APPLICATION OF ARABINOFURANOSYL CYTOSINE IN THE KINETIC ANALYSIS AND QUANTITATION OF DNA REPAIR IN HUMAN CELLS AFTER ULTRAVIOLET IRRADIATION

RONALD D. SNYDER, W. L. CARRIER, AND JAMES D. REGAN, *Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830*

ABSTRACT We have developed a technique whereby 3-h pulses of arabinofuranosyl cytosine (ara-C) and hydroxyurea (HU) are used to analyze the kinetics of repair with time after ultraviolet irradiation in human fibroblasts. We demonstrate that this technique offers a significant improvement over existing repair assays in its ability to visualize between 57 and 100% of all sites undergoing repair in a given period of time. In addition, kinetic analyses of repair are more easily made and yield more information than techniques such as repair replication or unscheduled DNA synthesis. We have also examined the nature of the inhibition event by ara-C and have determined that repair breaks accumulate in the presence of ara-C and HU only up to a certain time beyond which no further breaks appear. The time needed to reach this saturation point depends on the number of sites undergoing repair during the treatment time. This observation is discussed with respect to a possible mechanism of excision repair inhibition by ara-C and HU.

INTRODUCTION

It is now well established that excision repair constitutes a major mode of repair in mammalian cells (1, 2). Although a great deal is known about this process (see reference 3 for reviews), many uncertainties still remain concerning the kinetics and molecular nature of repair. Part of the problem in understanding these aspects of repair lies in the inability of most available repair assays to adequately visualize repairing sites. In the case of ultraviolet (UV) light damage, for example, it has been estimated (4) that repair at a dimer site is complete in ~3 min. This event thus does not give rise to many single-strand breaks measurable by alkaline sedimentation techniques except at relatively high UV light doses. It is probable that repair after chemical damage is similarly rapid and thus largely refractory to analysis by usual procedures. Although repair replication techniques largely circumvent this problem, they do not yield information concerning the number of sites undergoing repair. Assays designed to measure the disappearance of specific lesions, such as dimer chromatography, radioimmunoassay, and lesion specific enzyme probes (e.g., 3-methyladenine glycosylase) also yield little information concerning the nature of the repair event itself. It is thus clear that no one assay can give the total picture of DNA repair after UV light or chemical insult. It is, therefore, desirable to develop techniques whereby the maximum number of sites undergoing repair can be visualized and which at the same time yield information concerning the nature of the repair event.

Two techniques in particular have been used extensively toward this end. Bromodeoxyuridine photolysis (5, 6) allows a determination of both the number of sites undergoing repair and the number of bases inserted into each site. It is capable of measuring about 25–35% of all major repair events after UV irradiation. The recently developed arabinofuranosyl cytosine (ara-C) inhibition technique (7–10) holds repairing sites open and allows their visualization by alkaline sucrose sedimentation. This technique is also capable of detecting about 20–30% of all repair after UV treatment.

In this paper we demonstrate that through a modification of this ara-C assay we are able to detect between 57 and 100% of pyrimidine dimer sites undergoing repair. Moreover, our analysis allows insights into both the nature of the ara-C inhibitory effect and into the nature of the repair event itself.

MATERIALS AND METHODS

Cell Culturing and Labeling

Normal human skin fibroblasts (HSBP) and xeroderma pigmentosum complementation group A fibroblasts (Jay Tim, CRL 1223, ATCC) were grown in modified Eagle's minimal essential medium supplemented with 15% fetal calf serum and nonessential amino acids Grand Island Biological Co., Grand Island, N. Y.). Cells were maintained at 37°C in humidity- and carbon dioxide-controlled incubators. In all experiments $\sim 5.0 \cdot 10^4$ cells were inoculated into 60-mm plastic petri dishes, allowed to grow for 48 h, and then labeled for 24 h. Cells were prelabeled with 515 mCi/mmol [^{14}C]thymidine at 0.25 μCi/ml or 1.9 Ci/mmol [^{3}H]thymidine at 4 μCi/ml (Amersham/Searle, Amersham Corp., Arlington Hgts., Ill.) in modified Eagle's medium with 10% fetal calf serum.

