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Bleomycin-induced Damage in Prematurely Condensed Chromosomes and Its Relationship to Cell Cycle Progression in CHO Cells[1]

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SUMMARY

The phenomenon of premature chromosome condensation, which involves the fusion of mitotic with interphase cells, was applied to the study of bleomycin-induced chromosome damage in Chinese hamster ovary cells. Examination of the prematurely condensed chromosomes immediately after bleomycin treatment revealed a 5 to 9 times higher incidence of chromosome aberrations than the incidence observed in mitotic chromosomes. Attempts to understand the cause for the existence of such a wide difference between prematurely condensed and mitotic chromosomes revealed the following: (*a*) bleomycin treatment (25 μg/ml for 30 min) had no effect on the progression of cells from mitosis to G_1 or from G_1 to S; (*b*) the rate of progression of S and G_2 phase cells into mitosis was slower in the treatment than in the control; (*c*) nearly one-half (44%) of the G_2 cells failed to enter mitosis even at 7 hr after the treatment; (*d*) about one-third of the aberrations (gaps and breaks) observed in the prematurely condensed chromosomes were repaired within 60 min after treatment. There was an indication that cells with extensive chromosome damage failed to enter mitosis, and hence they were blocked in G_2 until the damage was repaired to tolerable levels. The data also suggested the existence of a maximum limit for the amount of chromosome damage that a cell can carry and still enter mitosis.

INTRODUCTION

BLM,[3] a complex glycopeptide antibiotic (20), has found increased usage in the treatment of a variety of neoplastic diseases in man. Nevertheless, the basis for its cytotoxic effect is not clearly understood. Like X-rays, BLM is capable of inducing DNA strand breaks (9, 17), G_2 delay (1, 10, 19, 21), and chromosome aberrations in mammalian cells either *in vivo* or *in vitro* (3, 11, 13). Some of the recent studies on a variety of cancer chemotherapeutic agents indicate a good correlation between the cytotoxic effects of these agents and the incidence of chromosome aberrations in the treated cells (2, 4, 15). However, BLM treatment severely inhibits the progression of cells into mitosis. The probability that the extensively damaged cells might not be reaching mitosis makes it difficult to assay the chromosome damage accurately by the conventional techniques.

Recently, we have shown that chromosome damage in interphase cells can be effectively observed by the technique of premature chromosome condensation, which involves the fusion of mitotic with interphase cells (6, 7). The premature chromosome condensation technique has some advantages over the conventional mitotic chromosome studies in these respects: (*a*) it makes it possible to visualize chromosome damage in interphase cells immediately after exposure to mutagenic or clastogenic agents; (*b*) there is no need for the cells to progress to mitosis in order to evaluate chromosome damage; (*c*) the repair of chromosome aberrations as a function of time can be measured directly; (*d*) the position of a cell in the cell cycle at the time of treatment becomes obvious by the morphology of its PCC.

Taking advantage of this technique, in this study we tried to answer why many of the BLM-treated cells failed to reach mitosis and what is the extent of chromosomal damage suffered by these cells. The results obtained by the premature chromosome condensation technique in this study revealed a 5- to 9-fold higher incidence of chromosome aberrations than the incidence observed by studying mitotic chromosomes. They also suggest that extensive damage to the genome may lead to the failure of cells to progress through the mitotic cycle.

MATERIALS AND METHODS

Cells. CHO cells, kindly supplied by Dr. T. T. Puck, were routinely grown as monolayer cultures in Falcon plastic dishes at 37° in a humidified CO_2 incubator in modified McCoy's Medium 5A (Grand Island Biological Co., Santa Clara, Calif.) supplemented with 20% heat-inactivated fetal calf serum (Grand Island Biological Co.) and 1% antibiotic mixture (penicillin, 10,000 units/ml, and streptomycin 10,000 μg/ml).

