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Hydroxyurea-Induced Accumulation of Short Fragments During Polyoma DNA Replication

I. Characterization of Fragments

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Hydroxyurea treatment of 3T6 mouse fibroblast cells infected with polyoma virus resulted within 15 min in more than a 20-fold reduction of the rate of both viral and cellular DNA synthesis. After the initial rapid inhibition, the rate of DNA synthesis remained essentially constant for at least 2 h. In the inhibited cells viral DNA accumulated as short chains with a sedimentation coefficient of about 4*S* (hydroxyurea fragments). A variable proportion of these fragments was released from the template strands when the viral DNA was extracted by the Hirt procedure. Reannealing experiments demonstrated that hydroxyurea fragments were polyoma-specific and probably synthesized on both parental strands at the replication forks.

Hydroxyurea is a specific inhibitor of DNA synthesis (22). The drug inhibits purified ribonucleotide reductase from either *Escherichia coli* (5) or from mammalian cells (11), and it appeared likely that this inhibition is the basis for the effect of hydroxyurea on DNA synthesis. Strong evidence supporting this concept comes from measurements of deoxyribonucleoside triphosphate pools in secondary mouse embryo cells (17). Addition of hydroxyurea to the medium gave a very rapid depletion of the intracellular dGTP pool followed by a somewhat slower decrease of the dATP pool. The inhibition of DNA synthesis paralleled the disappearance of the dGTP pool.

Recent experiments from this laboratory (8) showed that inhibition of DNA synthesis in polyoma virus-infected 3T6 mouse fibroblast cells by hydroxyurea resulted in the accumulation of newly synthesized short fragments of polyoma DNA with a sedimentation coefficient of about 4*S* (hydroxyurea fragments). Fragments of similar size were also found upon incubation of nuclei isolated from polyoma-infected cells and were shown to be intermediates during polyoma DNA replication. Both types of fragments contained, at least in part, short stretches of RNA which were attached to the 5'-end of the normal intermediates. It was suggested that the accumulation of short fragments in the presence of hydroxyurea might be caused by a preferential inhibition of one of the reactions involved in the joining of the fragments.

This communication describes the characterization of the 4*S* fragments formed in 3T6 cells after addition of hydroxyurea. In the accompanying paper, experiments with isolated nuclei obtained from hydroxyurea-treated cells are reported.

MATERIALS AND METHODS

For many of the details concerning cells, virus, and general methodology previous publications should be consulted (9, 21).

Cells and virus. Growing cultures of 3T6 cells were infected with polyoma virus at a multiplicity of 20 PFU per cell. All experiments were done 26 to 28 h postinfection.

Chemicals. ^{3}H-thymidine (^{3}H-dThd) (20 Ci/mmol) was obtained from New England Nuclear Corp. Bio-Gel P-30 was purchased from Bio-Rad Laboratories. Hydroxyapatite was prepared as described by Bernardi (1). Hydroxyurea was obtained from Squibb Institute for Medical Research and recrystallized from hot ethanol before use.

Enzymes. *E. coli* exonuclease I was purified as described by Lehman and Nussbaum (6) and included chromatography on DEAE-cellulose. *E. coli* exonuclease III, purified as described by Richardson and Kornberg (14), was a gift from Lambert Skoog. Both enzymes were free from endonuclease activity as judged from lack of inhibition by tRNA.

Hydroxyurea treatment of cells. A 0.2 M solution of hydroxyurea, freshly prepared in Tris-buffered saline (3), was added directly to the medium to give a final concentration of 0.01 M.

Labeling of cells with ^{3}H-dThd. Pulses with ^{3}H-dThd were performed by direct addition of the labeled nucleoside to the culture medium to a final

concentration of 1.0 μM. When pulse chases were done, a concentrated solution of dThd and dCyd was added directly to the medium to give a 100 μM final concentration of each nucleoside. After either the labeling or chase period, the medium was removed, and the cell monolayers were washed three times with ice-cold Tris-buffered saline. The cells were then immediately lysed with 0.7% (wt/vol) sodium dodecyl sulphate (SDS), 0.025 M Tris-hydrochloride, pH 7.6, and 0.005 M EDTA (2.5 ml/15-cm petri dish). Viral and cellular DNA were separated as described by Hirt (4). The DNA in the resulting Hirt supernatant fluids and precipitates will be referred to as viral and cellular DNA, respectively.

