-Cell Interactions Due to Actinomycin D Toxicity

In addition to the technical difficulty resulting from the binding of actinomycin D in dishes (Fig. 1), another difficulty arises which is biologically important. This involves the slow release of actinomycin D from lethally affected cells.

The three panels in Fig. 8 show the dependence on age of both actinomycin D bound in dishes and actinomycin D in cells. If we take as 200 the cell number equivalent of a 1X inoculum (i.e. the average number of colonies in control dishes), 10X stands for 2×10^3 cells, 100X for 2×10^4 cells, and so on. In panel *A*, each pair of points at a given time after synchronization was determined with inocula of the sizes indicated. The upper of the two curves resulted from two prompt rinses and one extended rinse; the lower curve, as in Fig. 1, shows in part the effect of drug release from the dishes. Panel *B* shows the effect of increasing cell inocula; the upper two curves are redrawn from panel *A*. In panel *C*, the upper three curves are from panel *B*, and the squares trace the additional loss in survival due to inadequate rinsing.

Part of the progressive loss in survival demonstrated in Fig. 8 reflects drug release from dishes. However, in Fig. 8 this release contributes to an appreciably smaller extent than in Fig. 1 because in the latter instance 10 ml was the final volume of medium throughout, whereas, in Fig. 8, 20 ml was the *smallest* volume used. Consistent with this, the drop in survival in Fig. 8, panel *A*, is everywhere smaller than the factor of 5 to 10 indicated at 3.8 μg/ml in Fig. 1.

In panel *B* (Fig. 8), both the uppermost and lowermost curves involved optimal rinsing. Here, then, it is clear that the drops in survival reflect the larger cell inocula. The magnitude of the effect in the first few hours is larger than thereafter. This could reflect the greater sensitivity of G_1 and early S cells. However, it probably represents simply a release of more drug to the medium, due to the greater cell numbers, in time periods of about one or a few cycle times since, in addition to the 2.3 hr half residence time of actinomycin D in cells which are at least morphologically intact (8), drug is probably released into the medium slowly as cells degenerate and lyse.

Thus we see that cell-to-cell toxicity mediated very likely by the release of drug which is still active into the medium can play a significant role. This is probably a tribute to the stability of actinomycin D and its strong binding to DNA. To obtain results in which cell-to-cell as well as dish-to-cell interactions are minimized, adequate rinsing, minimal cell inocula, and large final volumes of growth medium should be used.

DISCUSSION

Actinomycin D is well known for its ability to inhibit nucleic acid synthesis. Although biological effects may be attributed to it which may not appear to be causally related to its binding to DNA (e.g. ref. 19), effects of the drug relative to the functional capacity of a cell's genome would appear to be of critical importance where cell replication is involved.

In vitro, actinomycin D inhibits RNA synthesis at concentrations appreciably lower than those required to inhibit DNA synthesis (20), and in vivo the inhibition of the synthesis of ribosomal RNA sets in at drug concentrations lower than those required to inhibit other types of RNA (21). These inhibitions, particularly those related to RNA synthesis, may be viewed as expressions of the imposition of abnormal controls of DNA template activity (22). Still, they afford little insight relative to the ability of actinomycin D to compromise the functionaly capacity of the genome in its support of the overall process of cell division other than to suggest that some effect is likely.

Using the concepts of survival curve analysis commonly employed in radiobiology (9), we may describe the broad features of the cycle dependence of actinomycin D-induced killing of Chinese hamster cells as follows. G_1 cells are maximally sensitive; their survival curve is steep and displays at most a small shoulder. Such a curve is suggestive of a "single hit-to-kill" mechanism. As cells progress into the S phase, they not only appear to develop a capacity for sublethal damage, as suggested by the appearance of a shoulder in their survival curve, but they are more resistant to the drug even when saturated with sublethal damage. This trend continues into late S just after which it is reversed for an age interval too narrow to permit a detailed examination with our methods. In G_2 (or G_2-mitosis) cells are maximally resistant even though the contribution to this resistance from a capacity for sublethal damage is probably less than for early and late S cells.

If we assume that all of the foregoing age-dependent killing pattern involves drug interactions at the level of the DNA, the inferences follow that the cyclic susceptibility of the genome to actinomycin D reflects a cyclic ability of this drug to reach and interact with the genome; an expression of cyclic function of the genome; or both. In the case of radiation age-dependent effects (e.g. Fig. 3), the penetrability of this agent precludes any concern with an age-dependent ability of the radiation to interact with the genome. While we cannot assume this to be the case for drug effects, still it seems unlikely that the qualitative as well as quantitative changes in survival curves observed reflect simply a varying ability of the drug to at least penetrate into the nucleus. This view is supported by the facts that the age-response pattern of S cells after actinomycin D treatment is similar to that after irradiation (e.g. Fig. 3), and radiation and actinomycin D damage interact in S cells as we have shown (6–8). Age-dependent cell killing probably is due, therefore, to the combined effects of the ability of actinomycin D to penetrate chromatin and to bind to DNA, and to the functional capacity of DNA-actinomycin complexes.

In connection with the nature of the interaction of actinomycin D with DNA, some insight is available from our studies of combined radiation-drug action. We noted in the Introduction that, at least in Chinese hamster cells undergoing DNA synthesis, actinomycin D damage interacts with lethal as well as sublethal radiation damage (1, 6–8). In many respects, this antibiotic acts as though it is equivalent to a dose of radiation. In its action, it may be introducing lesions which act like radiation lesions (e.g. single or double-strand breaks) or it may be interfering with radiation repair processes. The latter possibility appears the more likely in view of the nucleic acid polymerase inhibitions, of which this drug is capable. Still, in mammalian cells interference with polymerases or other enzymes, whose actions are intimately involved with DNA replication as well as function, could lead operationally to effects which amount to lesion production. For example, if DNA replication normally involves single-strand breakage and/or single-strand rejoining, an interference with such processes would have the effect of producing strand breaks.

Thus, while a rational basis for a radiation-actinomycin interaction can be proposed, this explanation is limited largely to cells undergoing DNA synthesis. The similar shapes of the single-dose age-response patterns for each agent separately also support, in a general way, similar actions in S cells. But the age-response patterns differ at least for cells in G_2 (or G_2-mitosis) and, therefore, some obvious differences in action, such as the possibility of a limited ability of actinomycin D to penetrate chromatin particularly in G_2 cells, must also be kept in mind.

Some further insight into the mode of action of actinomycin D comes from other studies of the comparative effects of radiation and drugs. We find an absence of any interaction between nitrogen mustard damage and radiation damage in S cells.[1] If, indeed, this bifunctional alkylating agent is effective because it produces DNA cross-links (23), we may conclude that cross-linking plays only a minor role in actinomycin D action (24). Intercalation (22) would appear to be responsible for actinomycin-radiation interaction and possibly for actinomycin D action by itself.

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[1] K. Sakamoto and M. M. Elkind. Paper in preparation.

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