8, 363.
Capra, J. D., and Kehoe, J. M. (1975), *Adv. Immunol. 20*, 1.
Chesebro, B., and Metzger, H. (1972), *Biochemistry 11*, 766.
Claflin, J. L., and Davie, J. M. (1974a), *J. Exp. Med. 140*, 673.
Claflin, J. L., and Davie, J. M. (1974b), *J. Immunol. 113*, 1678.
Claflin, J. L., Lieberman, R., and Davie, J. M. (1974), *J. Exp. Med. 139*, 58.
Claflin, J. L., and Rudikoff, S. (1977), *Cold Spring Harbor Symp. Quant. Biol. 41*, 725.
Gearhart, P. J., Sigal, N. H., and Klinman, N. R. (1975), *J. Exp. Med. 141*, 56.
Gearhart, P. J., Sigal, N. H., and Klinman, N. R. (1977), *J. Exp. Med. 145*, 876.
Gotschlich, E. C. (1971), *J. Immunol. 107*, 910.
Gotschlich, E. C., and Edelman, G. M. (1967), *Proc. Natl. Acad. Sci. U.S.A. 57*, 706.
Greenwood, F. C., Hunter, W. M., and Glover, J. S. (1963), *Biochem. J. 89*, 114.
Gutman, G. A., Loh, E., and Hood, L. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72*, 5046.
Gutman, G. A., and Weissman, I. L. (1971), *J. Immunol. 107*, 1390.
Hansburg, D., Briles, D. E., and Davie, J. M. (1977), *J. Immunol. 119*, 1406.
Hood, L., and Prahl, J. (1971), *Adv. Immunol. 14*, 291.
Ju, S.-T., Gray, A., and Nisonoff, A. (1977), *J. Exp. Med. 145*, 540.
Keck, K. (1972), *Immunochemistry 9*, 359.
Keuttner, M. G., Wang, A. L., and Nisonoff, A. (1972), *J. Exp. Med. 135*, 579.
Kindt, T. J. (1975), *Adv. Immunol. 21*, 35.
Leder, P., Honjo, T., Packman, S., Swan, D., Nau, M., and Norman, B. (1974), *Proc. Natl. Acad. Sci. U.S.A.* 71, 5109.
Lieberman, R., Potter, M., Mushinski, E. B., Humphrey, W., Jr., and Rudikoff, S. (1974), *J. Exp. Med. 139*, 983.
Liu, T. Y., and Gotschlich, E. C. (1963), *J. Biol. Chem. 238*, 1928.
Michaelsen, T. E., Forre, O., and Natvig, J. B. (1977), *J. Immunol. 118*, 2275.
Osmand, A. P., Mortensen, R. F., Siegel, J., and Gewurz, H. (1975), *J. Exp. Med. 142*, 1065.
Perlmutter, R. M., Briles, D. E., and Davie, J. M. (1977), *J. Immunol. 118*, 2161.
Perlmutter, R. M., and Davie, J. M. (1977), *J. Immunol. 118*, 769.
Pierce, J. A., Jeppson, J. O., and Laurell, C. B. (1976), *Anal. Biochem. 74*, 227.
Riesen, W. F., Braun, D. G., and Jaton, J. C. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73*, 2096.
Schroer, J., and Davie, J. M. (1977), *J. Immunol. 118*, 1987.
Tomasz, A. (1967), *Science 157*, 694.
Tonegawa, S. (1976), *Proc. Natl. Acad. Sci. U.S.A. 73*, 203.
Volanakis, J. E., and Kaplan, M. H. (1971), *Proc. Soc. Exp. Biol. Med. 136*, 612.
Weber, K., and Osborn, M. (1975), *Proteins, 3rd Ed. 1*, 129.
Williamson, A. R. (1976), *Annu. Rev. Biochem. 45*, 567.

Inhibition of Deoxyribonucleic Acid Chain Initiation: A New Mode of Action for 1-β-D-Arabinofuranosylcytosine in Human Lymphoblasts†

Arnold Fridland

ABSTRACT: A novel biochemical effect of 1-β-D-arabinofuranosylcytosine (*ara*-C) on DNA replication in cultured human lymphoblasts is described. By incubating the cells with [^{3}H]thymidine for 5 min and analyzing the nascent DNA by velocity sedimentation in alkaline sucrose gradients, it was possible to discern the initial effect of a very low concentration of drug (5 nM) on DNA replication. During the first 30 min of incubation, *ara*-C inhibited the initiation of new replicating units of DNA but did not affect the elongation of previously initiated units. A later effect was the reduction of the rate of DNA chain elongation. A model, based on the incorporation of *ara*-C into nascent DNA, is presented to account for a differential effect of the drug on DNA chain initiation and elongation in mammalian cells.