Ultraviolet Irradiation, ara-C Treatment, and Alkaline Sucrose Sedimentation

The details of the ara-C inhibition assay have been presented elsewhere (7). Briefly, prelabeled cells were chased for 2 h in medium containing no radioisotopes. Those prelabeled with [^{3}H]thymidine were irradiated with a General Electric Co. (Cleveland, Ohio) germicidal lamp with peak emission at 254 nm at an incident dose of 5–70 J/m^2. ara-C (Calbiochem-Behring Corp., San Diego, Calif.; 20 μM final concentration) and hydroxyurea (HU; Sigma Chemical Co., St. Louis, Mo.; final concentration 2 mM) were added to both ^{3}H- and ^{14}C-labeled cells either immediately after irradiation or after an appropriate incubation period with inhibitors in modified Eagle's medium with 10% fetal calf serum. After appropriate periods of incubation with the inhibitors, the treated and untreated cells were suspended together in cold saline (0.14 M NaCl with 0.12% EDTA) and 50 μl of cells were lysed for 1 h at room temperature in 200 μl of 1 N NaOH overlayed on 5–20%, 4-ml alkaline sucrose gradients containing 2 M NaCl. Centrifugation was in a SW56 rotor (Beckman Instruments, Inc. Fullerton, Calif.) at 20°C for 150 min at 30,000 r/min. Seven-drop fractions were collected on filter paper strips (Whatman, Inc., Clifton, N. J., No. 17), which were then given a 20-min wash in 5% trichloroacetic acid and two 15-min washes in 95% ethanol. Radioactivity in acid-insoluble material was measured in a liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Molecular weight data were analyzed by means of a PDP-11 (Digital Equipment Corp., Maynard, Mass.) computer system. Twice the difference in the reciprocal weight average molecular weights of the ^{3}H- and ^{14}C-labeled DNA is a direct measure of excess breaks formed in the DNA due to repair.

Pyrimidine Dimer Assays

The pyrimidine dimer content of DNA was determined by two-dimensional paper chromatography as previously described (11).

Endonuclease-sensitive Site Assay

This assay was performed as previously described (12), with the following modifications: DNA was isolated from cells by phenol extraction. An aliquot (0.2 ml) of the isolated DNA was transferred to a small centrifuge tube, 0.5 ml of cold 95% ethanol was added, and the DNA was collected on the wall of the tube by centrifugation. The ethanol solution was decanted, and 0.2 ml of assay buffer (0.02 M Tris [pH 8.0], 0.03 M NaCl, and 0.001 M EDTA) was added. The sample was immediately analyzed or quickly frozen for later assay. About 10 μl (1,000 cpm) of sample was added to 100 μl of assay buffer and 10 μl of *Micrococcus luteus* dimer-specific UV endonuclease. After 15 min at 37°C the sample was layered onto a gradient as described above and spun at 50,000 r/min for a time calculated to sediment the DNA halfway down the gradients. The gradients were collected onto strips and counted as described above. The profiles were then analyzed by computer for molecular weights. The number of enzyme-sensitive sites was calculated as

$$\frac{2}{M_w \text{ of DNA in presence of endonuclease}} - \frac{2}{M_w \text{ of DNA in absence of endonuclease}}$$

from $M_n = M_w/2$ when M_w is random. M_w is random if one assumes that pyrimidine dimers and, hence, UV-endonuclease-induced breaks, are randomly distributed in the DNA.

RESULTS

In a previous paper (7) we reported that breaks accumulate in UV-irradiated cellular DNA in the presence of ara-C and HU (ara-C, HU) and that those breaks persist for at least 18–20 h in the presence of the inhibitors. To examine this accumulation of breaks more closely, we conducted a series of experiments in which ara-C, HU was added to cells at various periods before and during repair of damage induced by UV light. We find that breaks accumulate in the presence of the inhibitors in a manner dependent upon the number of lesions undergoing repair during the period of incubation. Representative data are shown in Fig. 1. During the period 0–6 h after irradiation different UV light doses result in different kinetics of break accumulation. After 20 J/m^2 or 50 J/m^2 of UV light, for example, break accumulation saturates at 3 h, whereas after 5 J/m^2 of UV light, full saturation does not occur until after ~5 h of ara-C, HU treatment. Data from a variety of experiments with different doses and

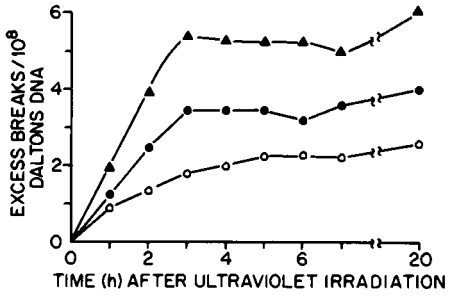

FIGURE 1 Kinetics of break accumulation in the presence of ara-C, HU. Cells were irradiated with UV light, treated with ara-C and HU (see Materials and Methods), and allowed to incubate in the presence of the inhibitors for 1, 2, 3, 4 h, etc., before being harvested and lysed on alkaline sucrose gradients. Excess breaks were calculated as described in Materials and Methods. ●, UV light dose of 20 J/m^2; ○, UV light dose of 5 J/m^2; ▲, UV light dose of 50 J/m^2.

