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Induction of Cellular Deoxyribonuleic Acid Synthesis by Simian Virus 40

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The incorporation of ^{3}H-thymidine (^{3}H-dT) into deoxyribonucleic acid (DNA) has been studied in uninfected confluent monolayer cultures of monkey kidney and mouse kidney cells, simian virus 40 (SV40)-infected cells, and in SV40-transformed mouse kidney cells. Radioautographic measurements revealed that during the period from 28 to 51 hr after productive SV40 infection of monkey kidney cultures about 80% of the cells synthesized DNA, compared to about 16% in uninfected cultures. At 28 to 43 hr after abortive SV40 infection of mouse kidney cultures, 24 to 37% of the cells synthesized DNA, compared to about 6 to 8% in uninfected cultures. The infected monkey kidney and mouse kidney cultures, respectively, incorporated about 5 to 10 times and 3 to 5 times as much ^{3}H-dT into DNA as did uninfected cultures. Moreover, the net DNA synthesized by SV40-infected monkey kidney cultures, estimated by colorimetric methods, substantially exceeded that of uninfected cultures.

Nitrocellulose chromatography and band centrifugation experiments were performed to elucidate the kinds of DNA synthesized in the cultures. In uninfected monkey kidney cultures and at 2 to 12 hr after SV40 infection, almost all of the ^{3}H-dT labeled DNA sedimented more rapidly than SV40 DNA, and the radioactive DNA was denatured by heating for 12 min at 100 C (cellular DNA). Almost all of the labeled DNA obtained from abortively infected mouse kidney cultures and from SV40-transformed cells also had the properties of cellular DNA. However, approximately one-third to one-half of the labeled DNA obtained from monkey kidney cultures 28 to 51 hr after infection sedimented more slowly than cellular DNA and was not denatured by the heating (SV40 DNA). It is concluded that cellular DNA synthesis was induced during either the productive or abortive SV40 infections.

Productive infection by simian virus 40 (SV40) results in a pronounced increase in the incorporation of ^{3}H-thymidine (dT) and ^{3}H-deoxyadenosine into deoxyribonucleic acids (DNA) of either primary green monkey kidney (GMK) or the CV-1 established line of GMK cells. Radioautographic experiments have shown that the percentage of cells with labeled nuclei also increases sharply beginning at about 16 to 20 hr after infection (23). Studies by Hatanaka and Dulbecco (16) and by Westphal and co-workers (38, 39) indicated that SV40 induces synthesis not only of virus DNA but also of cellular DNA. In the experiments of Hatanaka and Dulbecco (16), resting confluent GMK cell cultures were used. Immunofluorescence measurements showed that about 50 to 60% of the cells were positive for the SV40-intranuclear-"tumor"-antigen at 40 hr after infection. In contrast to the above findings, Gershon, Sachs, and Winocour (13) observed that in contact-inhibited or X-irradiated BSC-1 monkey kidney cells, where nearly all of the cells synthesized virus, there was *no* induction of cellular DNA synthesis. Moreover, experiments by Sauer et al. (31), with proliferating GMK cell cultures, also suggested that SV40 does not induce cellular DNA synthesis.

Interpretation of experiments with the closely related polyoma virus has also been controversial. Using mouse kidney cultures in which about half of the cells were productively infected with polyoma virus, several laboratories (1, 2, 10, 12, 36, 37, 40) have concluded that an induction of cellular DNA synthesis occurs. However, Sheinin (32) has reported that in exponentially growing

mouse embryo cells, in which nearly all infected cells make virus, cellular DNA synthesis is inhibited after polyoma virus DNA synthesis begins. On the other hand, there appears to be agreement that cellular DNA synthesis is induced after *abortive* infection of 3T3 cells by SV40 (13, 17) or of rat embryo cells by polyoma virus (12, 33).

In the investigation of DNA synthesis in monkey kidney cultures, colorimetric estimations revealed that the net DNA synthesized 30 to 50 hr after SV40 infection greatly exceeded that of uninfected cultures (23). It seemed unlikely that the substantial net DNA increase could be accounted for solely as viral DNA. The question of whether SV40 induces cellular DNA synthesis has therefore been investigated both in primary GMK cultures and in the established CV-1 cell line, under conditions where at least 75% of the cells were infected (6, 23). The possible induction of cellular DNA synthesis in mouse kidney cultures abortively infected by SV40 has also been studied. In addition, it was of interest to learn whether closed circular-SV40 DNA (W. Bauer and J. Vinograd, Abstr. Ann. Meeting Biophys. Soc., 11th, Houston, Tex., p. 133, 1967) was made in cells transformed by SV40 (25).

MATERIALS AND METHODS

Cell cultures. CV-1 cells, an established strain of GMK cells, primary cultures of GMK cells, primary cultures of mouse kidney cells, and SV40-transformed mouse kidney cells (mKS-A) were grown in monolayer cultures as described previously (22, 23, 25).

Preparation and assay of virus stocks. SV40 was propagated and assayed in monolayer cultures of CV-1 cells as previously described (23).

