JOURNAL OF VIROLOGY, Oct. 1967, p. 968-979
Vol. 1, No. 5

Printed in U.S.A.

Virogenic Properties of Bromodeoxyuridine-sensitive and Bromodeoxyuridine-resistant Simian Virus 40-transformed Mouse Kidney Cells

DEL R. DUBBS, SAUL KIT, RAMON A. DE TORRES, AND MILTON ANKEN
Division of Biochemical Virology, Baylor University College of Medicine, Houston, Texas 77025

Received for publication 3 July 1967

When simian virus 40 (SV40)-transformed mouse kidney cells (mKS) were grown in the presence of susceptible indicator cells, SV40 was readily recovered from: (i) 15 transformed cell lines, (ii) transformed cells subcultured 45 times over a 7-month period in medium containing antiviral serum and bromodeoxyuridine (dBU), (iii) 45 of 46 clonal lines isolated in the presence of antiviral serum, (iv) 19 of 19 secondary clones isolated from two clonal lines, and (v) dBU-resistant transformed cell lines. dBU-resistant SV40-transformed mouse kidney cell lines were selected and shown to contain the T antigen and to have normal levels of thymidylate kinase and deoxyribonucleic acid (DNA) polymerase, but to be deficient in thymidine (dT) kinase. Radioautographic and biochemical experiments demonstrated that very little ^{3}H-dT was incorporated into DNA of dBU-resistant cells during a 6-hr labeling period. After infection of dT kinase-deficient mKS cells with vaccinia virus, high levels of dT kinase were induced. The properties of SV40 recovered from dBU-sensitive and dBU-resistant cells were studied. SV40 recovered from transformed cells was shown to express in CV-1 cells at least six functions characteristic of parental virus: synthesis of capsid antigen, synthesis of T antigen, synthesis of viral DNA, induction of dT kinase, induction of DNA polymerase, and induction of host cell DNA synthesis. In addition, SV40 recovered from the transformed cells induced T antigen, dT kinase, deoxycytidylate deaminase, thymidylate kinase, and DNA polymerase in abortively infected mouse kidney cultures, and the virus was also capable of transforming primary cultures of mouse kidney cells.

The fact that cells transformed by simian virus 40 (SV40) contain virus-induced antigens (3, 11, 28, 29, 32) has provided evidence that at least part of the SV40 genome persists in the majority of transformed cells. Recovery of SV40 from transformed cells, however, was often difficult and sporadic. In 1962, Gerber and Kirchstein reported that SV40 recovery could be enhanced by seeding SV40 tumor cells directly onto sensitive indicator cells (10). Studies by Sabin and Koch, which showed that minute amounts of virus could be recovered from tumors induced by inoculation of 2 to 10 tumor cells into adult hamsters, provided strong evidence that SV40 tumor cells were carrying the entire SV40 genome (30). More conclusive evidence that the majority of transformed cells carry the viral genome was obtained by the studies of Black and co-workers (1, 2), who isolated single-cell clones of SV40-transformed cells in the presence of antiserum and showed that those clones yielded infectious SV40. Studies by Tournier et al. (33) showed by neutralization and immunofluorescence with antiviral sera that the SV40 recovered from a clonal strain of SV40-transformed hamster tumor cells was similar to the parental SV40. The virus recovered from transformed cells was also oncogenic for Syrian hamsters and transformed hamster cells in vitro.

The present study provides evidence that, in SV40-transformed mouse kidney lines, nearly every cell carries in a noninfectious state the SV40 genome. Cloning experiments, as well as experiments in which the cells were subcultured in antiviral sera and bromodeoxyuridine (dBU), have ruled out a host cell-virus relationship in which extracellular virus plays an essential role in maintaining the latent infection. Moreover, a study of the properties of SV40 recovered from transformed cells strongly suggests that essentially the entire SV40 genome is maintained in the transformed cell.

MATERIALS AND METHODS

Cell cultures. CV-1 cells, an established strain of green monkey kidney cells (12), were grown in monolayer cultures as previously described (17).