TABLE I
KINETICS OF SATURATION OF BREAK ACCUMULATION AS A FUNCTION OF THE NUMBER OF SITES BEING REPAIRED

Ultraviolet light dose	Repair period*	Breaks repaired‡	Saturation at 3 h
J/m^2	*h*		%
5	0–6	2.6	80
5	3–9	0.4	40
10	0–6	3.4	100
20	0–6	5.1	100
20	6–12	2.6	88
20	15–21	1.0	56
30	0–6	4.8	100
30	21–27	1.8	68

*ara-C and HU were added at appropriate times after UV irradiation, and cells were collected and put on gradients after 1, 2, 3, 4, 5, or 6 h of incubation with the inhibitors.
‡This number represents the number of sites per 10^8 daltons DNA expected to be undergoing repair during the first 3 h of each repair period and is taken from data in Table II.

different time periods are presented in Table I and Fig. 2. The percent saturation seen at 3 h of continuous inhibitor treatment at various selected periods after insult is directly correlated with the amount of repair during that time period. This suggests that the inhibition is not simply the result of binding to cellular repair enzymes and the resulting closure of repair gaps but that it is occurring at the site of repair.

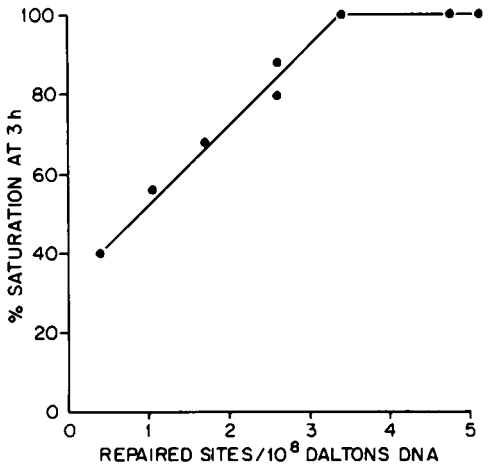

FIGURE 2

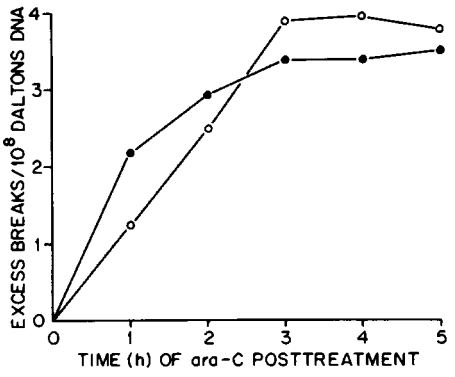

FIGURE 3

FIGURE 2 Kinetics of saturation of break accumulation as a function of number of sites undergoing repair. Experiments were conducted as described in the legend to Table I.
FIGURE 3 Effect of pretreatment of cells with inhibitor. Cells either were pretreated with ara-C, HU for 6 h before UV irradiation at 20 J/m^2 or were irradiated without a pretreatment. ara-C and HU were replaced after irradiation, and cells were harvested and placed on alkaline sucrose gradients after 1, 2, 3, 4, or 5 h of exposure to the inhibitors. ●, pretreatment; ○, no pretreatment.

To examine the effects of increased cellular concentrations of ara-C at the time of repair, we performed experiments in which cells were pretreated with ara-C, HU for 6-h periods before the UV light insult and then further incubated in the presence of the same concentrations of inhibitors for various times before being harvested and analyzed. Fig. 3 shows that cells pretreated in this manner initially displayed a higher number of breaks, which saturated in a shorter period of time, suggesting that cellular levels of ara-C (ara-CTP) are important in determining the level of inhibition seen. Studies of antagonism of repair inhibition by deoxycytidine demonstrate that the level of inhibition is also governed to a great extent by deoxycytidine pools in the cell (data to be presented elsewhere). Attempts at using higher concentrations of ara-C (up to 160 μM) in the usual posttreatment application were not effective in changing either the number of breaks observed or the kinetics of break accumulation. This suggests either that the membrane transport system is already loaded at 20 μM ara-C or that conversion of ara-C to the triphosphate is a rate-limiting step.

Because, as we have demonstrated above, under no conditions of dose or repair period does saturation of break accumulation take less than 3 h, we are able to monitor the number of breaks produced after various doses of UV light during a given 3-h period and be fairly confident that we are not excluding many breaks that would have been made had saturation not occurred. Although we expected that this procedure would not be sensitive enough to measure all putative dimer repair events, it will be seen below that a good correlation exists between observed ara-C, HU-induced breaks and the number of dimers expected to be removed from the DNA in 24 h.

Table II shows the break induction with time after various doses of UV irradiation as measured at 3-h intervals over the first 27 h after insult; Fig. 4 displays this same data graphically. At doses up to 50 J/m^2 the dose response is such that the higher the UV light dose, the more breaks are produced in the DNA for a given 3-h period. At 70 J/m^2, however, the number of breaks actually decreases from that observed at 50 J/m^2. The other general feature of these experiments is that, for all doses employed, the number of breaks per hour decreases with time after the initial insult. Moreover, at low doses (5 and 10 J/m^2) breaks are no longer observed to any extent after 9 and 21 h, respectively, whereas with higher doses (up