Complement-fixation tests. SV40 tumor antigen was demonstrated by complement fixation with ascitic fluid from hamsters bearing SV40 transplant (virus-free) tumors (Flow Laboratories, Inc., Rockville, Md.). A semi-microtest was employed which involved the use of 0.05 ml of each ingredient in disposable plastic trays (28). Reference antigens were prepared from either SV40-transformed mouse kidney cells (mKS) or CV-1 cells 40 to 44 hr after inoculation of SV40. Packed cells were resuspended in 4 or 9 volumes of modified barbital buffer (6) and treated at 4 C with a Raytheon sonic oscillator at 10 kc for 1 min. The sonic-treated material was centrifuged for 1 hr at either 34,000 $\times$ *g* or 100,000 $\times$ *g*. Supernatant fluids were collected and stored at -20 C. Antigen titers were determined as the reciprocal of the highest dilution of antigen giving 3+ or 4+ fixation in the presence of 4 units of antibody and 2 full units of complement. For comparative purposes, antigen titers were expressed as complement-fixing (CF) units per 10^7 cells.

Infection of cells for biochemical experiments. Confluent monolayer cultures fed 24 to 48 hr prior to infection were used. The culture medium was discarded, and 2 to 5 ml of virus suspension was added to each culture. The input multiplicities for each experiment are shown in the legends to the tables and figures. The cultures were incubated for 2 hr at 37 C to permit virus absorption, during which time they were rocked approximately every 20 min. The fluid was discarded and 20 ml of prewarmed R5a medium (8) plus 10% calf serum was added to the CV-1, mKS-A, or mouse kidney cultures; 30 ml of Melnick's medium B was added to GMK cell cultures.

At various times after infection, cultures were inoculated with 0.1 or 0.2 ml of ^{3}H-dT (New England Nuclear Corp., Boston, Mass.). The cultures were incubated at 37 C for the times indicated in Tables 1 and 2. Noninfected cultures were treated similarly, except that either R5a medium or spent medium (medium removed from 4- to 7-day-old cultures) was substituted for the virus preparations. After the ^{3}H-dT-labeling period, the cultures were washed with saline-glucose solution and removed from the glass with saline-glucose solution containing 0.05% trypsin (1:300; Nutritional Biochemicals Corp., Cleveland, Ohio) and 0.05% ethylenediaminetetraacetic acid.

Cells from 18 to 30 cultures were pooled for each experimental group. Portions of the resulting cell suspension were withdrawn for radioautography and for the determination of the total DNA content and specific activity of the DNA (24). The remainder of the cells (about 300 million cells) were sedimented by centrifugation, washed with saline-glucose, and used for the extraction of cellular and viral DNA.

Extraction and purification of DNA. The *p*-amino-salicylate-phenol method (18, 20) was modified as follows. To each volume of centrifuged cells was added 12 volumes of 6% (w/v) sodium *p*-amino-salicylate (J. H. Delamar and Son, Inc., Chicago, Ill.) and 12 volumes of 90% (v/v) redistilled phenol. The viscous suspension was stirred for 1 hr at room temperature and centrifuged for 1 hr at 10,000 rev/min (by use of an SS-34 rotor and a Servall RC-2 automatic refrigerated centrifuge). The phenol phase was re-extracted with 4 ml of 6% *p*-aminosalicylate per ml of centrifuged cells and recentrifuged; the aqueous phases were then combined. The aqueous solutions were gently shaken with 4 ml of phenol per ml of centrifuged cells and were centrifuged to break up the emulsion. DNA was precipitated from the *p*-amino-salicylate solution by the addition of an equal volume of redistilled 2-ethoxyethanol (J. T. Baker Chemical Co., Phillipsburg, N.J.). The DNA was dissolved in 4% sodium acetate and reprecipitated four times with 2-ethoxyethanol. Finally, the DNA was dissolved in 1$\times$ SSC (0.15 M sodium chloride, 0.015 M sodium citrate, *p*H 7.0) and dialyzed for about 24 hr against 2 liters of 1$\times$ SSC-buffer solution. The dialysate solution was changed four or five times during this interval. Then the DNA solution was diluted with 1$\times$ SSC to a concentration of about 200 to 500 μg/ml, and this stock solution was stored at 4 C until needed for nitrocellulose-column chromatography or velocity gradient-centrifugation experiments.

With the *p*-aminosalicylate-phenol method, over 90% of the DNA is extracted from cells. There are small losses during the transfer and reprecipitation steps. In the present experiments, the final DNA yields,

determined either by radioactive isotope labeling or by colorimetric methods, were 66 to 95%.

The possibility that DNA preparations of either high or low specific activity were being selected by the extraction and purification procedure was considered. Contrary to this hypothesis, it was found that the specific activity of the purified DNA agreed within 10% with the specific activity of the DNA determined directly on portions of the cells.

Heat denaturation of cellular DNA. DNA in 1× SSC at a concentration of about 10 μg/ml was heated in a boiling-water bath for 12 min, and then was transferred to an ice bath. After cooling, the ionic strength of the solution was adjusted to 2× SSC by the addition of 10× SSC, and the DNA was then used immediately for nitrocellulose-column chromatography.

Separation of double-stranded from heat-denatured DNA by nitrocellulose chromatography. The method of Klamerth (27) was used to separate double-stranded DNA from heat-denatured (single-stranded DNA). Serva NitroCel-S (Gallard-Schlesinger Chemical Manufacturing Corp., Carle Place, N.Y.) was tightly packed in columns (5 by 1 cm) and equilibrated with 2× SSC. Approximately 40 to 90 μg of native or heat-denatured DNA dissolved in 2× SSC, was applied to each column. Figure 1 illustrates the elution from the columns of DNA from uninfected and from SV40-infected GMK cell cultures. Native DNA was not retained by the columns; approximately 93% of the DNA was eluted with the first 12 ml of 2× SSC. However, 95 to 98% of the heat-denatured DNA from uninfected cells was retained. This DNA could be recovered quantitatively from the columns by successive elutions with 0.1× SSC and 0.01 N NaOH. Figure 1 also illustrates the fact that about 40% of the heat-treated DNA from SV40-infected GMK cells was eluted from the nitrocellulose by 2× SSC. The latter DNA apparently remains double-stranded after heating and has other properties which suggest that it is SV40 DNA.