1-β-D-Arabinofuranosylcytosine (*ara*-C; generic name, cytarabine)[1] is a potent inhibitor of DNA replication in a variety of cell types, bacterial (Cohen, 1966) as well as mammalian (Roy-Burman, 1970), and in DNA viruses (Ch'ien et al., 1973). In clinical trials the drug has been quite effective in the treatment of acute granulocytic leukemia (Gee et al., 1969; Fleming et al., 1974). Although *ara*-C has been actively studied for over a decade, its mode of action within the cell nucleus remains controversial. Two alternative hypotheses for its inhibitory effect on DNA synthesis rest with the replicative DNA polymerase. In the first model the 5′-triphosphate of

† From the Division of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101. *Received July 1, 1977.* This work was supported by research grants CH52 from the American Cancer Society and CA-18446 from the National Cancer Institute and by ALSAC. A preliminary account was presented at the 68th Annual Meeting of the American Association for Cancer Research, May 16, 1977, Denver, Colorado (Abstract No. 256).

[1] Abbreviations used: *ara*-C, 1-β-D-arabinofuranosylcytosine; *ara*-CTP, the 5′-triphosphate of *ara*-C; SSC, 0.15 M NaCl–15 mM sodium citrate (pH 7.4); BrdUrd, bromodeoxyuridine; FdUrd, fluorodeoxyuridine.

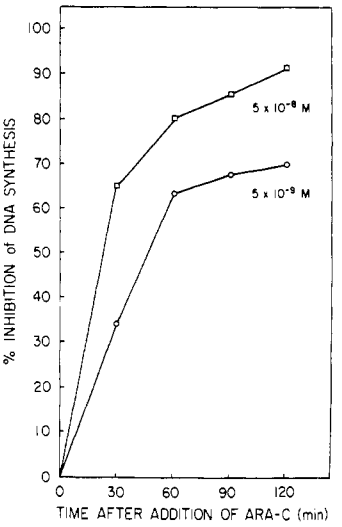

FIGURE 1: Effect of *ara*-C on DNA synthesis in CCRF-CEM cells. Cell suspensions (6×10^5 cells/mL) were incubated with either 5 nM (○) or 50 nM (□) of drug for the indicated times. At different intervals replicate 2-mL samples were collected and pulse labeled with [^{3}H]dThd for 5 min at 37 °C and the subsequent incorporation of radioactivity was determined as described previously (Fridland, 1977). The data are expressed as percent inhibition of DNA synthesis, determined by comparing the incorporation of [^{3}H]dThd in drug-treated cells with that of control cells (approximately 28 000 dpm were incorporated in a 5-min pulse).

ara-C (ara-CTP) inhibits competitively the utilization of deoxycytidine triphosphate during DNA chain elongation (Furth and Cohen, 1968; Graham and Whitmore, 1970; Rama-Reddy et al., 1971). In the second model *ara*-CTP is incorporated into the growing DNA chain, where it prevents further chain elongation catalyzed by the polymerase (Momparler, 1969, 1972; Kornberg, 1974).

Recently, I reported preliminary studies that implicated a new mode of action for *ara*-C, namely, the inhibition of initiation of replication units in DNA (Fridland, 1977). Presented here is a more detailed analysis of the drug's inhibitory effect on replicon initiation as well as chain elongation. The results indicate that *ara*-C preferentially affects the former process without an immediate effect on the latter.

Materials and Methods

Cells. Human lymphoblasts (CCRF-CEM line) were cultured in suspension as reported earlier (Fridland, 1977).