Drug Treatment. BLM (Bristol Laboratories, Syracuse, N. Y.) was dissolved in 0.9% NaCl solution to give a stock solution of 1000 μg/ml. This stock solution was later diluted with culture medium to give the desired concentrations. In

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[3] The abbreviations used are: BLM, bleomycin; PCC, prematurely condensed chromosomes; CHO, Chinese hamster ovary cells; TdR-^{3}H, tritiated thymidine.

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most of these experiments 2 dishes of exponentially growing monolayer cultures were first pulse labeled for 15 min with TdR-^{3}H (1 μCi/ml; specific activity, 6.7 Ci/mmole). Upon removal of the isotope, medium containing BLM (25 μg/ml) was added to one culture while regular medium was added to the other. After a 30-min incubation both the cultures were rinsed twice with regular medium and reincubated until they were harvested for chromosome preparation or cell fusion.

Cell Fusion. The procedures for the collection of synchronized populations of mitotic and interphase cells and the technique of cell fusion to induce PCC have been described in our earlier publication (6, 14). Briefly, this involves the mixing of an equal number of untreated mitotic cells with BLM-treated interphase cells in serum-free medium, centrifugation of the mixture, and resuspension of the cell pellet in 0.5 ml of phosphate-buffered saline containing about 2000 hemagglutinating units of UV-inactivated Sendai virus. The fusion mixture was placed at 4° for 15 min and then transferred to 37° water bath for a 45-min incubation. By this time cell fusion and the induction of premature chromosome condensation were completed.

Preparation and Scoring of Slides. For chromosome preparations cells were expanded by hypotonic treatment (0.7% sodium citrate for 20 min at room temperature), fixed in methanol:glacial acetic acid (3:1), and dropped on wet slides. The air-dried slides usually were stained with Giemsa. In experiments involving radioautography the slides were scored first on aceto-orcein-stained preparations. After radioautography and restaining with Giemsa the same cells were rescored for the presence of label.

For autoradiography, slides were extracted 3 times (for 20 min each) in cold (4°) 5% trichloroacetic acid to remove nonspecific label and then rinsed in distilled water. Dried slides were dipped in Kodak nuclear track emulsion (diluted 1:3), allowed to dry and stored in light-tight boxes for 2 days. The slides were developed in D19 for 2 min and in Kodak fixer for 2 min, washed, and then stained in Giemsa.

Of the 3 types of PCC (G_1, S, and G_2) observed, the G_2 PCC are the most favorable for scoring chromosome aberrations and were used exclusively in these studies. Well-spread PCC or mitotic cells on coded slides were chosen at low power and then scored at high power. The coding of the slides was done to avoid any bias in scoring aberrations. Aberrations were divided into categories of gaps, breaks, and exchanges. In this study a gap is defined as an apparent discontinuity or an achromatic lesion the linear dimensions of which are smaller than the width of a chromatid.

RESULTS

Comparative Evaluation of Chromosome Damage by Premature Chromosome Condensation and Mitotic Methods. This experiment was designed to compare the levels of chromosome damage in G_2 cells evaluated immediately after BLM treatment in PCC or when the G_2 cells reached mitosis. Monolayer CHO cells were treated with BLM (25 μg/ml) for 30 min at 37°. One-half of these cells were immediately fused with an equal number of mitotic CHO cells to induce premature chromosome condensation. The 2nd half of the treated cells was reincubated in fresh medium for 30 min before adding Colcemid (0.25 μg/ml), allowing those cells already in mitosis at the time of treatment to complete division. After 1 hr of exposure to Colcemid, these cells were trypsinized and chromosome preparations were made. Cells that were not treated with BLM served as control. Both mitotic chromosomes and PCC were scored for aberrations. Chromatid-type gaps, breaks, and exchanges were observed both in G_2 PCC and the mitosis of cells treated during G_2 period (Fig. 1). The comparative frequencies of aberrations scored in 2 such experiments are shown in Table 1. These results are striking in that 5 to 9 times as much chromosome damage was observed in the G_2 PCC as in the mitotic chromosomes.