Sedimentation velocity analysis (band centrifugation) of DNA. Band centrifugation (35) of DNA was carried out as described by Weil et al. (37). The lamella consisted of 0.05 or 0.1 ml (2 to 5 μg) of DNA in 1× SSC. The bulk solution was 3 ml of CsCl, density 1.503 g/cc. The DNA was centrifuged at 20 C in the SW39 rotor of a Spinco model L-2 centrifuge for 3 hr at 35,000 rev/min. Fractions consisting of 8 drops were collected (Density Gradient-Sedimentation Device, Buchler Instruments, Inc., Fort Lee, N.J.), and the radioactivity was determined with a Packard Tri-Carb model 314EX liquid scintillation spectrometer.

SV40-DNA marker. To isolate SV40 DNA, advantage was taken of the fact that the twisted-circular form of this DNA is resistant to thermal denaturation under conditions where cellular DNA is denatured. Therefore, DNA from SV40-infected CV-1 cells was heated for 12 min in a boiling-water bath, cooled, and applied to a nitrocellulose column in 2× SSC. The DNA eluted from the column with 2× SSC was utilized as an SV40-DNA marker.

To verify that the marker DNA had the characteristics of SV40 DNA, the following experiments were performed.

(i) The marker DNA was heated for a second time at 100 C, cooled, and applied to a nitrocellulose column. Over 90% of this DNA was recovered by eluting with 2× SSC, as would be expected for double-stranded DNA;

(ii) The marker DNA was treated with beef pancreatic deoxyribonuclease to learn whether deoxyribonuclease treatment would convert the marker DNA to a form that would not be resistant to heat denaturation (9, 35). A solution of beef pancreatic deoxyribonuclease (2 × 10^{-4} μg/ml; Worthington Biochemical Corp., Freehold, N.J.), containing 5 × 10^{-3} M $MgCl_2$, was added to the SV40-marker DNA for 90 min at 37 C, and then the DNA was heated for 10 min at 100 C. Nitrocellulose chromatography experiments showed that 92% of this deoxyribonuclease-treated marker DNA now was denatured by heating, as would be expected if closed circular DNA were converted by the deoxyribonuclease treatment to a linear structure.

(iii) Native SV40 marker-DNA sedimented more slowly than cellular DNA during band centrifugation in CsCl density gradients. Some of the marker-DNA was isolated from the peak fractions, dialyzed against 1× SSC, and heated at 100 C. The heated DNA was then subjected to nitrocellulose column chromatography; 89% of the DNA was eluted from the columns with 2× SSC.

(iv) During velocity sedimentation in CsCl density gradients, SV40 marker-DNA banded at the same position as labeled DNA isolated from purified SV40 particles.

RESULTS

Incorporation of ^{3}H-dT into DNA of SV40-infected monkey kidney cell cultures. In confirmation of previous studies (23), SV40 infection markedly stimulated the incorporation of ^{3}H-dT into DNA of GMK or CV-1 cell cultures 28 to 51 hr after infection. This stimulation was not observed, however, when the cultures were incubated with ^{3}H-dT at 2 to 12 hr after infection (Experiment VII, Table 1). Radioautographic measurements showed that at 33 to 51 hr postinfection 81 to 87% of the cells of the infected cultures were synthesizing DNA, whereas only 9 to 16% of the cells in the uninfected cultures were doing so. These observations support the conclusion that virtually all of the cells were productively infected.

From the right-hand column of Table 1, it may be seen that the net DNA synthesis of SV40-infected cultures greatly exceeded that of uninfected cultures. Thus, it is unlikely that the increased incorporation of ^{3}H-dT merely represents an enhancement of DNA turnover or synthesis of DNA by repair mechanisms in the infected cultures.

Induction of dT kinase activity and "tumor"-antigen by SV40. For control purposes, the induc-

TABLE 1. *Incorporation of ^{3}H-dT into the DNA of uninfected, SV40-infected, and SV40-transformed cell cultures*[a]

Expt	Cells	Interval of ^{3}H-dT incorporation (hr postinfection)[b]	Radioautography (% Nuclei labeled)		Counts per min per μg of DNA		Total DNA per culture[c] (infected/uninfected)
			Uninfected	SV40-Infected	Uninfected	SV40-Infected	
I	mKS-A	0–10	—	—	3,040	—	—
II	Mouse kidney	32–42	5.7	23.9	420	1,230	1.20
III	Mouse kidney	28–43	8.3	36.9	714	3,320	1.06
IV	GMK	28–38	—	—	212	660	1.26
		38–48	—	—	196	594	1.54
V	CV-1	28–38	16.2	80.9	582	2,280	1.25
		38–48	13.4	87.0	437	1,990	1.79
VI	CV-1	31–41	16.3	71.8	573	2,110	1.30
		41–51	14.0	80.8	497	1,880	1.60
VII	CV-1	2–12	19.4	20.7	721	722	1.04
		33–43	9.2	80.6	392	2,390	1.37