Sucrose gradient centrifugation of viral DNA. Analytical sucrose gradient centrifugations were done as described previously (21), except for the use of a Spinco SW56 rotor. Centrifugations were for 2.5 h at 55,000 rpm at 4 C. For preparative purposes, 2.0-ml samples were sedimented through 35-ml, neutral 5 to 20% sucrose gradients for 18 h at 27,000 rpm in a Spinco SW27 rotor at 4 C.

Purification of replicating viral DNA. In a typical experiment, 11 ml of Hirt supernatant fluid was treated three times with 10 ml of a mixture of water-saturated phenol and chloroform (1:1). The deproteinized aqueous phase (10 ml) was then desalted by filtration through a column of Bio-Gel P-30 (75-ml bed volume) equilibrated with 0.01 M Tris-hydrochloride, pH 7.6, 0.25 M NaCl, and 0.001 M EDTA. After elution, the viral DNA (13.5 ml) was adsorbed to a 2-ml column of benzoylated-naphthoylated DEAE-cellulose (BND-cellulose). Closed, circular (form I) DNA (20) was eluted with buffer containing 1.0 M NaCl and replicative intermediates (RI) (2, 7, 10, 16) of polyoma DNA were then eluted with 2.0 ml of buffer containing 2% caffeine. Hydroxyurea fragments could be further purified by preparative neutral sucrose centrifugation of the heat-denatured (100 C for 5 min) caffeine eluate. Fractions containing the short fragments were pooled (8 ml) and precipitated with two volumes of isopropanol at −20 C. The resulting precipitate was collected by centrifugation and dissolved in 0.50 ml of 0.40 M sodium phosphate buffer, pH 6.8.

Reannealing of DNA. Reannealing experiments were done with the purified and concentrated 4S fragments after heating for 10 min at 100 C. Annealing to DNA on filters was performed as described previously (21). Self-annealing experiments were carried out in 0.40 M phosphate buffer by incubating 10- or 20-μliter portions of the concentrated preparation for different time periods at 68 C in sealed glass ampoules. To terminate the reaction, samples were diluted with 1 ml of cold 0.12 M phosphate buffer. Single-stranded and native DNA were separated after adsorption to hydroxyapatite (100-μliter packed volume) by using the procedure of Thoren et al. (19). Single-stranded DNA was eluted at 60 C, and native DNA was eluted at 100 C with 0.12 M phosphate buffer.

Exonuclease digestion of viral DNA. Hirt supernatant fluids were used immediately after dialysis against 0.01 M Tris-hydrochloride, pH 8.0, and 0.15 M NaCl. The reactions were carried out in a total volume of 0.50 ml with 0.40 ml of dialyzed Hirt supernatant fluid. The reaction mixture for exonuclease I contained 0.067 M glycine buffer, pH 9.5, 6.7 mM $MgCl_2$, 1.0 mM β-mercaptoethanol, 0.025 M $(NH_4)_2SO_4$, 20 μg of bovine serum albumin, and three units of enzyme. The exonuclease III reaction mixture contained 0.01 M Tris-hydrochloride, pH 8.0, 1.0 mM $MgCl_2$, 1.0 mM β-mercaptoethanol, 20 μg of bovine serum albumin, and 10 units of enzyme. After 30 min of incubation at 37 C, 0.1-ml portions were removed and analyzed for acid-insoluble radioactivity and sedimentation properties.

RESULTS

Kinetics of hydroxyurea inhibition. Hydroxyurea was added to 3T6 cultures 26 h after infection with polyoma virus, and DNA synthesis was measured by pulse-labeling with ^{3}H-dThd for 10-min periods at different times after addition of the drug (Fig. 1). Viral and cellular DNA were separated by the Hirt procedure and analyzed for radioactivity. As measured by this method the rate of polyoma DNA synthesis decreased to about 3% of the control value within 25 min. During the following 2 h, there was only a small additional decrease of the rate