Analysis of DNA Size. Cells were incubated, with or without *ara*-C, at 37 °C and at various intervals pulse labeled with [^{3}H]dThd (10 μCi/mL, spec act. 60 Ci/mmol) for 5 min. The pulse was terminated by washing the cells with ice-cold SSC (0.15 M NaCl–15 mM sodium citrate) containing 10 M KCN and 8 mM EDTA. In pulse-chase experiments, medium containing [^{3}H]dThd was removed, and the cells were washed with SSC and incubated for the desired time in fresh medium containing unlabeled dThd (10 μM) with or without *ara*-C. After these incubations, the cells were washed with ice-cold SSC containing KCN and EDTA, resuspended in the same buffer, and layered on a lysing solution on top of a 36-mL, 15–30% alkaline sucrose gradient containing 0.5 M NaCl, 0.25 M NaOH, and 0.001 M EDTA. About 4×10^5 cells in 0.1 mL of SSC were layered onto 0.4 mL of lysing solution (0.2 M NaOH, 0.02 M EDTA, 0.1% Nonidet NP-40) and allowed to lyse on the gradient in the dark at 37 °C for 6 h. Gradients were centrifuged at 26 000 rev/min for 4 h in a Beckman SW 27 rotor at 15 °C and fractionated with an ISCO density gradient fractionator. The DNA of each fraction was precipitated on Whatman GF/A filters, washed 4–5 times with 5% trichloroacetic acid, once with ethanol, and dried.

Weight-average molecular weights ($\overline{M}_w$) of the labeled DNA in gradients were calculated from the relationship $\overline{M}_w = \Sigma(C_i \times M_i)/\Sigma C_i$, where C_i is dpm per min in the ith fraction and M_i the molecular weight of molecules in the ith fraction as calculated by Studier's relationship (Studier, 1965). A variable amount of radioactivity (15–20%) was often found in the bottom of the gradients, probably as a result of wall effects during sedimentation (Schumaker, 1967). These bottom fractions were always excluded from the determination of molecular weights.

Isopycnic Centrifugation in Alkaline CsCl Gradients. For these gradients about 4.2 g of CsCl was dissolved in 4.5 mL of sodium phosphate buffer, pH 12. About 0.1 mL of a solution containing 100 μg of DNA, isolated as described previously (Fridland, 1977), was added to the CsCl solution in polyallomer tubes. The specific gravity of the gradient was adjusted to 1.6850 g/mL and the tubes were centrifuged for about 30 h at 40 000 rev/min in a Beckman SW 50.1 rotor at 20 °C. Fractions of 0.3 mL were collected from the bottom of the gradient, and each fraction was precipitated on Whatman GF/A filter discs, washed 4–5 times with 5% trichloroacetic acid, once with ethanol, and dried, and the radioactivity in each filter was determined by liquid scintillation spectrometry.

Results

Kinetics of Inhibition of DNA Synthesis in CCRF-CEM Cells. When CCRF-CEM cells were incubated with 5 nM or 50 nM *ara*-C, the overall rate of DNA synthesis decreased rapidly and remained depressed for at least 2 h (Figure 1). Addition of 10 μM deoxycytidine to the medium with *ara*-C prevented inhibition of DNA synthesis (data not shown), presumably because it blocked the formation of *ara*-CTP from *ara*-C by competing for deoxycytidine kinase. Neither cytidine or uridine at concentrations of 100 μM prevented the inhibitory effects of *ara*-C.

Analysis of DNA Sizes by Velocity Sedimentation. Drug-induced inhibition of DNA synthesis in growing cells is mediated either by the rate of polymerization within individual replicating units (replicons) or by a decrease in the number of replicons operating per unit time, or both. The sedimentation properties of pulse-labeled DNA were therefore analyzed in alkaline sucrose gradients as a means of distinguishing between these possibilities. CCRF-CEM cells were incubated in the presence or absence of *ara*-C for 5 min with [^{3}H]dThd and DNA was analyzed by velocity sedimentation in 15–30% alkaline sucrose gradients. In untreated cells, the main peak of radioactivity was associated with DNA corresponding to a molecular mass of about 2.6×10^7 daltons (49 S); more than 50% of the total radioactivity was found in fragments that sedimented between 10^7 to 10^8 daltons (Figure 2). This distribution of radioactivity is typical of a growing population of cells, in which there are nascent DNA strands of all sizes, ranging from a few linked nucleotides to completed replicons. Similar radioactive profiles have been observed in other cell lines pulse labeled with [^{3}H]dThd, and their origin was interpreted in a similar manner (Gautschi et al., 1974; Huberman and Horwitz, 1973).