[a] Experiment I: 3-day-old cultures (30×10^6 cells/culture) of mKS-A cells (passage 72 after initial infection by SV40); ^{3}H-dT, 0.5 μc and 2 μg/ml. Experiment II: 6-day-old cultures (8.2×10^6 cells per culture); SV40 input multiplicity, 200 plaque-forming units (PFU)/cell. ^{3}H-dT, 0.5 μc and 1 μg/ml. Experiment III: 8-day-old cultures (5.9×10^6 cells per culture); SV40 input multiplicity, 218 PFU/cell; ^{3}H-dT, 1 μc and 2 μg/ml. Experiment IV: 11-day-old cultures (27.1×10^6 cells per culture); SV40 input multiplicity, 52 PFU/cell; ^{3}H-dT, 0.66 μc and 2.6 μg/ml. Experiment V: 8-day-old cultures (18×10^6 cells per culture); SV40 input multiplicity, 62 PFU/cell; ^{3}H-dT, 0.75 μc and 2 μg/ml. Experiment VI: 7-day-old cultures (15.3×10^6 cells/culture); SV40 input multiplicity, 77 PFU/cell; ^{3}H-dT, 0.75 μc and 2 μg/ml. Experiment VII: 7-day-old cultures (14.1×10^6 cells per culture); SV40 input multiplicity, 84 PFU/cell; ^{3}H-dT, 0.75 μc and 2 μg/ml.

[b] For mKS-A cultures, interval of ^{3}H-dT labeling denotes time after medium change. For uninfected cultures, interval of labeling refers to time after "mock infection."

[c] Total DNA per culture measured by diphenylamine reaction (4).

tion of dT kinase and the complement-fixing intranuclear "tumor"-antigen were determined on replicate monkey kidney cell cultures at about 46 hr after SV40 infection. In all of the experiments, the dT kinase activity of infected cultures was about 12 times greater than that of uninfected cultures. The "tumor"-antigen titers varied from about 100 to 250 CF units per 10^7 cells. These values are in the same range as was observed previously (23, 25).

Fractionation of heated DNA on nitrocellulose columns. Figures 1 through 3 show that heating DNA from uninfected monkey kidney cells at 100 C for 12 min converted 95 to 99% of that DNA to a single-stranded form which was strongly retained by the nitrocellulose. The ^{3}H-dT-labeled DNA from SV40-infected monkey kidney cells consisted of both cellular and viral DNA. To estimate the amount of radioactive SV40 DNA present, the heated DNA from the infected cultures was also chromatographed. From one-third to one-half of this DNA had the properties of double-stranded DNA. Some of the SV40 DNA might have been converted to single-stranded DNA by the heat treatment. Therefore, the experiment provides an estimate of the *minimal* amount of SV40 DNA present in the samples from SV40-infected monkey kidney cells.

Figures 2 and 3 show that the total ^{3}H-dT incorporated into DNA of SV40-infected cultures was 5 to 10 times greater than that of the uninfected cultures. Since one-half to two-thirds of the total labeled DNA was denatured to the single-stranded form by heating, these figures provide an upper limit to the amount of radioactive cellular DNA. It would appear from Fig. 2 and 3 that SV40 markedly increased the incorporation of ^{3}H-dT into *cellular DNA*.

Sedimentation velocity analysis of ^{3}H-dT-labeled DNA from SV40-infected monkey kidney cells. SV40 DNA has a molecular weight of about 3×10^6 daltons (7). This molecular weight is considerably smaller than that of the bulk of the cellular DNA (19, 20).

The SV40 DNA can be separated from most of the native-cellular DNA by band centrifugation in CsCl gradients (1, 7, 37). Thus, a lower limit to the amount of ^{3}H-dT-labeled cellular DNA present in DNA purified from SV40-infected monkey kidney cultures can be obtained.

Results of sedimentation velocity analyses of DNA from uninfected and SV40-infected monkey

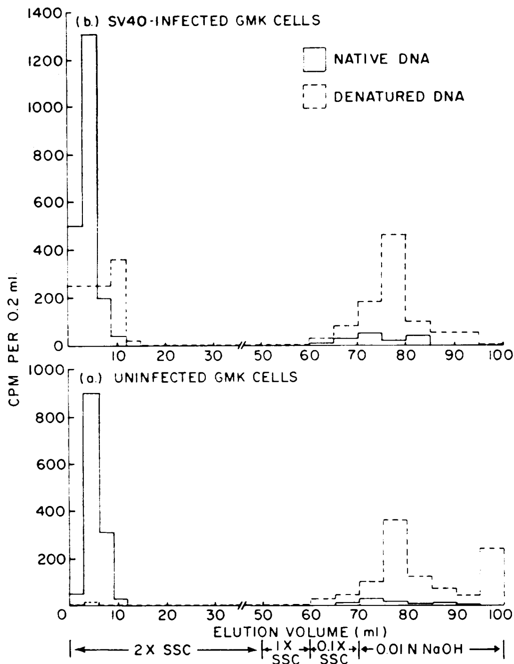

FIG. 1. *Nitrocellulose-column chromatography of native DNA and heat-denatured DNA from: (a) uninfected and (b) SV40-infected GMK cell cultures. SV40-input multiplicity: 75 PFU/cell. Cultures were incubated with ³H-dT (1.3 μc and 2 μg of dT/ml) from 48 to 54 hr after infection. Approximately 20 μg of DNA (24,000 counts per min for DNA from uninfected and 36,000 counts per min for DNA from SV40-infected cultures) was applied to each of the columns.*

kidney cells are shown in Fig. 4. The DNA obtained from cultures pulse-labeled with ³H-dT at 33 to 43 hr after SV40 infection, exhibits a peak of radioactivity at the same position as that of the SV40 DNA marker. This radioactive peak was not present in the DNA of cultures labeled with ³H-dT from 2 to 12 hr after infection nor in the DNA of cultures from uninfected cells. About 90% of the DNA from uninfected cell cultures sediments more rapidly than the SV40 DNA marker or the slowly sedimenting radioactive peak from SV40-infected cultures. From 50 to 60% of the radioactivity is present in the more rapidly sedimenting DNA of infected cultures. Thus, this DNA is too large to be SV40 DNA, and at least half of the ³H-dT-labeled DNA from the infected cultures must be cellular DNA. The findings were the same when DNA samples described in experiments V and VI of Table 1 were employed for band centrifugation. From these results, it can be concluded that cellular DNA synthesis is indeed induced by SV40 in productively infected CV-1 or GMK cell cultures.