Transformed mouse kidney lines (mKS) were obtained by inoculating primary cultures (6 to 7 days old) of mouse kidney with SV40 clone 307L at an input multiplicity of approximately 150 plaque-forming units (PFU) per cell. The mKS-A and mKS-B lines were obtained from cultures which displayed colonies of transformed cells 22 and 30 days, respectively, after inoculation with SV40. Lines mKS-1, mKS-4, mKS-8, mKS-11, mKS-14, mKS-17, mKS-21, mKS-24, and mKS-28 were obtained by subculturing 1, 4, 8, 11, 14, 17, 21, 24, and 28 days, respectively, after infection with SV40. In this series, colonies of transformed cells were not evident until 21 days postinoculation. The second passage of each line was made when the cells reached a density of about 10 million cells per 55 cm². Subsequently, the lines were subcultured at 3- or 4-day intervals.

The mKS-BU cell lines were obtained from mKS-B by growing the cells in medium containing dBU as shown in Fig. 1. After three passages in 25 μg of dBU per ml (passage 32, dBU 15), a critical point was reached, and cultures had to be fed for 2 weeks with medium lacking dBU. At passage 36, dBU 19, the concentration of dBU was increased to 50 μg per ml (mKS-BU 50). After two passages in 50 μg of dBU per ml, a second critical stage was reached, during which the cultures were fed for 2 weeks with medium lacking dBU and were subcultured once in the absence of dBU. Again at passage 49, dBU 30, it was essential to subculture the lines four consecutive times in the absence of dBU. Finally, at passage 52, dBU 35, the concentration of dBU was increased to 100 μg of dBU per ml, and the cells were grown in this concentration of dBU for 21 passages.

mKS cells were cloned without a feeder layer by the Puck technique (27) in medium containing 1% SV40 antisera in 60-mm plastic petri dishes. Clones were picked from plates containing less than five

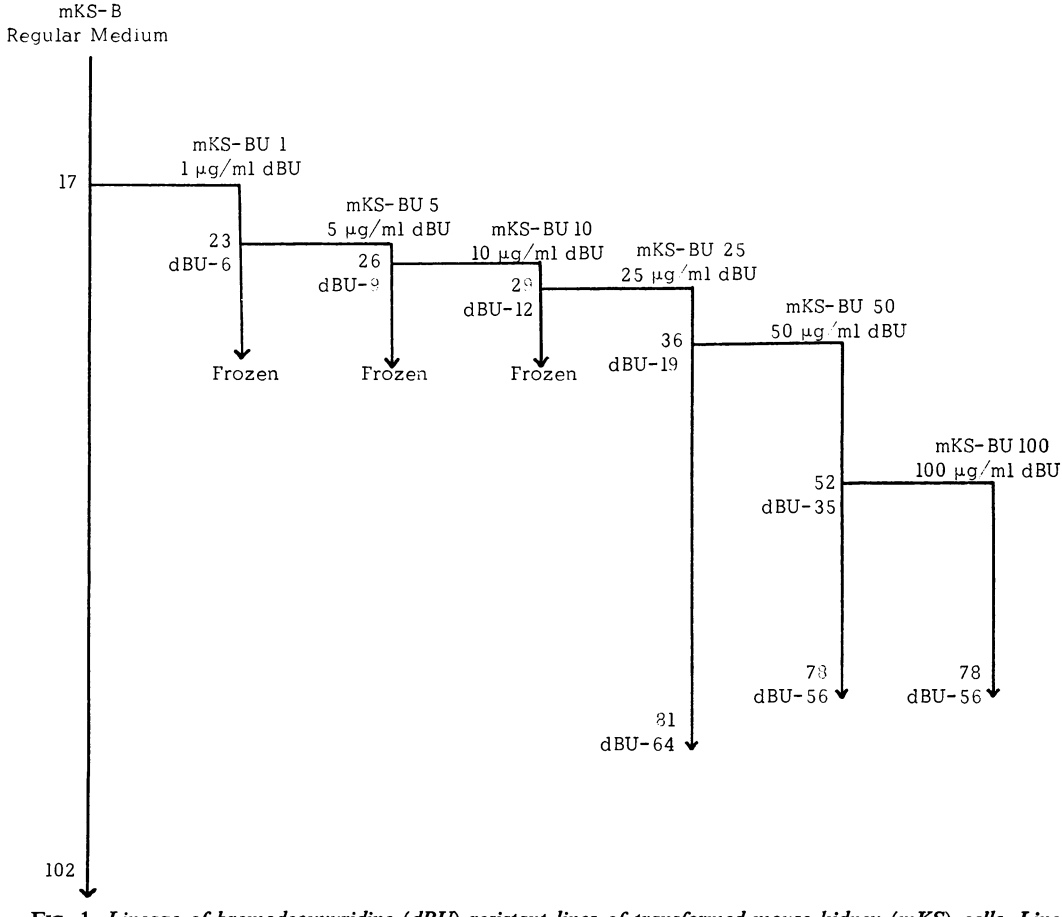

FIG. 1. *Lineage of bromodeoxyuridine (dBU)-resistant lines of transformed mouse kidney (mKS) cells. Lines of mKS cells were designated with the concentration of dBU in which they were grown. Hence, mKS-BU 1 was grown in medium containing 1 μg of dBU per ml, and mKS-BU 5 was grown in medium containing 5 μg of dBU per ml. The numbers at the left indicate passage levels at which the concentration of dBU was increased.*