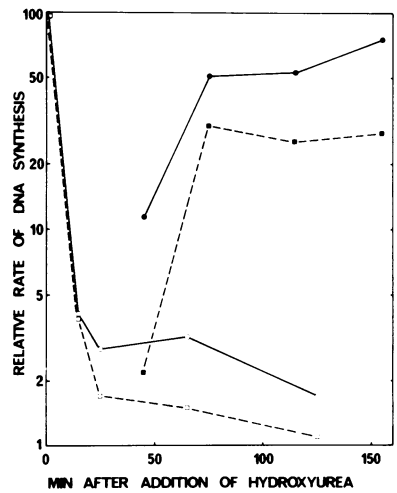

FIG. 1. *Time course of inhibition of viral and cellular DNA synthesis by hydroxyurea. The rate of DNA synthesis was measured by 10-min pulses of ^{3}H-dThd at different times after addition of hydroxyurea to polyoma virus-infected cells. In one set of cultures, the inhibitor was removed by a washing 35 min after addition of the drug in order to study reversal of inhibition. Acid-insoluble radioactivity was measured in viral (○) and cellular (□) DNA, and the amount of radioactivity from each point was normalized by relating it to the total DNA content of the cells. 100% corresponds to 733 counts per min per μg of DNA for viral DNA and 1,402 counts per min per μg of DNA for cellular DNA. Filled symbols represent cultures after removal of hydroxyurea.*

of ^{3}H-dThd incorporation. The synthesis of cellular DNA was still more sensitive to hydroxyurea. After 25 min cellular DNA synthesis had decreased to less than 2% and then remained almost constant during the next 2 h. When cells were labeled for 20 to 120 min with ^{3}H-dThd, the amount of ^{3}H-label incorporated into both viral and cellular DNA showed a linear relationship with time (data not shown).

The inhibition of DNA synthesis was reversible. Removal of hydroxyurea after 35 min and continued incubation in fresh medium resulted in rapidly resumed synthesis of DNA (Fig. 1).

Sedimentation properties of viral DNA synthesized during hydroxyurea inhibition. Viral DNA synthesized in the presence of hydroxyurea was characterized by both alkaline and neutral sucrose gradient centrifugations. After a 10-min pulse with ^{3}H-dThd, the viral DNA from control cultures showed two radioactive peaks in alkaline sucrose gradients with *S* values of 53 and 16, corresponding to polyoma form I DNA and replicating single-stranded chains, respectively (Fig. 2A). In neutral sucrose gradients (Fig. 2D), radioactive peaks were found at 25*S* (RIs) and 20*S* (form I). Similar labeling experiments during hydroxyurea inhibition gave very different results. Under alkaline conditions no form I was found, and the majority of the replicating labeled chains was found as a sharp peak at the top of the gradient (Fig. 2B and C). This peak had a sedimentation coefficient of close to 4*S*, as was determined in a separate experiment by using 8 h of centrifugation through alkaline sucrose with 16*S* polyoma DNA as a reference. A second, smaller radioactive peak had an *S* value of close to 16. The sedimentation profile of DNA labeled between 10 to 20 min after addition of hydroxyurea (Fig. 2B) was similar to that obtained from material labeled between 120 and 130 min (Fig. 2C). There was, however, a clear