When cells were pulse labeled for 5 min, immediately after addition of 5 nM *ara*-C, the radioactivity profiles of gradient fractions of control and drug-treated cells were essentially alike. As the time between drug addition and pulse labeling was increased, the peaks of radioactivity shifted gradually toward longer DNA strands. Thirty minutes after drug addition (Figure 2A), the incorporation of radioactivity into fractions

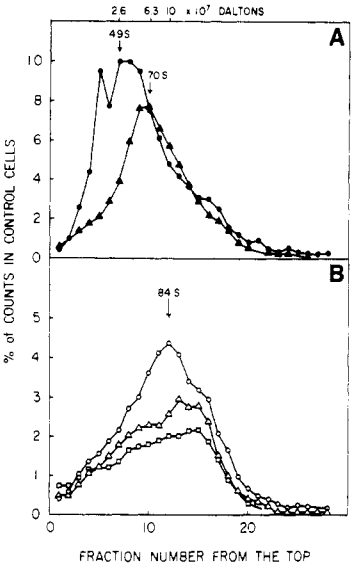

FIGURE 2: Velocity sedimentation profiles of alkaline sucrose gradients of DNA from CCRF-CEM cells. Cell suspensions (6×10^5 cells/mL) incubated in the absence (●—●) or presence of 5 nM *ara*-C were pulse labeled with 10 μCi of [^{3}H]dThd per mL (spec act. 60.7 Ci/mmol) at different intervals: 30 min (▲—▲); 60 min (○—○); 120 min (△—△); and 150 min (□—□). After each pulse the cells were washed with ice-cold SSC, resuspended in the same buffer and the DNA analyzed in alkaline sucrose gradients as described in Materials and Methods. The incorporation of total [^{3}H]dThd into DNA was 0.71 of the control value after 30 min, 0.52 after 60 min, 0.38 after 120 min, and 0.32 after 150 min. The percentages of radioactivity present in the gradient fractions for cells incubated for these four time periods were therefore multiplied by either 0.71, 0.52, 0.38, or 0.32 to yield the data points shown in the figure.

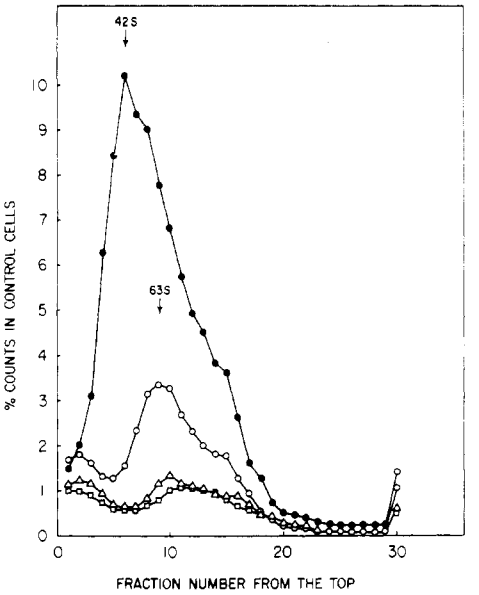

FIGURE 3: Velocity sedimentation profiles of alkaline sucrose gradients of pulse-labeled DNA. Cell suspensions (6×10^5 cells/mL) incubated without (●—●) or with 50 nM cytarabine were pulse labeled with 10 μCi [^{3}H]dThd for 5 min at different time intervals: 30 min (○—○); 60 min (△—△); and 120 min (□—□). After each pulse the DNA was analyzed in alkaline sucrose gradients as described for Figure 2. At this drug concentration, [^{3}H]dThd incorporation into DNA was 0.38 of the control value after 30 min, 0.2 after 60 min, and 0.17 after 120 min.

near the tops of the gradients (fractions 2–8), where the most recently initiated replicons should be found, was suppressed by more than 50%. On the other hand, there was no detectable effect on the incorporation of radioactivity into the region of the gradient (fractions 10–16) that contained the larger DNA fragments. After 1 h, the incorporation of radioactivity into smaller DNA strands was suppressed further, while incorporation into higher molecular weight DNA strands was, by comparison, only slightly affected (Figure 2B). By 2 h or 2.5 h, the peak incorporation of label was near fraction 16, the position where bulk-size DNA is found in these gradients. This result suggests that replicons present in the cells when *ara*-C is added continue to elongate and to join with each other despite exposure to the drug.

Similar kinetic studies were performed with cells treated with 50 nM *ara*-C. Again, with increasing periods of drug treatment, the average size of newly synthesized DNA increased (Figure 3). At this concentration of drug, the incorporation of radioactivity into fractions of newly initiated replicons was suppressed almost entirely. However, besides inhibiting initiation, *ara*-C at 50 nM also inhibited chain elongation, as shown by the reduced amount of radioactivity in the high-molecular-weight region of the gradients (Figure 3, fractions 10–16).