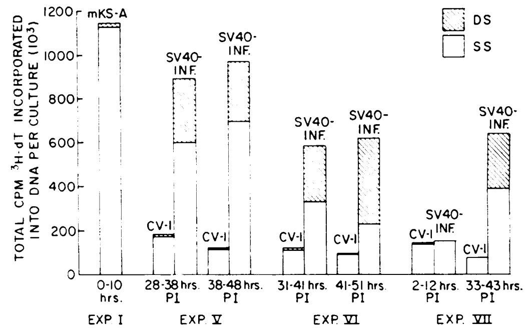

FIG. 2. *Estimation by nitrocellulose-column chromatography of the relative amounts of SV40 DNA and cellular DNA synthesized by mKS-A, and by uninfected and SV40-infected CV-1 cell cultures. DNA samples were heated at 100 C for 12 min prior to chromatography. Single-stranded (SS) DNA is the denatured DNA which requires 0.1× SSC and 0.01 N NaOH for elution. Double-stranded (DS) DNA is that DNA which is not denatured by the heating and is eluted from the columns with 2× SSC (circular-SV40 DNA). The intervals of ³H-dT incorporation into DNA are shown below each column. The experiment numbers refer to the experiments depicted in Table 1.*

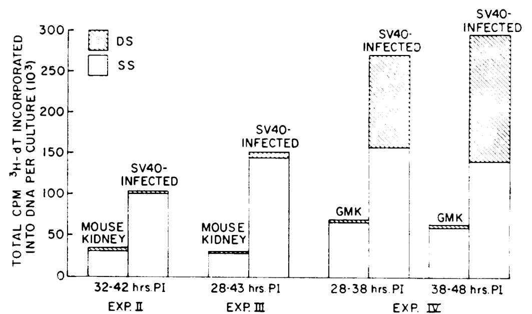

FIG. 3. *Estimation by nitrocellulose-column chromatography of the relative amounts SV40 DNA and cellular DNA synthesized by SV40-infected mouse kidney and GMK cell cultures. DNA samples were heated at 100 C for 12 min prior to chromatography. Single-stranded (SS) DNA is the denatured DNA which requires 0.1× SSC and 0.01 N NaOH for elution. Double-stranded (DS) DNA is that DNA which is not denatured by the heating and is eluted from the columns with 2× SSC (circular-SV40 DNA). The intervals of ³H-dT incorporation into DNA are shown below each column. The experiment numbers refer to the experiments depicted in Table 1.*

Abortive-SV40 infection of mouse kidney cell cultures. Infection of mouse kidney cell cultures by SV40 is abortive (25). Experiments II and III in Table 1 confirmed previous findings that SV40 infection of the mouse kidney cells results in a three- to fivefold stimulation of the incorporation

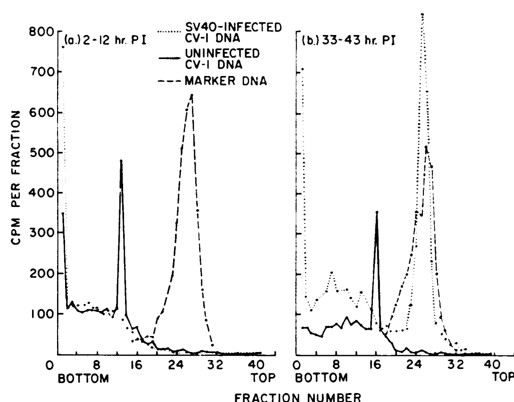

FIG. 4. *Sedimentation-velocity analysis (band centrifugation) of ^{3}H-dT-labeled DNA from uninfected and SV40-infected CV-1 cell cultures (see Table 1, experiment VII).*

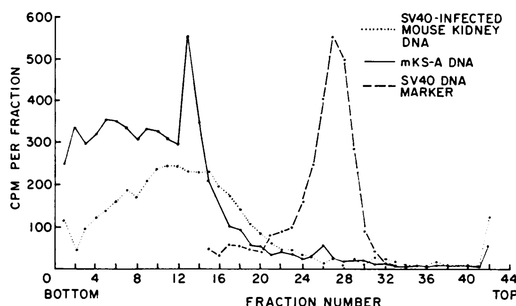

FIG. 5. *Sedimentation velocity analysis (band centrifugation) of ^{3}H-dT-labeled DNA from mKS-A cell cultures and from SV40-infected mouse kidney cell cultures. For mKS-A DNA and SV40-infected mouse kidney DNA, respectively, see Table 1, experiments I and II.*

of ^{3}H-dT into DNA and that the percentage of cells in the culture with labeled nuclei increases 28 to 43 hr after infection. In the same experiments, it was shown that a threefold increase in dT kinase activity and an induction of 2 CF units per 10^{7} cells of SV40-tumor antigen occurred 46 hr after the SV40 infection.