Why do the G_2 PCC exhibit such a high incidence of aberrations in comparison to the mitotic chromosomes? It is difficult to answer this question unless we know the effect of a 30-min BLM treatment on the progression of cells from mitosis to G_1, G_1 to S, and G_2 to mitosis. The following experiments were designed to measure the effects of BLM on the movement of cells through the mitotic cycle.

Table 1

Comparative estimates of BLM (25 μg/ml) damage to CHO cells by scoring PCC and mitotic chromosomes

BLM treatment	Type of chromosomes scored	No. of cells scored	Aberrations/cell[a]		
			Gaps	Breaks	Exchanges
Experiment 1					
+	PCC	50	3.94	4.00	0.80
–	PCC	50	0.26	0.08	0
+	Mitotic	100	0.77	0.58	0.13
–	Mitotic	100	0.04	0.01	0
Experiment 2					
+	PCC	38	3.32	3.03	0.37
–	PCC	35	0.14	0.09	0
+	Mitotic	100	0.66	0.34	0.09
–	Mitotic	100	0.06	0.01	0.02

[a] The ratio of aberrations (PCC/mitotic) in Experiments 1 and 2 are 5.0, 5.3 for gaps; 6.9, 8.9 for breaks; and 6.1, 5.3 for exchanges, respectively.

Effect of BLM on the Mitotic-G_1 Transition. In order to be certain that those cells that were in mitosis at the time of BLM treatment did complete cell division, the following experiment was done. We obtained a synchronized population of mitotic cells by a 4-hr Colcemid block and the selective detachment technique (18). These cells having a mitotic index of about 95% were divided into 3 parts, and they were exposed to different concentrations of BLM, *i.e.*, 0, 5, and 25 μg/ml plus Colcemid (0.25 μg/ml) for 30 min. After treatment, the cells were washed free of drugs and plated in dishes with fresh medium to allow them to progress into G_1 phase. Samples were taken at hourly intervals until 4 hr after treatment. The rates at which the cells completed mitosis and entered G_1 are shown in Chart 1. From these data it appears that BLM, at a concentration of 25 μg/ml or below, has no effect on the progression of cells from mitosis to G_1.

Effect of BLM on the Progression of Cells to Mitosis. Exponentially growing cultures of CHO cells in 16 dishes (35 mm) were selected. BLM (25 μg/ml) was added to a set of 8 dishes while the other set of 8 dishes served as control without the drug. After a 30-min exposure to BLM all the treated and untreated cultures were rinsed twice with fresh medium and reincubated with medium containing TdR^3H (0.2 μCi/ml) and Colcemid. At hourly intervals 1 dish from each of the sets was trypsinized, and the cells were fixed and processed for radioautography. The slides were scored for labeled and unlabeled mitoses and interphases, which were expressed as a percentage of the total number of cells scored.

As seen from the mitotic accumulation and labeling index curves the BLM treatment slowed the progression of cells into mitosis but apparently had no effect on the rate of progression of G_1 cells into S phase (Chart 2). The total mitotic index (*i.e.*, both labeled and unlabeled mitoses) in the treated cells was always lower than the index in the control, suggesting some sort of G_2 delay (Chart 2). At this point it is important to know whether all the G_2 cells are entering into mitosis or not. The kinetics of the unlabeled G_2 cell progression into mitosis has been plotted in Chart 3. This chart shows that the treated G_2 cells were not only slower in reaching mitosis, but also nearly one-half of them (44%) failed to enter mitosis. This fact was also confirmed by the percentage of unlabeled interphase cells in the population (Chart 3). The difference of about 6% in the frequencies of unlabeled interphase cells between the treatment and the control correlates very well with a similar difference in the frequencies of unlabeled mitosis noticeable at the end of the experiment. This difference is a measure of the fraction of the G_2 population that failed to reach mitosis. An examination of the rate of progression of labeled population into mitosis showed that the treated cells exhibited some delay over the control (Chart 4). This may suggest that those cells that were either in S or G_1 at the time of treatment also suffered some delay similar to those in G_2.