colonies. Plating efficiencies of mKS-A and mKS-B cells were 45 to 55%, and clones were picked from plates receiving 5 or 10 cells. Plating efficiencies of mKS-BU and mKS-BU antisera-treated (mKS-BUAs) cells were 2.5 and 5.0%, respectively, and clones were picked from plates which initially received 10 and 100 cells.

Virus. SV40 clone 307L was propagated and assayed in monolayer cultures of CV-1 as previously described (17). SV40 clone 307L was used to transform mouse kidney cultures and is referred to as "parental" virus. SV40 strains recovered from transformed mouse kidney cell strains are designated SV40 (mKS-A), SV40 (mKS-BU 25), and SV40 (mKS-BU 100). Vaccinia (IHD) was grown in monolayer cultures of LM or LM(TK$^-$) cells (5) and titrated by plaque assay on CV-1 monolayers.

Antisera. SV40 antiviral sera prepared in horses and SV40 antitumor sera from hamsters bearing SV40 transplant (virus-free) tumors were obtained from Flow Laboratories, Inc., Rockville, Md. The horse antiviral serum had a log neutralization of 3.67 at 1:10 dilution against SV40 clone 307L and a titer of 1:640 against 50 TCD_{50}. SV40 antiviral sera were also prepared in New Zealand white rabbits by immunization with partially purified SV40 clone 307L. For neutralization tests, serial dilutions of virus were made in medium lacking calf serum. Equal volumes of virus dilutions were mixed with equal volumes of antisera or control sera, and the mixtures were incubated at 37 C for 1 hr. Samples of each dilution (0.1 ml) were plated on CV-1 monolayers in 60-mm petri dishes, and unneutralized virus was permitted to adsorb 1 hr at 37 C before overlaying with agar medium.

Isolation of SV40 from mKS cells. For direct assay, 4- or 5-day-old cultures containing 10 million to 30 million cells were used. Cells were scraped into the growth medium with a rubber policeman. The cell suspension was treated by sonic oscillation at 10 kc for 1 min, and was then assayed on CV-1 monolayers for plaque formation. mKS-BU cells were grown for at least one passage in medium lacking dBU prior to assay for SV40.

In a mixed-culture method, 1 million freshly trypsinized mKS cells were mixed with 1 million freshly trypsinized CV-1 cells in 20 ml of growth medium and were permitted to grow together for 3 to 6 days. Then the cells were scraped into the supernatant fluid, and the suspension was treated with sonic oscillation at 10 kc for 1 min and assayed on CV-1 monolayers.

The capacity of mKS cells to produce infectious centers when plated on CV-1 cells under agar was determined as previously described (17). Three-day-old cultures of mKS cells were trypsinized, and the cells were incubated in 2.5 ml of SV40 antiserum (Flow Laboratories, Inc., Rockville, Md.) prior to diluting and plating.

Complement-fixation (CF) tests. SV40 tumor antigen was demonstrated by CF with ascitic fluid from hamsters bearing SV40 transplant (virus-free) tumors and with 2 full units of complement as previously described (24). Cell extracts were prepared by resuspending washed, centrifuged cells in 4 or 9 volumes of modified barbital buffer (4), treating them at 4 C with a Raytheon sonic oscillator at 10 kc for 1 min, and centrifuging them for 1 hr at 34,000 $\times$ *g* or, occasionally, at 100,000 $\times$ *g*. Antigen titers were determined as the highest dilution of antigen giving 3+ or 4+ fixation in the presence of 4 units of antibody (CF titer). For comparative purposes, the antigen titer values were divided by the protein content of the 50-μliter samples used in the CF assay and are expressed as CF units per milligram of protein.