Figure 3 shows that, in contrast to the results with SV40-infected monkey kidney cell cultures, little, if any, heat-resistant, closed circular-SV40 DNA was made in mouse kidney cultures abortively infected with SV40. Figure 5 illustrates a typical band-centrifugation experiment with native DNA from SV40-infected mouse kidney cultures. About 90% of the DNA sedimented more rapidly than the SV40 DNA marker. The same result was obtained with DNA from uninfected mouse kidney cultures. The sedimentation velocity experiments further demonstrate that circular-SV40 DNA synthesis is blocked in SV40-infected mouse kidney cultures. Since there was a three- to fivefold increase in the total ^{3}H-dT incorporated into DNA after SV40 infection (Fig. 3), it would appear that cellular DNA synthesis was induced by SV40 during the abortive infection.

DNA synthesis by SV40-transformed mKS-A cells. Growth and DNA synthesis are rapid in mouse kidney lines transformed by SV40. In comparison with normal mouse kidney cell cultures, the transformed cell lines all contain elevated levels of dT kinase, DNA polymerase, deoxycytidine monophosphate (dCMP) deaminase, and deoxythymidine monophosphate (dTMP) kinase activities. The SV40-transformed cell lines also contain the SV40-"tumor" antigen, but no detectable SV40-capsid antigen or virus particles. Neither intact cells nor extracts of the transformed cells produced plaques when plated on CV-1 cells. However, small amounts of SV40 could be recovered routinely when the transformed cells were grown in mixed cultures with CV-1 cells (25, 26).

One strain (mKS-A) of the SV40-transformed mouse kidney cells (Table 1; experiment I) has been tested for the possible presence of twisted-circular SV40 DNA. Nitrocellulose-chromatography (Fig. 2) and sedimentation-velocity (Fig. 5) analyses of the DNA from mKS-A cells show that at least 98% of the DNA synthesized by mKS-A cells, does not have the properties of twisted-circular SV40 DNA.

DISCUSSION

The incorporation of ^{3}H-dT into DNA has been studied in confluent monolayer cultures of GMK and CV-1 cells infected with SV40 at input multiplicities of about 50 to 85 PFU per cell. It was shown previously that 60 to 80% of the cells in these cultures are virus producers (23). In the present series of experiments, about 80% of the cells in the infected cultures were synthesizing DNA, compared with about 15% of the cells in uninfected cultures, supporting the conclusion that the majority of the cells were infected with SV40.

In SV40-infected cultures, the incorporation of ^{3}H-dT into DNA was markedly stimulated. Since the activities dT kinase, DNA polymerase, dTMP synthetase, and FH_2 reductase are also enhanced after SV40 infection (11, 23, 25, 26), it would not be unreasonable to suppose that labeled DNA precursor pools increase in infected cultures and that an increase in ^{3}H-dTTP pools partly explains the increased incorporation of ^{3}H-dT into DNA. Another possibility is that repair replication of DNA partly accounts for the increased incor-

poration of ^{3}H-dT into DNA (15, 29). Although these possibilities have not been ruled out, it seems unlikely that enlarged precursor pools or repair replication are alone responsible for the stimulated ^{3}H-dT labeling of DNA in infected cultures. As shown previously (24) and in the present study (Table 1), the net amount of DNA synthesized in infected cultures, estimated by colorimetric methods, substantially exceeds that of noninfected cultures.

The kind of DNA synthesized has been elucidated by nitrocellulose column chromatography and band centrifugation experiments. These experiments show that only one-third to one-half of the ^{3}H-dT labeled DNA has the properties of SV40 DNA. Since there is 5 to 10 times more ^{3}H-dT incorporated into DNA of infected than uninfected cultures, the present findings confirm the conclusions of Hatanaka and Dulbecco (16) and Westphal and workers (38, 39) that SV40 infection induces cellular DNA synthesis in confluent cultures of monkey kidney cells. This induction can be demonstrated in the established CV-1 cell line of GMK cells as well as in primary GMK cultures. The present results differ, however, from those of Gershon et al. (13), who used the BSC-1 established line of monkey kidney cells. Perhaps the divergent findings are attributable to differences in the metabolism and response of BSC-1 and CV-1 cells to SV40 infection.

In SV40-infected monkey kidney cultures, SV40-DNA synthesis probably begins at about 12 to 16 hr and continues through 51 hr after infection. This may be inferred from the following considerations: (i) the radioactive DNA made 2 to 12 hr after SV40 infection does not have the properties of SV40 DNA, and uptake of ^{3}H-dT into DNA at that time is not enhanced; (ii) radioautographic and isotope incorporation studies show that stimulated uptake of ^{3}H-dT usually commences at about 16 hr; (iii) single-step growth curves show that the SV40 eclipse period is of 21 to 24 hr duration (23); and (iv) a substantial proportion of the labeled DNA at 28 to 51 hr after infection is SV40 DNA (Table 1, Fig. 2, 3). It is therefore noteworthy that enhanced incorporation of ^{3}H-dT into cellular DNA was observed even during the time that virus maturation and SV40 DNA synthesis were proceeding. Thus, under the conditions of the present study, the onset of SV40 DNA synthesis was not accompanied by a shutoff of cellular DNA synthesis, as has been observed in exponentially growing mouse embryo cultures infected with the closely related polyoma virus (32).