Effects of ara-C on Nascent Strand Elongation. The effect of *ara*-C on nascent strand elongation was examined in greater detail in pulse-chase experiments. Exponentially growing CCRF-CEM cells were pulse-labeled for 5 min with [^{3}H]dThd in the absence or presence of drug and chased with unlabeled thymidine. After each period of pulse or chase, the DNA was analyzed by alkaline sucrose gradient centrifugation. The results, depicted in Figures 4A–E, are typical of the kinetics obtained when pulse-labeled DNA is converted to high-molecular-weight strands in control cells. To facilitate the presentation of data for a large number of gradients, the weight-average molecular weight of [^{3}H]DNA was calculated for each time interval as described in Materials and Methods. Calculations were done for both control and drug-treated CCRF-CEM cultures. As shown in Figure 5, the time required to convert pulse-labeled DNA of control cells to high-molecular-mass DNA strands of 1.7×10^8 daltons is about 60 min, similar to the result obtained for the completion and fusion of adjacent replicons, as determined by autoradiography (Huberman and Tsai, 1973; Hori and Lark, 1973; Hand and Tamm, 1974).

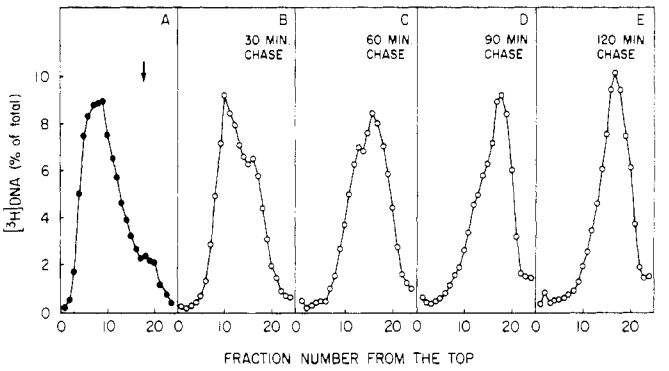

FIGURE 4: Sedimentation profiles of [^{3}H]dThd-dThd pulse-chase labeled cells. Cells were pulse labeled for 5 min (●—●) (A) and [^{3}H]dThd, washed and chased with unlabeled dThd (1 μM) for 30 min (B), 60 min (C), 90 min (D), or 120 min (E). Before incubation with [^{3}H]dThd, the cells were grown for 16 h in the presence of [^{14}C]dThd (0.02 μCi/mL) to label bulk DNA. The arrow shows the position of the ^{14}C-prelabeled DNA. Labeled DNA was analyzed by velocity sedimentation as described in Materials and Methods.

In the presence of 5 nM *ara*-C, there was no detectable effect on the size of DNA synthesized during the pulse and during the first 30 min of chase (Figure 5). However, as the

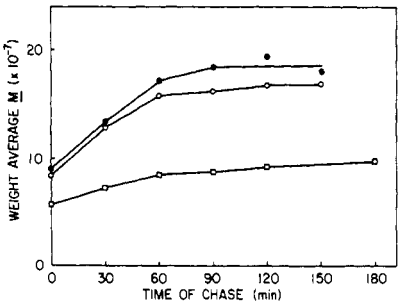

FIGURE 5: Rate of formation of high-molecular-weight nascent DNA strands. Each symbol represents the average value of two separate experiments in which [^{3}H]dThd–dThd pulse-chase labeled cells were incubated in the absence (●—●) or presence of 5 nM (○—○) or 50 nM (□—□) *ara*-C as described for Figure 4. The weight-average molecular weights were calculated as described in Materials and Methods.

time of incubation increased, the elongation of nascent DNA appeared to slow, beginning at about 60 min and continuing to at least 150 min. Increasing the concentration of *ara*-C to 50 nM caused a more pronounced inhibition of chain elongation: the size of DNA made after a 5-min pulse was about one-third the size of DNA made at lower drug concentrations, and strand elongation proceeded at a very slow rate. Residual DNA synthesis dropped to nearly zero after 1 h and nascent DNA could not be chased into bulk-size strands.