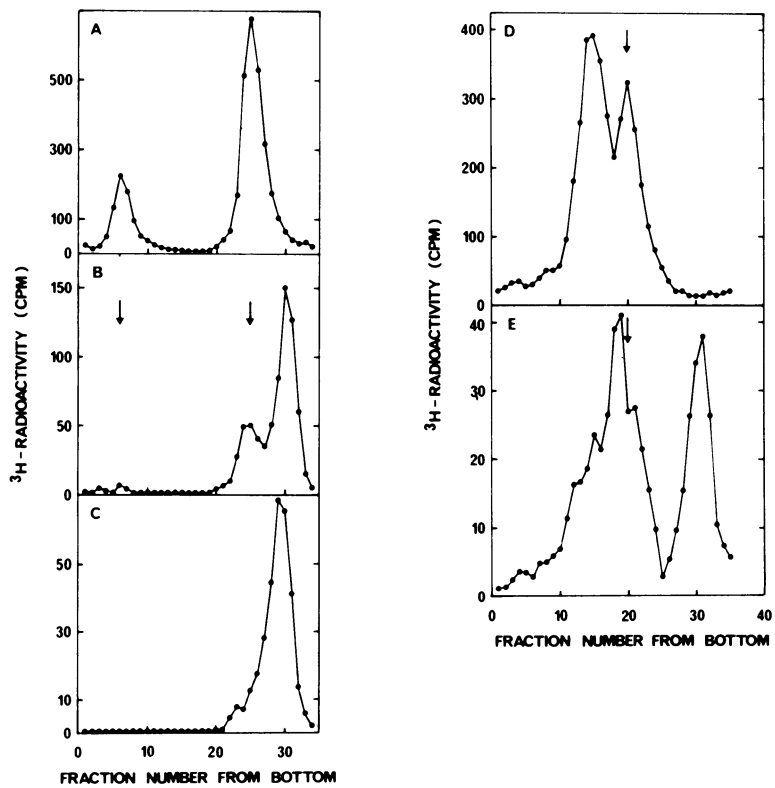

FIG. 2. *Characterization by sucrose gradient centrifugation of viral DNA formed during hydroxyurea inhibition. Viral DNA labeled for 10 min with ^{3}H-dThd was centrifuged through alkaline (A, B, C) or neutral (D, E) sucrose gradients. Panels A and D represent controls in the absence of hydroxyurea; panels B and E give data from a pulse 10 to 20 min after addition of hydroxyurea; and panel C represents a pulse 120 to 130 min after onset of inhibition. The arrows give the positions of the markers (20S at neutral pH and 53S and 17S at alkaline pH).*

difference in the relative amount of radioactivity in the 16S region, which was less after the longer inhibition time.

In neutral sucrose gradients pulse-labeled viral DNA from inhibited cultures was recovered in two peaks. Part of the radioactivity sedimented like the RI (20 to 25S), but a considerable amount of radioactivity was found at the top of the gradients with a sedimentation coefficient of about 4S (Fig. 2E). The relative amounts of material in the two peaks showed large, unexplained variations between different experiments. The single-stranded nature of the DNA in the 4S peak will be demonstrated below.

In the experiment depicted in Fig. 2, the viral DNA was labeled with 10-min pulses of ^{3}H-dThd at different times after the onset of inhibition. In a second experiment (Fig. 3) ^{3}H-dThd was supplied to the cultures 10 min after addition of hydroxyurea, and viral DNA was analyzed by alkaline sucrose gradient centrifugation after 20, 40, and 120 min. With longer labeling times, the viral DNA became increasingly heterogenous in size. DNA labeled for 120 min contained radioactive chains varying in size from full-length strands (16S) to slowly sedimenting material. Some radioactivity was also recovered as form I (53S). A comparison of this result with that obtained from 10-min pulse labeling after 2 h of inhibition (Fig. 2C) suggested that 4S fragments are synthesized first and then grow slowly to form longer chains.

Pulse chase of ^{3}H-labeled 4S hydroxyurea fragments. A pulse chase experiment was done to investigate if hydroxyurea fragments were converted into longer DNA chains.

Ten minutes after addition of hydroxyurea to infected cells, ^{3}H-dThd was added to the medium for an additional 10 min. The ^{3}H-label was then chased in the presence of hydroxyurea for 20 or 40 min by addition of 0.1 mM unlabeled dThd and 0.1 mM dCyd. A parallel set of cultures was pulse chased after removal of hydroxyurea. The sedimentation profile of the DNA before the chase shows the accumulation of hydroxyurea fragments (Fig. 4A). During the chase in the presence of hydroxyurea, the isotope was transferred to longer chains (Fig. 4B and C). The data from the chase without hydroxyurea (Fig. 4D and E) were similar to those obtained with hydroxyurea. In both cases a small amount of label (12%) sedimented at 53S. The total amount of acid insoluble radioactivity in the Hirt supernatant fluid increased by 40% during the 40-min chase without hydroxyurea, whereas the corresponding value for the chase in the presence of the drug was only 4%.