Measuring the Repair of Chromosome Damage following BLM Treatment. The difference in the aberration frequencies between mitotic chromosomes and PCC might be due to the repair of damage that could occur during the interval between the time of treatment and the entry of the cells into mitosis. How much of the BLM-induced damage could be repaired and

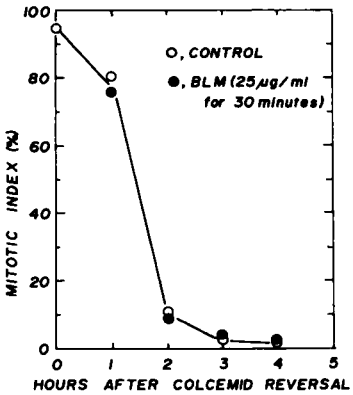

Chart 1. Effect of BLM on the progression of cells from mitosis to G_1. The decrease in mitotic index was plotted as a function of time after the removal of Colcemid and BLM. In this experiment, cells were exposed to Colcemid for 4 hr with BLM present during the last 30 min.

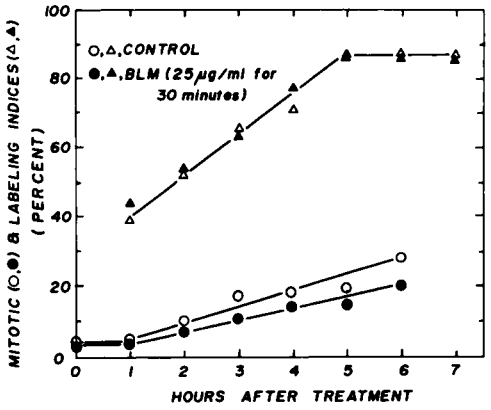

Chart 2. Effect of BLM on the rate of entry of cells into S phase and mitosis as a function of time after treatment. At the end of a 30-min treatment, BLM-containing medium was replaced with fresh medium containing thymidine-^{3}H and Colcemid, which were present throughout the experiment. The number of labeled cells and mitosis were expressed as a percentage of the total number of cells scored.

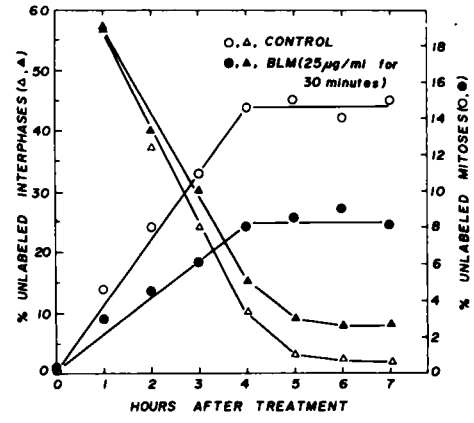

Chart 3. Effect of BLM on the progression of G_2 cells into mitosis. The increase in the labeled mitosis and a decrease in the number of unlabeled interphases are plotted as a function of time after BLM treatment. TdR-^{3}H and Colcemid were present in the medium throughout the experiment.