TABLE II

BREAKS INDUCED/10^8 DALTONS DNA DURING VARIOUS 3-h POSTIRRADIATION INCUBATION PERIODS IN THE PRESENCE OF ara-C, HU

Time of ara-C, HU addition	Breaks induced at an ultraviolet light dose (J/m^2) of					
	5	10	20	30	50	70
h						
0–3	2.6	3.4	5.1	4.8	5.6	4.6
3–6	0.4	2.7	3.0	3.3	3.8	3.6
6–9	0.1	2.3	2.7	2.6	3.7	2.4
9–12	0.1	1.9	2.3	2.3	3.1	2.1
12–15	0.1	1.4	1.3	2.0	2.5	1.9
15–18	0.0	0.8	1.0	1.8	2.2	1.7
18–21	0.0	0.2	0.6	1.8	1.9	1.2
21–24	0.0	0.2	0.6	1.7	1.5	0.5
Total, 0–24	3.3	12.9	16.6	20.3	24.3	18.0

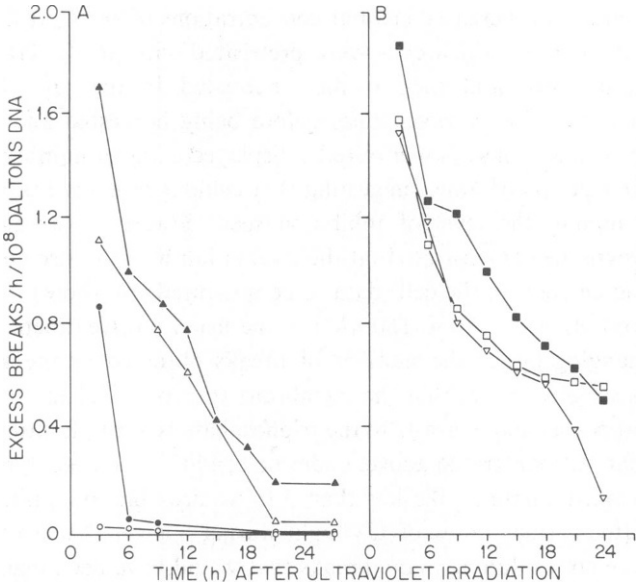

FIGURE 4 DNA strand breaks produced per hour in the presence of ara-C, HU at various times after increasing doses of ultraviolet irradiation. Cells were irradiated with UV light, and ara-C, HU was added for 3-h pulses from 0–3, 3–6, 6–9 h, etc., for the first 27 h after insult. Immediately after the 3-h pulse, cells were collected and sedimented in alkaline sucrose gradients as described. Data are from Table II. (*A*) ○, Xeroderma pigmentosum, 20 J/m^2; ●, HSBP, 5 J/m^2; △, HSBP, 10 J/m^2; ▲, HSBP, 20 J/m^2. (*B*) □, HSBP, 30 J/m^2; ■, HSBP, 50 J/m^2; ▽, HSBP, 70 J/m^2.

to 50 J/m^2) breaks are still quite evident even when ara-C, HU is added 33–35 h after insult (data not shown). Xeroderma pigmentosum fibroblasts fail to show any break induction in this assay. Dimer removal as measured by two-dimensional paper chromatography as a function of time after insult and dose of irradiation appears to continue through the time periods used in the ara-C study (Fig. 5). One may calculate the percent dimers removed from the DNA at various times after insult for various doses and from this number calculate the number of dimers expected to be removed from the DNA per 10^8 daltons. Data in Tables II and III show that a summation of breaks produced over the first 24 h after irradiation yields a number that correlates well with the number of dimers expected to be removed during that period (the number is an underestimate but probably still reflects all repaired sites; see Discussion), at least for doses up to 50 J/m^2. Above this dose, there clearly are fewer total breaks than expected.

To demonstrate that ara-C, HU is primarily blocking dimer removal and not the repair of some other lesions, we analyzed dimer removal directly, both with two-dimensional paper chromatography and by loss of UV-endonuclease-sensitive sites in cells that had been given a continuous inhibitor treatment immediately after irradiation until the time of analysis. Fig. 6 shows that, although dimer removal can be followed for at least 48 h after insult in the absence of inhibitors, within the limits of resolution of this assay, dimers are no longer removed after 6 h from cells treated with ara-C, HU. Fig. 7 demonstrates this same inhibition when endonuclease-sensitive sites are measured. No further loss of sites is observed after ~6 h

TABLE III

COMPARISON OF RATE OF DIMER REMOVAL AND APPEARANCE OF INHIBITOR-INDUCED SINGLE-STRAND BREAKS

UV light (254 nm) dose	Total dimers induced/10^8 daltons DNA*	Dimers Removed in 24 h‡	Dimers removed/ 10^8 daltons DNA	ara-C breaks§ / Dimers removed
J/m^2		%		
5	12.5	47	6	0.6
10	25	48	12	1.1
20	50	48	24	0.7
30	75	47	35	0.6
50	125	34	43	0.6
70	175	6	11	1.6

*Derived from dosimetry data in our system, which indicate that the number of dimers produced per 10^8 daltons of DNA is linear at least up to 70 J/m^2.