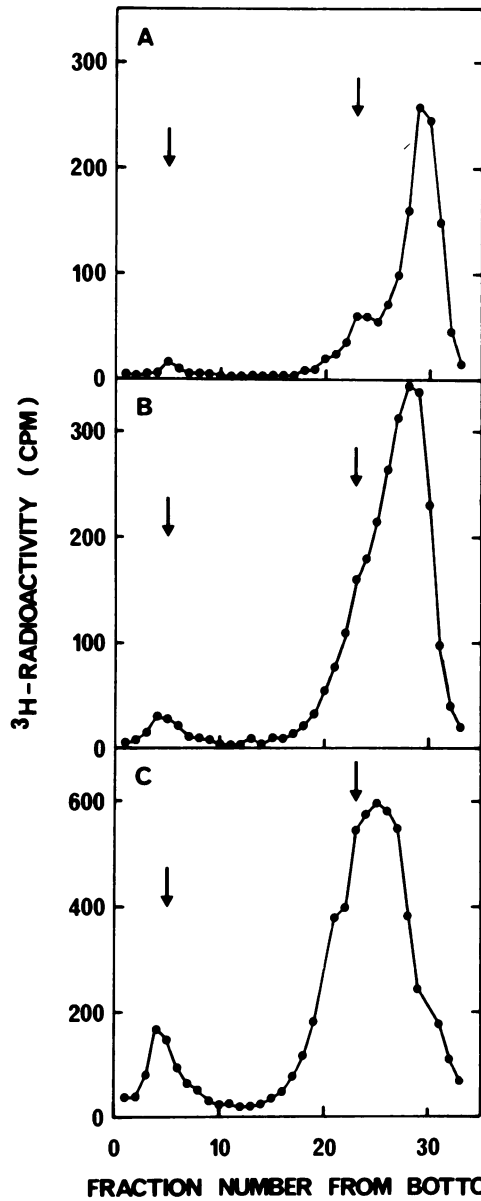

FIG. 3. *Viral DNA labeled for longer time periods during hydroxyurea inhibition analyzed by alkaline sucrose gradient centrifugation. ^{3}H-dThd was added 10 min after the onset of inhibition, and the viral DNA was labeled for 20 (A), 40 (B), or 120 min (C). The arrows indicate the positions of the markers (53S and 17S).*

Fate of prelabeled polyoma DNA during incubation with hydroxyurea. Addition of hydroxyurea to cells conceivably could result in an activation of nucleases, and the hydroxyurea

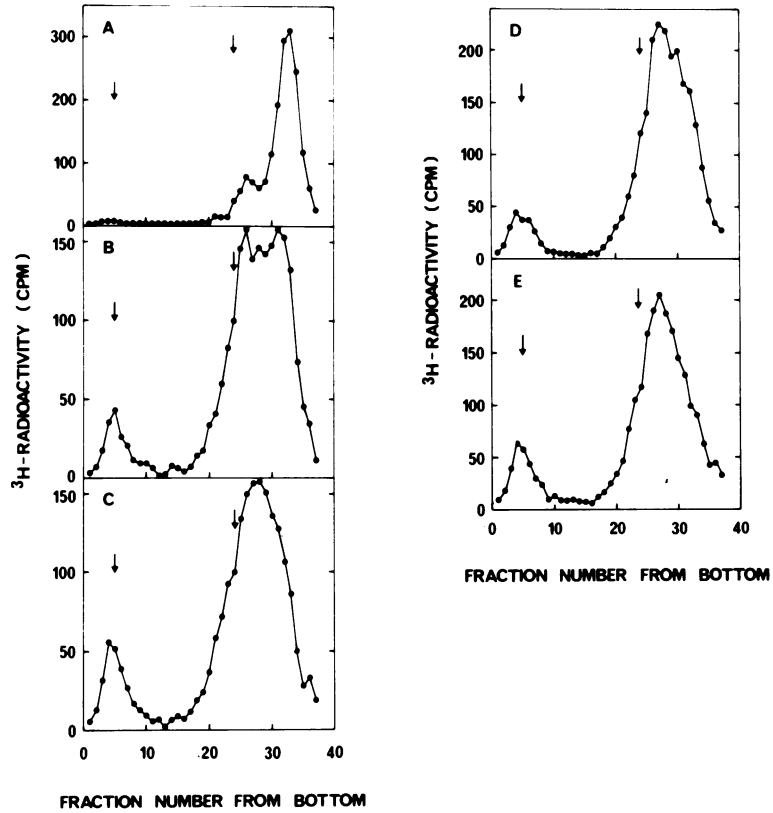

FIG. 4. *Pulse chase of ^{3}H-labeled 4S fragments. The cells were labeled with a 10 min pulse of ^{3}H-dThd 10 min after addition of hydroxyurea. The cell monolayers were then washed twice with warm medium and reincubated for either 0 (A), 20 (B, D), or 40 min (C, E) with 0.1 mM nonlabeled dThd and 0.1 mM dCyd in the presence (B, C) or absence (D, E) of 10 mM hydroxyurea. In each case, the labeled viral DNA was analyzed by alkaline sucrose gradient centrifugation.*