Chain elongation in the presence of *ara*-C was further analyzed by assessing the density distribution of DNA molecules synthesized after incubation with [^{3}H]bromodeoxyuridine (BrdUrd). Substituting BrdUrd for dThd during DNA synthesis in cells will raise the buoyant density of newly synthesized DNA in isopycnic gradients, thus providing a method to determine relative rates of chain elongation (Gautschi et al., 1973; Bonhoeffer and Gierer, 1963).

CCRF-CEM cells were incubated in the absence or presence of *ara*-C for 20 min with [^{3}H]BrdUrd. The cells were washed free of radioactivity and then incubated for 3 h with unlabeled thymidine. The DNA was isolated from cells, sheared to fragments of about 26 × 10^6 daltons, and analyzed by equilibrium centrifugation in alkaline CsCl gradients. As shown in Figure 6, the incorporation of [^{3}H]BrdUrd by control cells caused a sharp increase in the buoyant density of newly synthesized DNA strands. When [^{3}H]BrdUrd was added during the first 20 min of incubation with 5 nM *ara*-C, DNA synthesis was inhibited by 20%, but the density of pulse-labeled DNA shifted to the same position as that of the control cells (Figure 6). These results demonstrate that DNA synthesized during the first 20 min in the presence of 5 nM *ara*-C is the same size as that of control cells, and confirm the finding (Figure 2) that some step of the replication process is more sensitive than chain elongation to inhibition by *ara*-C.

In the presence of 50 nM *ara*-C, the density of [^{3}H]DNA was substantially reduced (Figure 6), indicating that the rate of chain elongation was rapidly inhibited by this concentration of drug. Finally, when CCRF-CEM cells were pretreated with *ara*-C for 1 h before pulse-chase analyses with [^{3}H]BrdUrd and unlabeled thymidine, the rate of chain elongation was inhibited more strongly by either drug concentration. As shown in Figure 7, 5 nM *ara*-C caused a 60% reduction of the density shift in the peak fraction, whereas an 85% reduction was obtained with 50 nM of drug.

Discussion

In this study, DNA replication in CCRF-CEM cells was immediately inhibited when low concentrations of *ara*-C were added to the growth medium. During the first 30 min of incubation with 5 nM of drug, the initiation of new DNA chains decreased sharply, but the process of chain elongation was not affected (cf. Figure 2 with Figure 5 or 6). Earlier investigators (Graham and Whitmore, 1970; Magnusson et al., 1974; Wist et al., 1974) of the mode of action of *ara*-C concluded that the drug acts primarily by inhibiting the process of chain elongation. These studies, however, made use of drug concentrations ranging from 5 μM to 0.1 mM, so that any effects on the initiation of replicons would likely be masked by the drastic reduction of chain elongation.

DNA replication in the nucleus of mammalian cells is thought to involve time-dependent, bidirectional synthesis of replicons which, after completion, join together to form complete DNA molecules (Huberman and Horwitz, 1973; Huberman and Tsai, 1973; Hori and Lark, 1973; Hand and Tamm, 1974). From autoradiographic studies, the average size of replicons in mammalian cells has been estimated to range from 30 to 60 × 10^6 daltons (Hori and Lark, 1973; McFarlane and Callan, 1973; Hand, 1975; Housman and Huberman, 1975). In alkaline sucrose gradients, the most frequent size of

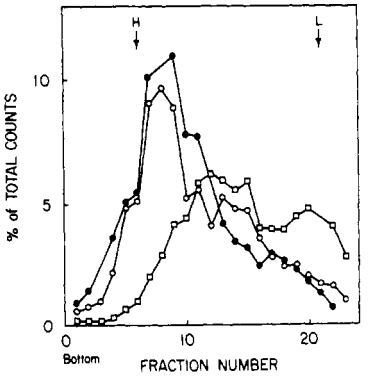

FIGURE 6: Density distribution of DNA in cells pulse labeled with [^{3}H]BrdUrd. Cells (6 × 10^5 cells/mL) were incubated without (●—●) or with 5 nM (○—○) or 50 nM (□—□) *ara*-C and pulse labeled for 20 min with 12 μCi of [^{3}H]BrdUrd per mL (spec act. 14 Ci/mmol) and unlabeled 1 μM FdUrd to deplete the thymidine nucleotide pools. After incubation, the cells were washed and reincubated for 5 min in medium devoid of radioactivity. Radioactivity in DNA was chased for 3 h in medium containing unlabeled 1 μM dThd. The arrows indicate the position of normal-density light strands (L) and BrdUrd-containing DNA strands (H) obtained from cells incubated for about 8 h with unlabeled BrdUrd (10 μM) and dCyd (10 μM). DNA isolation and centrifugation were carried out in alkaline CsCl gradients as described in Materials and Methods.