‡Based on two-dimensional paper chromatography data from Fig. 5.

§Summation of breaks produced in the first 24 h, from Table II.

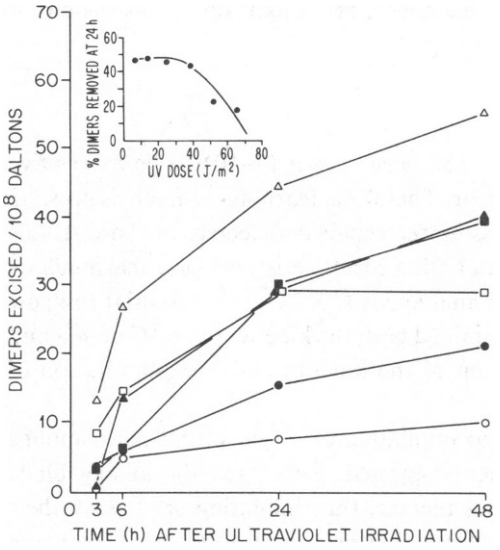

FIGURE 5

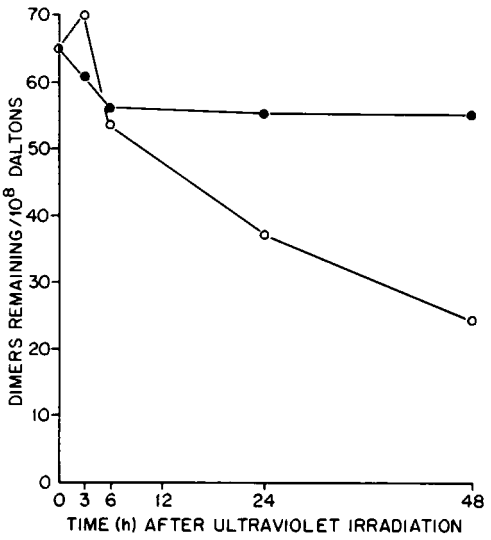

FIGURE 6

FIGURE 5 Removal of pyrimidine dimers from cellular DNA as measured by two-dimensional paper chromatography. The experimental procedures were as outlined in Materials and Methods. ○, 6.5 J/m^2; ●, 13 J/m^2; ▲, 26 J/m^2; △, 39 J/m^2; ■, 52 J/m^2; □, 65 J/m^2. The *inset* shows percent dimers removed at 24 h as a function of UV light dose.

FIGURE 6 Effects of ara-C, HU on the removal of pyrimidine dimers from cellular DNA. Cells were given 26 J/m^2 of 254-nm UV light, ara-C, HU was added at zero time, and the cells were incubated for various lengths of time before being harvested and analyzed for pyrimidine dimer content as detailed in Materials and Methods. Data points represent the average of two determinations. ●, With ara-C, HU; ○, no ara-C, HU.

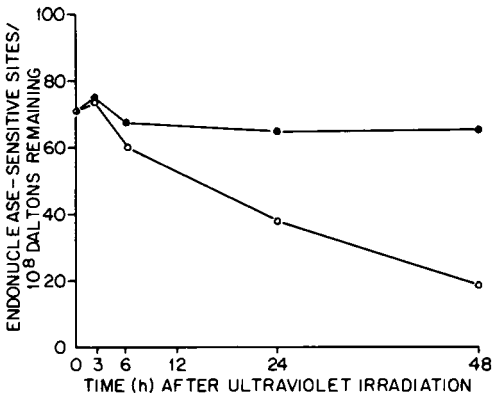

FIGURE 7 Effects of ara-C, HU on the loss of endonuclease-sensitive sites from cellular DNA. Cells were given 26 J/m^2 of 254-nm UV light, ara-C, HU was added at zero time, and cells were incubated for various lengths of time before being harvested and analyzed for endonuclease-sensitive sites as detailed in Materials and Methods. Data points represent the average of two determinations. •, With ara-C, HU; ○, No ara-C, HU.

of inhibitor treatment, whereas, there is a continuing loss of sites for up to at least 48 h in the untreated controls. This suggests that, whatever else ara-C, HU might do in this system, it inhibits loss of dimers from the DNA.

DISCUSSION

The use of ara-C, HU inhibition of DNA repair has been shown (7–10) to be a sensitive method for measuring the capacity of a cell for repair. Therefore, learning as much as possible about the basis for this inhibition will aid in assessing the repair deficiencies in those strains that show decreased repair after chemical and radiation insult. Furthermore, inasmuch as ara-C has been shown to be an effective antileukemia agent (13, 14), it is essential to know about its mode of action. In this paper we have examined both the kinetics of ara-C action and the usefulness of this technique for the elucidation of the kinetics and the quantitation of repair in mammalian cells.