Incorporation of ^{3}H-dT into cellular DNA was also stimulated in primary mouse kidney cultures abortively infected with SV40 (Fig. 3, 5). However, in abortively infected cultures and in SV40-transformed cells, little, if any, closed-circular SV40 DNA was made. Nor does it appear likely that a linear form of SV40 DNA was synthesized and then partly fragmented. If this were true, radioactivity should have been found in the band centrifugation experiments in a region sedimenting more slowly than the SV40 marker DNA (Fig. 5). However, almost all of the ^{3}H-dT-labeled DNA from SV40-infected mouse kidney or from SV40-transformed cells sedimented more rapidly than SV40-DNA marker, a result signifying that this DNA had a higher molecular weight than either the closed circular or the linear form of double-stranded SV40 DNA (9, 34; Bauer and Vinograd, Abstr. Ann. Meeting Biophys. Soc., 11th, Houston, Tex., p. 133, 1967).

From the present study and previous findings (11, 16, 23, 25, 26, 38, 39), the following description of events in confluent monolayer cultures of SV40-infected cells emerges. In contrast to poxvirus (21, 24), herpesvirus (3), and possibly adenovirus infections (14, 30), SV40 infection *does not* shutoff cellular DNA synthesis during the time that SV40 DNA and SV40 capsid proteins are being synthesized and for at least 20 hr after virus maturation has begun.

In SV40-infected GMK or CV-1 cell cultures, T-antigen, capsid protein, and SV40 DNA are synthesized, cellular DNA synthesis is induced, and the activities of four enzymes of DNA metabolism are enhanced. Two of these four enzymes, namely, dT kinase and DNA polymerase, have altered properties (23, 26).

After abortive SV40-infection of mouse kidney cells, the T antigen is made and cellular DNA synthesis is induced, but capsid protein and SV40 DNA synthesis are blocked. The activities of dT kinase, DNA polymerase, dTMP kinase, and dCMP deaminase are enhanced. In contrast to the enzymes of productively infected monkey kidney cells, the dT kinase and the DNA polymerase induced by SV40 in mouse kidney cells do not exhibit altered properties, suggesting that they are derepressed cellular enzymes (S. Kit, *In* Y. Ito [ed.], Subviral carcinogenesis, *in press*).

In SV40-transformed mKS cells, the T antigen is made but not SV40 DNA or capsid protein. The properties of the dT kinase and the DNA polymerase purified from mKS cells also resemble those of uninfected mouse kidney cells, so that the block in the expression of SV40 genetic functions in mKS cells resembles that in abortively infected mouse kidney cells (S. Kit, *In* Y. Ito [ed.], Subviral carcinogenesis, *in press*).