fragments then might originate from a degradation of preexisting polyoma DNA. This question was investigated by prelabeling infected cultures with ^{3}H-dThd for either 10 or 60 min before addition of hydroxyurea. The former labeling regimen gave radioactive, replicative DNA strands, whereas in the latter case, the bulk of the label was recovered in form I DNA. In both experiments, the radioactivity was diluted by addition of 0.1 mM cold dThd before the cultures were washed and reincubated for 20 min in medium containing 10 mM hydroxyurea. Viral DNA was then extracted and analyzed by alkaline sucrose centrifugation. Only the result from the 10-min pulse is shown here (Fig. 5). The sedimentation profile before addition of the drug showed the expected radioactive peaks at 53*S* and 16*S*. After hydroxyurea treatment, a similar profile was obtained, but the material at 53*S* had increased from 20 to 40% of the total radioactivity, and the radioactive peak at 16*S* was narrower. No radioactivity was found in the 4*S* region, which demonstrated that the hydroxyurea fragments did not arise from a hydroxyurea-induced degradation of replicating DNA. When cells were prelabeled for 60 min and then were incubated with hydroxyurea, again there was no formation of labeled 4*S* fragments, demonstrating that breakdown of form I was not the source of the fragments.

Properties of hydroxyurea fragments. The polyoma-specific nature of the hydroxyurea fragments was demonstrated by reassociation experiments. Short fragments of viral DNA obtained after a 10-min pulse with ^{3}H-dThd in the presence of hydroxyurea were purified as described in Materials and Methods and used for reassociation experiments. As shown in Table 1, 62% of the hydroxyurea fragments reassociated with polyoma DNA bound to filters (Table 1). In a parallel experiment, authentic sonically treated polyoma DNA reassociated to about the same extent (68%). When the purified hydroxyurea fragments were allowed to self-anneal for 48 h at 68 C, almost 40% of the radioactivity behaved as double-stranded DNA

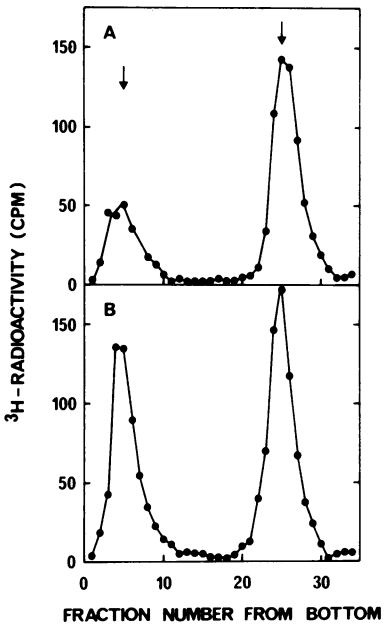

FIG. 5. *Effect of hydroxyurea on prelabeled DNA. Infected 3T6 cultures were labeled for 10 min with ^{3}H-dThd in the absence of hydroxyurea. After a 30-s chase with 0.1 mM nonlabeled dThd and 0.1 mM dCyd, 10 mM hydroxyurea was added to the medium. Viral DNA was extracted after the 30-s chase (A) or after 20 min of additional incubation with hydroxyurea (B) and analyzed by centrifugation through alkaline sucrose gradients. The arrows give the positions of 53S and 17S marker DNA.*

as judged from its adsorption to hydroxyapatite (Table 2). About the same amount of reassociation was observed after 72 h of incubation of a fivefold-diluted sample. In separate experiments, an excess of sheared polyoma DNA was added to hydroxyurea fragments both before and after incubation at 68 C. In both cases, 60% of the labeled molecules behaved as double-stranded DNA after 5 h of incubation at 68 C (Table 2). The self-annealing properties of purified hydroxyurea fragments imply that both parental strands serve as templates for their synthesis.

As described above, a large part of the radioactive viral DNA synthesized during hydroxyurea inhibition sedimented with low *S* values in neutral sucrose gradients (Fig. 2E). The strandedness of this material was investigated by treatment of a dialyzed Hirt supernatant fraction with either exonuclease I or exonuclease III from *E. coli*. Exonuclease I digestion resulted in the loss of approximately 50% of the total acid-insoluble radioactivity, whereas the corresponding figure after exonuclease III treatment was 10%. Figure 6B and C depict the results from neutral sucrose gradient centrifugations of the two digested samples. The slowly sedimenting material was selectively and completely degraded by exonuclease I, but was resistant to digestion by exonuclease III. Because exonuclease I only hydrolyzes single-stranded DNA (6) and exonuclease III is specific for native DNA (15), the results demonstrate that the short fragments found after centrifugation through neutral sucrose gradients were single stranded.