how long does it take to reach the minimum levels? In the following experiment, 6 dishes of CHO cells in exponential growth were pulse labeled first with TdR-^{3}H for 15 min and then exposed to BLM (25 μg/ml) for 30 min. After the drug treatment, *i.e.*, at t = 0 hr, cells were washed and reincubated with fresh medium until they were harvested for cell fusion or chromosome preparation. Three of the cultures were trypsinized at t = 0, 1, and 4 hr and fused separately with mitotic cells to induce premature chromosome condensation. Colcemid was added to the other 3 cultures at t = 0.5, 1.5, and 4.5 hr. After 1 hr of incubation with Colcemid, these cultures were trypsinized and processed for chromosome preparations. The lag of 1.5 hr between the collection of cells for cell fusion and mitotic chromosome preparation for each of the 3 sampling times was provided to allow the G_2 cells to reach mitosis. For example, those cells that were in G_2 phase at t = 0, 1, and 4 hr were expected to be in mitosis by t = 1.5, 2.5, and 5.5 hr. The chromosome aberrations were scored first by staining the slides with aceto-orcein, and the same cells were rescored after radioautography for the incorporation of label to determine whether they were in S or G_2 phase at the time of BLM treatment. The aberration rates in PCC and mitotic chromosomes as a function of time after BLM treatment are shown in Table 2. This table revealed that the incidence of aberrations in PCC was always higher than the incidence in mitotic chromosomes. The ratios for gaps and breaks between PCC and mitotic chromosomes were approximately 4:1 and 6:1, respectively. In addition, there was a significant decrease in the incidence of aberrations both in PCC and mitotic chromosomes after an interval of approximately 1 hr following treatment, but the incidence remained about the same even 3 or 4 hr later. This is indicative of some repair processes at work. A direct measurement of chromosome repair in cells that were treated in G_2 can be obtained by comparing the aberration frequencies in G_2 PCC at t = 0 and t = 1 hr. The gap and break frequencies in G_2 PCC declined by 37.0 and 35.3%, respectively, within the 1st hr, while the exchange frequency remained the same. A similar but somewhat smaller (about 25%) decline in the rate of aberrations was seen even in the mitotic chromosomes (Table 2).

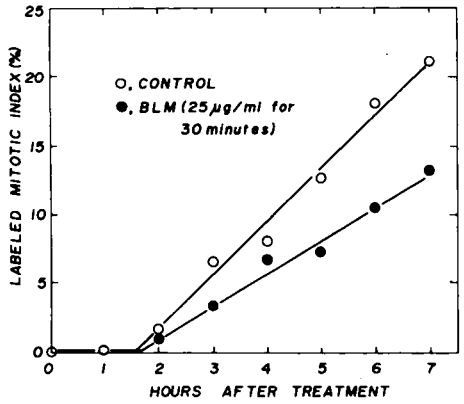

Chart 4. Effect of BLM on the progression of S-phase cells into mitosis with the continuous presence of TdR-^{3}H and Colcemid in the medium. The number of labeled mitoses is expressed as a percentage of the total cells scored for each sample.

Could this decrease in the aberration frequency be due to the flow of S-phase cells into G_2 and subsequently into mitosis? This leads to the question of whether S-phase cells suffered more or less chromosome aberrations than did the G_2 fraction. The data in Table 2 were rearranged in Table 3 on the basis of the radioautographic data. A comparison of the incidence of aberrations in the labeled and unlabeled PCC, *i.e.*, those that were in S and G_2, respectively, at the time of drug treatment, suggests that the S-phase cells suffered about the same or slightly greater damage than those in G_2 (Table 3). However, the aberration frequencies in the labeled and unlabeled mitosis appeared to be about the same. The data on the exchange frequency were quite variable, this could be due to the small number of cells carrying exchanges.

DISCUSSION

BLM has been shown to be a unique cancer chemotherapeutic agent because it damages cells in a manner much like X-rays. We have confirmed the findings of Paika and Krishan (13) that BLM is capable of producing chromatid-type aberrations directly in G_2 cells. Similarly, our preliminary studies indicate that BLM induces chromosome-type damage in cells treated in G_1 phase. These results are unique in that most other known chemical clastogens (and UV light) require the passage of a cell through S phase in order for structural aberrations to be formed (5, 7, 8) and even then only chromatid-type aberrations are observed at mitosis.

Table 2

Chromosome aberration frequency as a function of time after BLM treatment

Sample	BLM treatment[a]	PCC/M[b] (hr after treatment)	No. of aberrations/cell[c]					
			Gaps		Breaks		Exchanges	
			PCC	M	PCC	M	PCC	M
1	+	0/1.5	3.05	0.86	3.00	0.57	0.43	0.13
2	+	1.0/2.5	1.96	0.64	1.96	0.41	0.42	0.08
3	+	4.0/5.5	2.24	0.60	2.14	0.48	0.42	0.26
	−		0.10	0.15	0.08	0.11	0	0.03

[a] Cells were exposed to BLM (25 μg/ml) for 30 min.
[b] M, mitotic chromosomes.
[c] In each of the treatments the number of cells scored were 50 for PCC and 100 for mitotic chromosomes.