Two possible mechanisms that could lead to accumulation of single-strand interruptions during repair in the presence of ara-C have been suggested. Either (*a*) the analog binds competitively to repair enzymes such as DNA polymerase, thus inhibiting or slowing their normal action (15–18), or (*b*) inhibition results from the incorporation of ara-C into the growing DNA chain at repairing sites, somehow altering the helix and preventing further repair synthesis, resulting in a gap (7, 8, 13, 14, 19–21). Since both of these events are envisioned as occurring at the site of repair, it might be predicted that the kinetics of inhibition would depend on the number of sites undergoing repair. That is, the greater the number of sites undergoing repair during a given period of time, the faster would the saturation of break accumulation be expected to be (assuming that once the repair enzyme is tied up by ara-C it is prevented from participating in other repair events). This is exactly what we observed, as shown in Table I and Fig. 2, which, supports the contention that ara-C operates at the site of repair. Note in Fig. 2 that the saturation of breaks is complete in 3 h

under conditions under which approximately three or more sites are undergoing repair per 10^8 daltons of DNA. This number of repairing sites translates to ~120,000 sites per cell and is also a good approximation of the total estimated number of DNA polymerases in a mammalian cell (22), again supporting the notion that the DNA polymerase is the repair enzyme directly involved in the inhibition.

It might further be predicted that the higher the intracellular concentration of ara-CTP, the more effective the inhibition. It has been shown by Wawra and Dolejs (23) and Bell and Fridland (24) that ara-C is taken up into cells very slowly and that once in the cell it is phosphorylated at a very low efficiency in the first hour or so. Our pretreatment studies (Fig. 3) demonstrate that the saturation of break accumulation occurs earlier with pretreatment by inhibitors, suggesting that an initially higher analog concentration allows more early repair sites to be held open.

We interpret the saturation of break accumulation not as a steady state in which equal numbers of sites are closing and forming but rather as a point at which all further repair is completely blocked. This would seem to be substantiated by the data in Figs. 6 and 7. Since only the polymerization step has been implicated in the action of ara-C (7–8, 13–15, 18–21), it seems reasonable to suggest that the repair polymerase might be "tied up" during the repair process in the presence of ara-C. This might be envisioned as occurring in one of two ways, both of which are compatible with existing hypotheses concerning the mechanism of action of ara-C. In the first case, incorporation of ara-C into the growing DNA chain could lead to helical distortion and premature chain termination due to the inability of the polymerase to insert the next base. The polymerase might "stall" at this site in an effort to continue the polymerization and would thus be removed from the pool of polymerases that could be used at other sites. A second possibility is that the large excess of ara-C over deoxycytidine triphosphate (dCTP) in the cell at the saturation time so favors the competitive binding of the analog that the polymerase essentially is halted at the site of repair. The cessation of break accumulation beyond the time when we propose that all of the DNA polymerases are tied up is consistent with the notion of a repair complex, as has been suggested (see reference 25 for a review; 26–28). A block in any of the components of the complex could be envisioned as blocking all of the activities of the complex. Thus, incision would be blocked and no new repair sites would be initiated.

The nature of the enhancement of repair inhibition by ara-C seen with HU (7) is not clear. We have recently shown (29) that the presence of HU during UV repair increases the number of bases inserted into a repairing region, and Erixon and Ahnstrom (30) have shown that HU holds UV-repairing sites open five times longer than normal. These observations are consistent with the notion that HU allows a greater level of interaction of ara-C with the repair site.

With regard to the UV repair event itself, several conclusions may be drawn from Fig. 4 and Tables II and III. First, it is clear that for all doses studied the number of breaks (repaired sites) created per unit of time decreases with increasing length of incubation after UV irradiation. Second, in the cases of 5 and 10 J/m^2 of radiation, breaks are no longer observed after 9 and 21 h, respectively, even though a fairly large number of dimers remains in the DNA. Third, the higher the dose of UV light up to 50 J/m^2, the greater the repair during a given period of time. Fourth, if the breaks that accumulate per 3-h period per 10^8 daltons of DNA for doses up to 50 J/m^2 are totalled over the first 24 hrs after insult, the sum correlates

well with the number of dimers expected to be removed during that period as measured by direct chromatographic analysis. The numbers are actually underestimates, probably due to uninhibited closure of sites repaired very rapidly after insult (8, 30) before a critical concentration of ara-C can enter the cells. We are currently developing methods by which more of this early repair may be inhibited. At 70 J/m^2, the number of breaks observed over the 24-h period drops from that seen at 50 J/m^2 and reflects the lowered dimer loss at high doses as measured chromatographically. This apparent inhibition of excision repair of pyrimidine dimers in the absence of inhibitors at high UV doses will be discussed in more detail elsewhere.