DISCUSSION

Hydroxyurea treatment of mouse embryo cells resulted in a rapid depletion of the dGTP pool and a parallel decrease in the rate of DNA

TABLE 1. *Reannealing to DNA bound to filters*[a]

Origin of labeled DNA	Input radioactivity (counts/min)	Input bound to filters (%)	
		Polyoma DNA (2 μg)	Mouse DNA (10 μg)
Hydroxyurea fragments[b]	671	62	
Hydroxyurea fragments	671		4.8
Polyoma DNA[c]	606	68	
Polyoma DNA	606		0.9

[a] The conditions for reannealing were the same as previously described (21).

[b] Hydroxyurea fragments were prepared as described in Materials and Methods.

[c] Radioactively labeled form I DNA was sheared and denatured before the experiment.

TABLE 2. *Self-annealing of hydroxyurea fragments*[a]

Incubation time (h)	DNA concn (counts per min per ml)	Reannealing (%)
0	$8.0 \cdot 10^4$	4.1
1	$7.5 \cdot 10^4$	20
2	$8.0 \cdot 10^4$	25
5	$6.8 \cdot 10^4$	29
24	$6.8 \cdot 10^4$	35
48	$6.5 \cdot 10^4$	38
72	$6.7 \cdot 10^4$	38
72 + 5[b]	$7.2 \cdot 10^4$	57
0 + 5[b]	$1.5 \cdot 10^4$	58
5	$1.6 \cdot 10^4$	20
24	$1.6 \cdot 10^4$	31
72	$1.4 \cdot 10^4$	35

[a] The preparation of hydroxyurea fragments and the conditions for reannealing are described in Materials and Methods.

[b] After 0 or 72 h of incubation, respectively, 0.54 μg of sheared, denatured polyoma DNA was added, and incubation continued for 5 h.

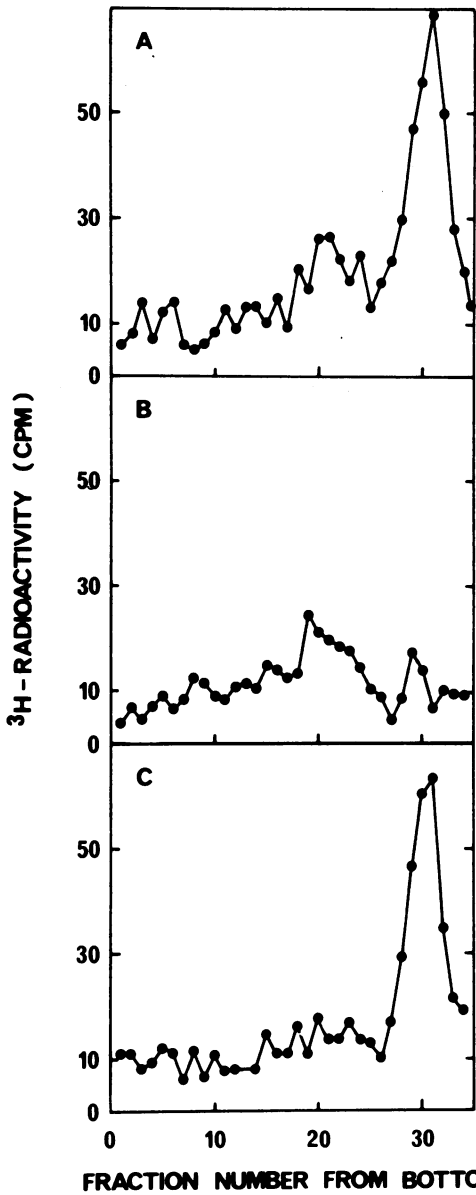

FIG. 6. *Digestion of 4S fragments by exonuclease I and III. The Hirt supernatant fluid from hydroxyurea-treated cells labeled with a 10 min ^{3}H-dThd pulse 10 min after addition of the drug was dialyzed against 0.01 M Tris-hydrochloride, pH 8.0, and 0.25 M NaCl (A), treated with either exonuclease I (B) or exonuclease III (C), and analyzed by neutral sucrose gradient centrifugation.*

synthesis (17). Hydroxyurea also inhibited the DNA synthesis in polyoma virus-infected 3T6 cells. After the initial decrease, the rate of DNA synthesis remained constant for at least 2 h at a level of 2 to 3% of the original value. Inhibition by hydroxyurea was reversible, for removal of the drug resulted in the recovery of both viral and cellular DNA synthesis (Fig. 1).