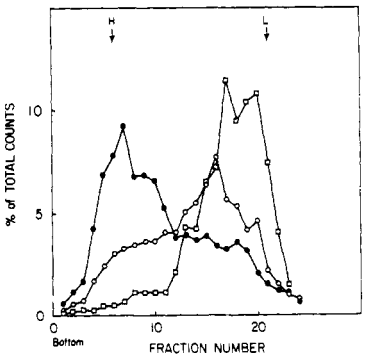

FIGURE 7: Density distribution of DNA in cells pulse labeled with [^{3}H]BrdUrd. Cells (6 × 10^5/mL) were incubated without (●—●) or with 5 nM (○—○) or 50 nM (□—□) *ara*-C and pulse labeled 1 h later for 20 min with [^{3}H]BrdUrd in the presence of unlabeled FdUrd. After incubation, the cells were washed and reincubated in medium devoid of radioactivity, and the radioactivity in DNA was chased for 3 h with unlabeled 1 μM dThd. The conditions of radiolabeling, isolation, and centrifugations are as described for Figure 6.

single-stranded pulse-labeled DNA of cells not treated with *ara*-C is 26×10^6 daltons (Figure 2). Thus, a 5-min pulse of [^{3}H]dThd in these human lymphoblasts labels DNA segments that are somewhat smaller than replicon size. During inhibition of chain initiation by *ara*-C, the radioactivity incorporated during a pulse is associated with DNA of much larger molecular weight than that of control cells. After a 30-min treatment with 5 nM *ara*-C, the peak of radioactivity was associated with DNA of about 6.3×10^7 daltons; at 1 h the peak was about 1×10^8 daltons; and by 2 h it was about 1.5×10^8 daltons (Figure 2). Thus, DNA strands that have been initiated before the addition of *ara*-C appear to elongate to DNA chains about three times the size of normal replicons. A similar alteration in the size distribution of pulse labeled DNA has been reported for Hela cells and Chinese hamster cells during inhibition of replicon initiation after exposure to low doses of ionizing radiation (Painter and Young, 1976).

These observations have several possible explanations. First, when the initiation of new DNA chains is blocked by agents such as *ara*-C or x-rays, the reduced number of growing DNA chains might replicate beyond their normal termination sites. Alternatively, the initiation or termination sites along the DNA chain might be variable (rather than fixed according to specific base sequences), so that drug or radiation effects on DNA could increase the distance between points of replicon formation (McFarlane and Callan, 1973). A third possibility is that larger DNA sizes reflect the existence of clusters of very large replicons in the mammalian genome (Painter and Young, 1976).

The biochemical route by which *ara*-C inhibits DNA replication in vivo is still unclear. Some workers have proposed the replicating DNA polymerase as a primary target of the drug (Furth and Cohen, 1968; Graham and Whitmore, 1970). The inhibition constant (K_i) of DNA polymerase from mammalian cells, however, occurs at drug concentrations ranging from 1 μM to 20 μM (Furth and Cohen, 1968, Graham and Whitmore, 1970; Rama-Reddy et al., 1971; Schrecker et al., 1974), at least 200-fold higher than the level of *ara*-CTP required for 50% inhibition of the overall rate of DNA synthesis in mammalian cells (Figure 1; also Graham and Whitmore, 1970; Chou et al., 1975). Moreover, a primary effect on DNA polymerase would not be expected to cause a differential effect on chain initiation and elongation.

The observations reported here are very similar to those reported recently for DNA exposed to x-rays or methylmethanesulfonate and for BrdUrd-substituted DNA exposed to 313 nm of light, in that all of these agents exerted a primary effect on replicon initiation (Painter and Young, 1976; Painter, 1977; Povirk and Painter, 1976). The main lesions produced by the agents are single-stranded DNA breaks and base damage. Povirk and Painter (1976) have suggested that such lesions, when occurring at or near the site of initiation of replicon clusters, can cause a change in the supercoiled structure of DNA, thus preventing the binding of initiation factors so that new replicons cannot be initiated.