Table 3
A comparison of BLM-induced chromosome damage in S- and G_2-phase cells

Sample	Hr after treatment	Chromosomes scored: Type	L[a] or UL	Frequency[b] (%)	No. of aberrations/cell: Gaps	Breaks	Exchanges
1	0	PCC	UL	100	2.95	2.92	0.43
2	1.0	PCC	UL	79	2.00	1.87	0.47
	1.0	PCC	L	21	1.81	2.18	2.0
	2.5	Mitotic	UL	55	0.71	0.46	0.1
	2.5	Mitotic	L	45	0.78	0.42	0.01
3	4.0	PCC	UL	25	1.17	1.75	0.5
	4.0	PCC	L	75	2.13	2.08	0.35
	5.5	Mitotic	UL	8	0.50	0.69	1.14
	5.5	Mitotic	L	92	0.46	0.35	0.17

[a] L, labeled; UL, unlabeled.
[b] The cells with labeled and unlabeled chromosomes were in S and G_2 phase, respectively, at the time of BLM treatment. From the data in this column it is evident that Sample 2 represents predominantly the G_2 fraction, whereas Sample 3 represents those in S phase at the time of drug treatment.

In the present study, the examination of G_2 PCC immediately following BLM treatment showed about 5 to 9 times as much chromosome damage as that observed for the same population when it reached mitosis (Table 1). The difference in the extent of observable damage between PCC and mitotic chromosomes does not appear to be dependent on the degree of chromosome condensation. This assumption is based on the data published earlier (6), which indicate that damage is hidden only if it is caused after the initiation of chromosome condensation either during premature chromosome condensation induction or the initiation of mitosis. However, these differences might be due to several other factors. First, the agent might prevent or delay those cells already in mitosis at the time of treatment from dividing; and since aberrations cannot be seen in chromosomes condensed prior to the treatment (6, 16), this could lead to a lower estimate of aberration frequency. Second, the more severely damaged cells might not progress into mitosis; thus aberrations apparent in G_2 PCC might not be represented in mitotic populations.

Chart 1 showed that BLM-treated mitotic cells completed division without any delay. Similar results have been reported (1, 21) that indicate that BLM has no effect on the progression of cells from mitosis to G_1. Likewise, BLM has no effect on the G_1-S transition, although the rate of mitotic accumulation (in the presence of Colcemid) in the treatment was lower than in the control (Chart 2). The lower rate of mitotic accumulation observed was due to: (*a*) the delay suffered by the G_2 cells, and (*b*) the failure of nearly one-half of the G_2 fraction to reach mitosis (Chart 3). Those cells that were in S phase at the time of the treatment also suffered similar delay in their cell cycle traverse like those in G_2 phase (Chart 4). These kinetic studies, which confirm the previous reports from other laboratories (1, 10, 19, 21), indicate that BLM (at 25 μg/ml for 30 min) essentially is producing a partial G_2 blockade, allowing only some cells to go into mitosis. However, for the 1st time this study provides a means to examine why some of the BLM-treated cells can enter mitosis while others do not. It appears that the ability of a cell to enter mitosis depends upon the degree of damage to its genetic material. The greater the chromosome damage, the lesser is the chance for such a cell to enter mitosis. This conclusion is based on the data in Table 2, which show that, in the treated cells, PCC had about 4 to 6 times more aberrations than did the mitotic chromosomes. In the untreated control no such difference is detectable.