When the ara-C, HU-generated curves from Fig. 4 are superimposed on curves plotting repair synthesis, such as those of Inoue and Takebe (31), the kinetics are nearly identical. It seems likely, therefore, that most, if not all, of the repair synthesis reflects the same molecular event as the ara-C inhibition. Several lines of evidence support the notion that this event is the excision repair of pyrimidine dimers. First, xeroderma cells show no break production (Fig. 4), and it has been clearly established that these cells lack excision repair of dimers (5, 6). Second, we have shown chromatographically that ara-C, HU blocks removal of dimers, making it likely that at least some of these breaks are due to aborted dimer repair. Third, data from Tables II and III demonstrate that the total number of breaks made in response to doses of up to 50 J/m^2 of UV light in 24 h is similar to the number of dimers expected to be removed in that period of time. Moreover, that number does not exceed 10 J-equivalents of dimers even at 50 J/m^2, a finding consistent with our earlier observation that the maximum number of dimers removed in 24 h is $\sim 10^6$/cell or $25/10^8$ daltons (32). Also, at doses >50 J/m^2 the total number of breaks induced decreases, thus mimicking chromatographic data. We are currently examining various vertebrate cell culture systems in an attempt to find one possessing both photoreactivation and excision ability, so that the relationship between ara-C-induced breaks and pyrimidine dimers may be more fully substantiated.

We feel that our results strengthen the notion that repair synthesis measures dimer repair rather than the repair of some other lesion. If this indeed is the case, it would confirm the observation that repair synthesis proceeds more rapidly than does dimer removal and support the "patch and cut" model of UV repair proposed for mammalian cells by Williams and Cleaver (33). We are currently examining the use of this ara-C pulse technique after insult by chemical carcinogens and find it to be a powerful tool in the kinetic analysis of any repair proceeding via a "long-patch" (UV-like) mechanism.

This research was sponsored jointly by the Office of Health and Environmental Research, U. S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corp.; National Aeronautics and Space Administration agreement 40-565-76; and National Cancer Institute agreement 40-5-63.

R. D. Snyder is a postdoctoral investigator supported by training grant CA-09336 from the National Cancer Institute.

Received for publication 23 December 1980 and in revised form 23 April 1981.

REFERENCES

1. Cleaver, J. E. 1974. Repair processes for photochemical damage in mammalian cells. *Adv. Radiat. Biol.* 4:1–75.
2. Painter, R. B. 1974. DNA damage and repair in eucaryotic cells. *Genetics.* 78:139–148.