There is now good evidence that *ara*-C is incorporated into nascent DNA in vivo (Graham and Whitmore, 1970; Chu and Fischer, 1968; Magnusson et al., 1974; Chou et al., 1975), which could cause a configurational change in the DNA helix (Silagi, 1965). The presence of any *ara*-C residues at the site of replicon origin in newly initiated DNA strands might be expected to alter the structure of the replication complex in this region, so that further growth of these short DNA chains would be blocked. The absence of an immediate drug effect on replicons that were already well into the process of elongation when *ara*-C was added to cell cultures indicates that the processes of elongation and joining are not appreciably affected by subtle structural changes in nascent DNA units. However, as more *ara*-C residues are incorporated into growing DNA chains, the structure of the DNA helix would become sufficiently altered to inhibit further polymerization, consistent with the reduced rate of chain elongation seen in these experiments after 30 min of incubation.

Acknowledgments

I am grateful to Mr. James McLellan for excellent technical assistance.

References

Bonhoeffer, R., and Gierer, A. (1963), *J. Mol. Biol. 7*, 534.
Ch'ien, L. T., Schabel, F. M., Jr., and Alford, C. A., Jr. (1973), in Selective Inhibitors of Viral Functions, Carter, W. A., Ed., Cleveland, Ohio, CRC Press, p 227.
Chou, T. C., Hutchison, D. J., Schmid, F. A., and Phillips, F. S. (1975), *Cancer Res. 35*, 225.
Chu, M. Y., and Fischer, G. A. (1968), *Biochem. Pharmacol. 17*, 752.
Cohen, S. S. (1966), *Prog. Nucleic Acid Res. 5*, 1.
Fleming, I., Simone, J., Jackson, R., Johnson, W., Walters, T., and Mason, C. (1974), *Cancer 33*, 427.
Fridland, A. (1977), *Biochem. Biophys. Res. Commun. 74*, 72.
Furth, J. J., and Cohen, S. S. (1968), *Cancer Res. 28*, 2061.
Gautschi, J. R., Kern, R. M., and Painter, R. B. (1973), *J. Mol. Biol. 80*, 393.
Gee, T. S., Yu, K.-P., and Clarkson, B. D. (1969), *Cancer 23*, 1019.
Graham, F. L., and Whitmore, G. F. (1970), *Cancer Res. 30*, 2636.
Hand, R. (1975), *J. Cell Biol. 64*, 89.
Hand, R., and Tamm, I. (1974), *J. Mol. Biol. 82*, 175.
Hori, T., and Lark, K. G. (1973), *J. Mol. Biol. 77*, 391.
Housman, D., and Huberman, J. A. (1975), *J. Mol. Biol. 94*, 173.
Huberman, J. A., and Horwitz, H. (1973), *Cold Spring Harbor Symp. Quant. Biol. 38*, 233.
Huberman, J. A., and Tsai, A. (1973), *J. Mol. Biol. 75*, 5.
Kornberg, A. (1974), DNA Synthesis, San Francisco, Calif., Freeman, p 226.
Magnusson, G., Craig, R., Narkhammar, M., Reichard, P., Staub, M., and Warner, H. (1974), *Cold Spring Harbor Symp. Quant. Biol. 39*, 227.
McFarlane, P. W., and Callan, H. G. (1973), *J. Cell Sci. 13*, 821.
Momparler, R. L. (1969), *Biochem. Biophys. Res. Commun. 34*, 464.
Momparler, R. L. (1972), *Mol. Pharmacol. 8*, 362.
Painter, R. B. (1977), *Mutat. Res. 42*, 299.
Painter, R. B., and Young, B. R. (1976), *Biochim. Biophys. Acta 418*, 146.
Povirk, L. F., and Painter, R. B. (1976), *Biochim. Biophys. Acta 432*, 267.
Rama-Reddy, G. V., Goulian, M., and Hendlers, S. S. (1971), *Nature (London), New Biol. 234*, 286.
Roy-Burman, P. (1970), *Recent Results Cancer Res. 25*, 1.
Schrecker, A. W., Smith, R. G., and Gallo, R. C. (1974), *Cancer Res. 34*, 286.
Schumaker, V. N. (1967), *Adv. Biol. Med. Phys. 11*, 245.
Silagi, S. (1965), *Cancer Res. 25*, 1446.
Studier, F. W. (1965), *J. Mol. Biol. 11*, 373.
Wist, E., Krakan, H., and Prydz, H. (1974), *Biochemistry 15*, 3647.