There is a possibility that some of the damage in the G_2 cells could be repaired by the time they are ready to enter mitosis. Indeed, there was a 35 to 37% decrease in the frequency of gaps and breaks within 1 hr, suggesting that these 2 types of aberrations can be repaired to some extent. It appears that the repair process is completed within 1 hr since no further decrease in the aberration frequency was noticed at 4 hr after treatment (Table 2). The fact that the exchange frequency in the PCC remained the same at different times suggests that exchanges, once formed, cannot be corrected. In a previous study (6) we had shown that most of the X-ray (217.5 rads)-induced damage could be repaired, whereas in this study it appears that only about one-third of the BLM-induced damage is repairable (Table 2).

Barranco and Humphrey (1) have shown that BLM was most effective against cells in mitosis and G_2 and less effective against cells in S and G_1. On this basis one would expect a higher incidence of chromosome damage in G_2 than in S-phase cells. Initially, the G_2 PCC (unlabeled) did exhibit a higher incidence of gaps and breaks at the 1st sampling time (Table 3). However, at the 2nd sampling, by which time most of the repair was completed, the incidence of aberrations in the unlabeled PCC dropped to the same level as that present in the labeled PCC (Table 3). This indicates that S-phase cells also receive considerable damage that could result in a prolonged G_2 or failure to enter mitosis.

These experiments suggest that only a certain amount of chromosome damage can be tolerated by a cell that is going to enter mitosis. Perhaps cells that cannot repair damage to permissible levels will be blocked in G_2 temporarily or permanently. Ohara and Terasima (12) reported a marked prolongation of S and G_2 phases in HeLa cells that were exposed to Mitomycin C. They assumed that the delay was due to irreversible damage to DNA. Their assumption seems to

find support in the data presented in this paper. As a working hypothesis we suggest that there is a maximum limit for the amount of chromosome damage that a cell can carry and still progress to mitosis. According to this we would expect the frequency of chromosome aberrations to increase as a function of dose and then to reach a plateau at higher drug concentrations. The data of Paika and Krishan (13) on chromosomal aberrations in BLM-treated HeLa and human lymphoblasts lend some credence to this hypothesis.

The premature chromosome condensation technique, as demonstrated in this and other studies (6, 7), has the distinct advantage over the conventional mitotic method in that the cells need not be in mitosis for the detection of chromosome damage. By this approach a more accurate estimate of chromosome damage due to chemotherapeutic drugs and its relationship to cytotoxicity can be obtained. The establishment of such a correlation between chromosome damage and cell lethality on a quantitative basis would provide a new parameter for the development of a system that can predict the response of human tumors to chromosome-breaking cancer drugs. Studies are in progress to develop such a predictive system.