When the inhibited cells were labeled for 10 min with ^{3}H-dThd, about 80% of the acid-insoluble radioactivity in the Hirt supernatant fluid sedimented with an *S* value of 4 in alkaline sucrose gradients (Fig. 2B and C). The short, hydroxyurea fragments could be annealed to filters containing polyoma DNA with almost the same efficiency as sonically treated polyoma DNA (Table 1). Even though some of the low-molecular-weight DNA might be of cellular origin the majority of the labeled 4*S* fragments appeared to be polyoma specific. The fragments were probably synthesized on the RI of polyoma DNA, as judged from the result of neutral sucrose gradient centrifugation (Fig. 2E). Even under neutral conditions, however, a considerable and variable proportion of the newly synthesized viral DNA was recovered as short fragments which were single stranded, as judged from their degradation by specific exonucleases. They were probably released from the parental strands during extraction of viral DNA by the Hirt procedure. It is known that extraction of newly synthesized bacterial DNA by buffers containing SDS results in the release of single-stranded DNA (12, 13).

The hydroxyurea fragments were synthesized de novo during the period of hydroxyurea inhibition, and preformed polyoma DNA was not used for their formation. This conclusion is derived from prelabeling experiments which demonstrated that ^{3}H-dThd incorporated into DNA before the onset of inhibition was not recovered in the 4*S* fragments.

Do hydroxyurea fragments represent synthesis of both or only one daughter strand at the replication forks? The finding that up to 80% of the radioactivity of pulse-labeled viral DNA can be recovered as hydroxyurea fragments by itself suggests synthesis from both of the parental strands. More substantiating evidence was obtained in self-annealing experiments in which 40% of the single-stranded hydroxyurea fragments could form double-stranded DNA. An additional 20% of the fragments formed double-stranded DNA when an excess of authentic polyoma DNA was added at the end point of the self-annealing experiment. Similarly, the direct addition of an excess of polyoma DNA to single-stranded, labeled hydroxyurea fragments resulted in the recovery of 60% of the radioactivity in double-stranded DNA.

It is not clear why only a total of 60% of the hydroxyurea fragments did reanneal in these experiments, even though the results of filter reannealing experiments suggest that essentially all of the DNA of hydroxyurea fragments

was polyoma specific. Nevertheless, the data provide evidence for the concept that hydroxyurea fragments are synthesized from both parental strands at the replication forks. The difference between the amount of hydroxyurea fragments which are able to self-anneal and the amount of fragments able to anneal to sheared polyoma DNA might mean that not all viral DNA strands are synthesized discontinously. It is also possible that the DNA fragments are joined to the progeny strands at different rates, depending on the overall direction of growth of the strands.

The given interpretation of the observed data depends upon the assumption that polyoma DNA replication, like the replication of simian virus 40 DNA, has a fixed origin (19). If polyoma DNA synthesis was initiated at different sites in different molecules and then proceeded in either or both directions, hydroxyurea fragments might be self-complementary without being synthesized from both parental strands at a given replication fork.

A source of error in the determination of self-complementarity of the purified hydroxyurea fragments might be a contamination by degraded polyoma-specific mRNA. Such a contamination would probably result in an overestimate of the amount of self-complementarity of the hydroxyurea fragments as measured by their adsorption to hydroxyapatite.

The short hydroxyurea fragments are slowly transformed to longer chains, both in the presence of and after removal of the drug. In pulse chase experiments, a small part of the labeled molecules was transformed to form I DNA, but most of the radioactivity was recovered as uncompleted strands, even after removal of hydroxyurea before the chase. Although it is clear that hydroxyurea fragments can be used for the formation of longer chains, and to some extent for formation of completed polyoma DNA strands, it also appears that the majority of the replicative intermediates formed during hydroxyurea inhibition do not mature to form I DNA.

There is a close similarity between the hydroxyurea fragments isolated from inhibited cells and the 4*S* fragments found during polyoma DNA synthesis in nuclei isolated from infected cells (9). It was suggested that these fragments represent intermediates in a discontinuous type of DNA synthesis similar to the "Okazaki fragments" in *E. coli* (13, 18). The similarity between the hydroxyurea fragments and the 4*S* fragments found in isolated nuclei is stressed by the finding that both types of fragments appear to contain stretches of RNA at their ends (8) and also that the fragments obtained from nuclei are synthesized from both strands of DNA (V. Pigiet et al., unpublished data).

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