3. Hanawalt, P. C., E. C. Friedberg, and C. F. Fox, editors. 1978. DNA repair mechanisms. Academic Press, Inc., New York. 291–337.
4. Erixon, K., and G. Ahnstrom. 1978. Kinetics of UV-induced excision repair in human fibroblasts: simultaneous study of incision-produced single-strand breaks and the removal of endonuclease-sensitive sites using the DNA unwinding technique. *In* DNA repair mechanisms. P. C. Hanawalt, E. C. Friedberg, and C. F. Fox, editors. Academic Press, Inc. New York. 319–322.
5. Regan, J. D., R. B. Setlow, and R. D. Ley. 1971. Normal and defective repair of damaged DNA in human cells: a sensitive assay utilizing the photolysis of bromodeoxyuridine. *Proc. Natl. Acad. Sci.* U. S. A. 68:708–712.
6. Regan, J. D., and R. B. Setlow. 1974. Two forms of DNA repair in the DNA of human cells damaged by chemical carcinogens and mutagens. *Cancer Res.* 34:3318–3325.
7. Dunn, W. C., and J. D. Regan. 1979. Inhibition of DNA excision repair in human cells by arabinofuranosyl cytosine: effects on normal and xeroderma pigmentosum cells. *Mol. Pharmacol.* 15:367–374.
8. Hiss, E. A., and R. J. Preston. 1977. The effect of cytosine arabinoside on the frequency of single-strand breaks in DNA of mammalian cells following irradiation or chemical treatment. *Biochim. Biophys. Acta.* 478:1–8.
9. Collins, A. R. S., S. L. Schor, and R. T. Johnson. 1977. The inhibition of repair of UV-irradiated human cells. *Mutat. Res.* 42:413–432.
10. Johnson, R. T., and A. R. S. Collins. 1978. Reversal of the changes in DNA and chromosome structure which follow the inhibition of ultraviolet-induced repair in human cells. *Biochem. Biophys. Res. Commun.* 80:361–369.
11. Carrier, W. L., and R. B. Setlow. 1971. The excision of pyrimidine dimers (the detection of dimers in small amounts). *Methods Enzymol.* 21:230–237.
12. Waters, R. 1979. Repair of DNA in replicated and unreplicated portions of the human genome. *J. Mol. Biol.* 127:117–127.
13. Cohen, S. S. 1976. Lethality of ara-nucleotides. *Med. Biol. (Helsinki).* 54:299–326.
14. Burman, R. P. 1970. Analogues of nucleic-acid components: mechanism of action. *Recent Results Cancer Res.* 25:1–111.
15. Dicoccio, E. A., and B. I. S. Srivastava. 1977. Kinetics of inhibition of deoxyribonucleic acid polymerase enzyme activities from normal and leukemic human cells by 9-β-D-arabinofuranosyl adenine 5'-triphosphate and 1-β-D-arabinofuranosyl cytosine 5'-triphosphate. *Biochemistry.* 79:411–418.
16. Plunkett, W., and S. S. Cohen. 1975. Metabolism of 9-β-D-arabinofuranosyl adenine by mouse fibroblasts. *Cancer Res.* 35:415–422.
17. Muller, W. E. G., H. J. Rohde, R. Beyer, A. Maidhof, M. Lachmann, H. Taschner, and R. K. Zahn. 1975. Mode of action of 9-β-D-arabinofuranosyl adenine on the synthesis of DNA, RNA and protein *in vivo* and *in vitro*. *Cancer Res.* 35:2160–2168.
18. Graham, F. L., and G. F. Whitmore. 1970. Studies in mouse L-cells on the incorporation of 1-β-D-arabinofuranosyl cytosine into DNA and on the inhibition of DNA polymerase by 1-β-D-arabinofuranosyl cytosine 5'-triphosphate. *Cancer Res.* 30:2636–2644.
19. Furth, J. J., and S. S. Cohen. 1968. Inhibition of mammalian DNA polymerase by the 5'-triphosphate of 1-β-D-arabinofuranosyl cytosine and the 5'-triphosphate of 9-β-D-arabinofuranosyl adenine. *Cancer Res.* 28:2061–2067.
20. Momparler, R. L. 1972. Kinetics and template studies with 1-β-D-arabinofuranosyl cytosine 5'-triphosphate and mammalian DNA polymerase. *Mol. Pharmacol.* 8:362–370.
21. Rashbaum, S. A., and N. R. Cozzarelli. 1976. Mechanism of DNA synthesis inhibition by arabinofuranosyl cytosine and arabinofuranosyl adenine. *Nature (Lond.).* 264:679–680.
22. Kornberg, A. 1980. DNA Replication, 2nd edition, W. H. Freeman & Co., San Francisco. 210.
23. Wawra, E., and I. Dolejs. 1979. Evidences for the function of DNA polymerase beta in unscheduled DNA synthesis. *Nucleic Acids Res.* 7:1675–1686.
24. Bell, D. E., and A. Fridland. 1980. Mode of action of 9-β-D-arabinofuranosyl-adenine and 1-β-D-arabinofuranosyl-cytosine on DNA synthesis in human lymphoblasts. *Biochim. Biophys. Acta.* 606:57–66.
25. Hanawalt, P. C., P. K. Cooper, A. K. Ganeson, and C. A. Smith. 1979. DNA repair in bacteria and mammalian cells. *Annu. Rev. Biochem.* 48:783–836.
26. Cleaver, J. E., G. M. Thomas, J. E. Trosko, and J. T. Lett. 1972. Excision repair (dimer excision, strand breakage and repair replication) in primary cultures of eukaryotic (bovine) cells. *Exp. Cell Res.* 74:67–80.
27. Kleijer, W. J., and D. Bootsma. 1971. Repair of DNA damaged by UV-and X-irradiation in cultivated normal human, xeroderma pigmentosum and bloom cells. Proceedings of the 1st European Biophysics Congress, Baden, Vol. II. 129–133.
28. Kleijer, W. J., M. L. Hoeksema, M. L. Sluyter, and D. Bootsma. 1973. Effects of inhibitors on repair of DNA in

normal human and xeroderma pigmentosum cells after exposure to x-rays and ultraviolet irradiation. *Mutat. Res.* 17:385–394.

29. Francis, A. A., R. D. Blevins, W. L. Carrier, D. P. Smith, and J. D. Regan. 1979. "Inhibition of DNA repair in ultraviolet-irradiated human cells by hydroxyurea. *Biochim. Biophys. Acta.* 563:385–392.
30. Erixon, K. and G. Ahnstrom (1979). Single-strand breaks in DNA during repair of ultraviolet-induced damage in normal human and xeroderma pigmentosum cells as determined by alkaline DNA unwinding and hydroxylapatite chromatography: effects of hydroxyurea, 5-fluorouracil and 1-β-D-arabinofuranosyl cytosine on the kinetics of UV repair. *Mutat. Res.* 59:257–271.
31. Inoue, M., and H. Takebe. 1978. DNA repair capacity and rate of excision repair in ultraviolet-irradiated mammalian cells. *Jpn. J. Genet.* 58:285–295.
32. Carrier, W. L., D. P. Smith, and J. D. Regan. 1978. Pyrimidine dimer excision in human cells. *J. Supramol. Struct.* 2 (Suppl.):77.
33. Williams, J. I., and J. E. Cleaver. 1978. "Excision repair of ultraviolet damage in mammalian cells. Evidence for two steps in the excision of pyrimidine dimers. *Biophys. J.* 22:265–279.