REFERENCES

1. Barranco, S. C., and Humphrey, R. M. The Effects of Bleomycin on Survival and Cell Progression in Chinese Hamster Cells *in Vitro*. Cancer Res., *31:* 1218–1223, 1971.
2. Bhambhani, R., Kuspira, J., and Giblak, R. E. A Comparison of Cell Survival and Chromosomal Damage Using CHO Cells Synchronized with and without Colcemid. Can. J. Genet. Cytol., *15:* 605–618, 1973.
3. Bornstein, R. S., Hungerford, D. A., Haller, G., Engstrom, P. F., and Yarbro, J. W. Cytogenetic Effects of Bleomycin Therapy in Man. Cancer Res., *31:* 2004–2007, 1971.
4. Dewey, W. C., Furman, S. C., and Miller, H. H. Comparison of Lethality and Chromosomal Damage Induced by X-rays in Synchronized Chinese Hamster Cells *in Vitro*. Radiation Res., *43:* 561–581, 1970.
5. Evans, H. J., and Scott, D. The Induction of Chromosome Aberrations by Nitrogen Mustard and Its Dependence on DNA Synthesis. Proc. Roy. Soc. London Ser. B., *173:* 491–512, 1969.
6. Hittelman, W. N., and Rao, P. N. Premature Chromosome Condensation. I. Visualization of X-ray-Induced Chromosome Damage in Interphase Cells. Mutation Res., *23:* 251–258, 1974.
7. Hittelman, W. N., and Rao, P. N. Premature Chromosome Condensation. II. The Nature of Chromosome Gaps Produced by Alkylating Agents and Ultraviolet Light. Mutation Res., *23:* 259–266, 1974.
8. Kihlman, B. A. Molecular Mechanisms of Chromosome Breakage and Rejoining. *In:* E. J. Dupraw, (ed.), Advances in Cell and Molecular Biology, Vol. 1, pp 59–107, New York: Academic Press, Inc., 1971.
9. Kuo, M. T., and Haidle, C. W. Characterization of Chain Breakage in DNA Induced by Bleomycin. Biochim. Biophys. Acta, *335:* 109–114, 1973.
10. Nagatsu, M., Richart, R. M., and Lambert, A. Effects of Bleomycin on the Cell Cycle of Ehrlich Ascites Carcinoma. Cancer Res., *32:* 1966–1970, 1972.
11. Ohama, K., and Kadotani, T. Cytologic Effects of Bleomycin on Cultured Human Leukocytes. Japan. J. Human Genet., *14:* 293–297, 1970.
12. Ohara, H., and Terasima, T. Lethal Effect of Mitomycin-C on Cultured Mammalian Cells. Gann, *63:* 317–327, 1972.
13. Paika, K. D., and Krishan, A. Bleomycin-induced Chromosomal Aberrations in Cultured Mammalian Cells. Cancer Res., *33:* 961–965, 1973.
14. Rao, P. N., and Johnson, R. T. Mammalian Cell Fusion. I. Studies on the Regulation of DNA Synthesis and Mitosis. Nature, *225:* 159–164, 1970.
15. Scott, D., Fox, M., and Fox, B. W. The Relationship between Chromosomal Aberrations, Survival, and DNA Repair in Tumor Cell Lines of Differential Sensitivity to X-rays and Sulphur Mustard. Mutation Res., *22:* 207–221, 1974.
16. St. Amand, W. Mitotic Inhibition and Chromosome Breakage Induced in Grasshopper Neuroblasts by X-irradiation at Known Mitotic Stages. Radiation Res., *5:* 65–78, 1956.
17. Suzuki, H., Nagai, K., Akutsu, E., Yamaki, H., Tanaka, N., and Umezawa, H. On the Mechanism of Action of Bleomycin. Strand Scission of DNA Caused by Bleomycin and Its Binding to DNA *in Vitro*. Biochim. Biophys. Acta, *174:* 309–314, 1969.
18. Terasima, T., and Tolmach, L. J. Growth and Nucleic Acid Synthesis in Synchronously Dividing Populations of HeLa Cells. Expt. Cell Res., *30:* 344–362, 1963.
19. Tobey, R. A. A Simple, Rapid Technique for Determination of the Effects of Chemotherapeutic Agents on Mammalian Cell-Cycle Traverse. Cancer Res., *32:* 309–316, 1972.
20. Umezawa, H., Maedoa, K., Takeuchi, T., and Okami, Y. New Antibiotics: Bleomycin A and B. J. Antibiotics Tokyo Ser. A, *19:* 200–209, 1966.
21. Watanabe, M., Takabe, Y., Katsumata, T., and Terasima, T. Effects of Bleomycin on Progression through the Cell Cycle of Mouse L-cells. Cancer Res., *34:* 878–881, 1974.

Fig. 1. Visualization of BLM-induced damage in CHO cells. *A*, G_2 PCC of an untreated cell. The PCC (*arrows*) with unseparated sister chromatids are much less condensed than are the mitotic chromosomes. × 1520. *B*, PCC of a G_2 cell immediately after BLM treatment. *Arrows*, various types of aberrations, *i.e.*, chromatid gaps, breaks, and exchanges. The mitotic chromosomes can be seen at the top of the picture. × 1300. *C*, metaphase plate of a cell that received BLM treatment when it was in G_2 phase. *Arrows*, gaps, breaks, and exchanges. × 1660.