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LITERATURE CITED

1. BASILICO, C., G. MARIN, AND G. DI MAYORCA. 1966. Requirement for the integrity of the viral genome for the induction of host DNA synthesis by polyoma virus. Proc. Natl. Acad. Sci. U.S. **56**:208-215.
2. BEN-PORAT, T., AND A. S. KAPLAN. 1963. The synthesis and fate of pseudorabies virus DNA in infected mammalian cells in the stationary phase of growth. Virology **20**:310-317.
3. BEN-PORAT, T., C. COTO, AND A. S. KAPLAN. 1966. Unstable DNA synthesized by polyoma virus-infected cells. Virology **30**:74-81.
4. BURTON, K. 1956. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. **62**:315-323.
5. CAMPBELL, D. H., J. S. GARVEY, N. E. CREMER, AND D. H. SUSSDORF. 1963. Methods in immunology, p. 246-247. W. A. Benjamin, Inc., New York.
6. CARP, R. I., AND R. V. GILDEN. 1966. A comparison of the replication cycles of simian virus 40 in human diploid and African green monkey cells. Virology **28**:150-162.
7. CRAWFORD, L. V., AND P. H. BLACK. 1964. The nucleic acid of simian virus 40. Virology **24**: 388-392.
8. DUBBS, D. R., AND S. KIT. 1965. The effect of temperature on induction of deoxythymidine kinase activity by herpes simplex mutants. Virology **25**:256-270.
9. DULBECCO, R., AND M. VOGT. 1963. Evidence for the ring structure of polyoma virus DNA. Proc. Natl. Acad. Sci. U.S. **50**:236-243.
10. DULBECCO, R., L. H. HARTWELL, AND M. VOGT. 1965. Induction of cellular DNA synthesis by polyoma virus. Proc. Natl. Acad. Sci. U.S. **53**:403-410.
11. FREARSON, P. M., S. KIT, AND D. R. DUBBS. 1966. Induction of dihydrofolate reductase activity by SV40 and polyoma virus. Cancer Res. **26**: 1653-1660.
12. GERSHON, D., P. HAUSEN, L. SACHS, AND E. WINOCOUR. 1965. On the mechanism of polyoma virus-induced synthesis of cellular DNA. Proc. Natl. Acad. Sci. U.S. **54**:1584-1592.
13. GERSHON, D., L. SACHS, AND E. WINOCOUR. 1966. The induction of cellular DNA synthesis by simian virus 40 in contact-inhibited and X-irradiated cells. Proc. Natl. Acad. Sci. U.S. **56**:918-925.
14. GINSBERG, H. S., AND M. K. DIXON. 1961. Nucleic acid synthesis in types 4 and 5 adenovirus-infected HeLa cells. J. Exptl. Med. **113**:283-299.
15. HANAWALT, P. C., AND R. H. HAYNES. 1965. Repair replication of DNA in bacteria: Irrelevance of chemical nature of base defect. Biochem. Biophys. Res. Commun. **19**:462-467.
16. HATANAKA, M., AND R. DULBECCO. 1966. Induction of DNA synthesis by SV40. Proc. Natl. Acad. Sci. U.S. **56**:736-740.
17. HENRY, P., P. H. BLACK, M. N. OXMAN, AND S. M. WEISSMAN. 1966. Stimulation of DNA synthesis in mouse cell line 3T3 by simian virus 40. Proc. Natl. Acad. Sci. U.S. **56**:1170-1176.
18. KIRBY, K. S. 1957. A new method for the isolation of deoxyribonucleic acids. Evidence on the nature of bonds between deoxyribonucleic acid and protein. Biochem. J. **66**:495-504.
19. KIT, S. 1960. Compositional heterogeneity of normal and malignant tissue deoxyribonucleic acids. Biochem. Biophys. Res. Commun. **3**: 361-367.
20. KIT, S. 1961. Equilibrium sedimentation in density gradients of DNA preparations from animal tissues. J. Mol. Biol. **3**:711-716.
21. KIT, S., AND D. R. DUBBS. 1963. Biochemistry of vaccinia-infected mouse fibroblasts (strain L-M). IV. ^{3}H-thymidine uptake into DNA of cells exposed to cold shock. Exptl. Cell Res. **31**: 397-406.
22. KIT, S., D. R. DUBBS, AND P. M. FREARSON. 1966. Enzymes of nucleic acid metabolism in cells infected with polyoma virus. Cancer Res. **26**: 638-646.
23. KIT, S., D. R. DUBBS, P. M. FREARSON, AND J. L. MELNICK. 1966. Enzyme induction in SV40-infected green monkey kidney cultures. Virology **29**:69-83.
24. KIT, S., D. R. DUBBS, AND T. C. HSU. 1963. Biochemistry of Vaccinia-infected mouse fibroblasts (Strain L-M). III. Radioautographic and biochemical studies of thymidine-H^3 uptake into DNA of L-M cells and rabbit cells in primary culture. Virology **19**:13-22.
25. KIT, S., D. R. DUBBS, L. J. PIEKARSKI, R. A. DE TORRES, AND J. L. MELNICK. 1966. Acquisition of enzyme function by mouse kidney cells abortively infected with papavavirus SV40. Proc. Natl. Acad. Sci. U.S. **56**:463-470.
26. KIT, S., L. J. PIEKARSKI, AND D. R. DUBBS. 1967. DNA polymerase induced by Simian Virus 40. J. Gen. Virol. **1**:163-173.
27. KLAMERTH, O. 1965. Separation of high-molecular weight deoxyribonucleic acid and ribonucleic acid. Nature **208**:1318-1319.
28. LENNETTE, E. H. 1964. General principles underlying laboratory diagnosis of viral and rickettsial infections. *In* Diagnostic procedures for viral and rickettsial diseases, 3rd ed., p. 51-57. American Public Health Association, Inc., New York.
29. PETTIJOHN, D. E., AND P. C. HANAWALT. 1963. Deoxyribonucleic acid replication in bacteria

following ultraviolet irradiation. Biochim. Biophys. Acta. **72**:127–129.

30. Rapp, F., L. A. Feldman, and M. Mandel. 1966. Synthesis of virus deoxyribonucleic acid during abortive infection of simian cells by human adenoviruses. J. Bacteriol. **92**:931–936.
31. Sauer, G., H. Fischer, and K. Munk. 1966. The effect of SV40 infection on DNA synthesis in *Cercopithecus* kidney cells. Virology **28**:765–767.
32. Sheinin, R. 1966. Deoxyribonucleic acid synthesis in cells replicating polyoma virus. Virology **28**:621–632.
33. Sheinin, R. 1966. DNA synthesis in rat embryo cells infected with polyoma virus. Virology **29**:167–170.
34. Vinograd, J., R. Bruner, R. Kent, and J. Weigle. 1963. Band-centrifugation of macromolecules and viruses in self-generating density gradients. Proc. Natl. Acad. Sci. U.S. **49**:902–910.
35. Vinograd, J., J. Lebowitz, R. Radloff, R. Watson, and P. Laipis. 1965. The twisted circular form of polyoma viral DNA. Proc. Natl. Acad. Sci. U.S. **53**:1104–1111.
36. Vogt, M., R. Dulbecco, and B. Smith. 1966. Induction of cellular DNA synthesis by polyoma virus. III. Induction in productively infected cells. Proc. Natl. Acad. Sci. U.S. **55**: 956–960.
37. Weil, R., M. R. Michel, and G. K. Ruschmann. 1965. Induction of cellular DNA synthesis by polyoma virus. Proc. Natl. Acad. Sci. U.S. **53**:1468–1475.
38. Werchau, H., H. Westphal, G. Maass, and R. Haas. 1966. Untersuchungen über den Nucleinsäuerestoffwechsel von Affennierengewebe-Kulturzellen nach Infektion mit SV40. II. Einfluss der Infektion auf den DNS-Stoffwechsel der Zelle. Arch. Ges. Virusforsch. **19**:351–360.
39. Westphal, H., H. Werchau, G. Maass, and R. Haas. 1966. Untersuchungen über den Nucleinsäuerestoffwechsel von Affennierengewebe-Kulturzellen nach Infektion mit SV40. I. Darstellung and Chromatographie der SV40-DNS. Arch. Ges. Virusforsch. **19**:342–350.
40. Winocour E., A. M. Kaye, and V. Stollar. 1965. Synthesis and transmethylation of DNA in polyoma infected cultures. Virology **27**:156–169.