Incorporation of tritium-labeled deoxythymidine (^{3}H-dT) into deoxyribonucleic acid (DNA). Cultures were seeded with 2.5 million cells; 48 and 72 hr after planting, ^{3}H-dT (1 μc and 0.5 μg/ml) was added to the cultures and they were incubated at 37 C for 6 hr. Then, they were washed with saline-glucose solution and were trypsinized (0.05% trypsin and 0.05% ethylenediaminetetraacetic acid). A portion of the resulting cell suspension was used for radioautography, and the specific activity of the DNA was determined on the remainder of the cells (18). Grains were counted with a phase-contrast microscope equipped with an oil immersion objective, and at least 500 cells were scored for each sample.

Enzyme assays. Tritium-labeled deoxyuridine was used as nucleoside substrate in the thymidine kinase assay (14). The assays for thymidylate (dTMP) kinase, DNA polymerase, and deoxycytidylate (dCMP) deaminase have been described (16, 20, 22). The protein content of the centrifuged extracts was determined by the method of Lowry et al. (25).

Isolation of cellular and SV40 DNA from SV40-infected cell cultures. CV-1 cell cultures, infected with 75 to 270 PFU per cell of SV40 clone 307L, SV40 (mKS-BU 25), and SV40 (mKS-BU 100), were incubated from 34 to 44 hr after infection with ^{3}H-dT (0.5 μc and 2 μg/ml). Tritium-labeled DNA was isolated from the infected cultures as described previously (24). Nitrocellulose chromatography and band centrifugation in CsCl density gradients were then carried out to determine the amounts of ^{3}H-labeled SV40 DNA and ^{3}H-labeled cellular DNA synthesized (24).

RESULTS

Transformation of primary mouse kidney cells by SV40. Dense multilayered colonies of cells became evident 15 to 21 days after infection of primary mouse kidney cultures with SV40, clone 307L. These colonies increased in size to 7 to 10 mm in diameter. When cultures containing the transformed colonies were trypsinized and seeded into new bottles, the transformed cells formed confluent monolayers very quickly, reaching cell densities of 20 million to 30 million cells per 55 cm^2, as compared to 5 million to 8 million cells per 55 cm^2 for primary mouse kidney cells. All of the transformed lines tested exhibited high levels of tumor antigen but no detectable capsid antigen or virus particles (20). Moreover, they displayed elevated levels of four enzymes which participate in DNA biosynthesis: dT kinase,

TABLE 1. *Recovery of SV40 from transformed mouse kidney cell lines*

Cell line	Cells required to produce one infectious center	Recovery of SV40 (PFU per culture)	
		Direct assay	Mixed culture
mKS-A	$>5 \times 10^5$ (8)[a]	2×10^{2}[b] (7) 0 (15)	4.0×10^4 (31)
mKS-B	$>5 \times 10^5$ (7)	0 (6)	9.1×10^4 (17)
mKS-1	$>5 \times 10^5$ (6)	0 (5)	7.4×10^3 (12)
mKS-4	$>5 \times 10^5$ (5)	2×10^2 (2) 0 (4)	1.6×10^3 (15)
mKS-8	$>5 \times 10^5$ (5)	1.0×10^2 (1) 0 (2, 4)	3.4×10^3 (13)
mKS-11	$>5 \times 10^5$ (5)	0 (2, 4)	2.2×10^3 (14)
mKS-14	5×10^5 (14)[c]	0 (2, 4)	3.3×10^4 (12)
mKS-17	5×10^5 (17)[c]	0 (2, 4)	2.0×10^2 (15)
mKS-21		0 (2, 4)	1.2×10^3 (4)
mKS-24			5.0×10^3 (10)
mKS-28			2.6×10^3 (12)
mKS-BU 10			1.6×10^4 (39, BU 19)
mKS-BU 15			1.3×10^4 (37, BU 20)
mKS-BU 25		0 (74, BU 57)	1.3×10^4 (74, BU 57)
mKS-BU 100		0 (71, BU 49)	4.8×10^4 (71, BU 49)

[a] Numbers in parentheses refer to passage levels tested.
[b] One or two plaques were produced on plates inoculated with undiluted cell extracts.
[c] One or two plaques were produced on one of five plates receiving 10^5 cells.

dCMP deaminase, dTMP kinase, and DNA polymerase (13, 20).

Recovery of SV40 from mKS cells. Attempts to isolate infectious SV40 from cell extracts and supernatant fluid from mKS cultures were rarely successful (Table 1). Only mKS-A at passage 7, mKS-4 at passage 2, and mKS-8 at passage 1 yielded infectious SV40. Only one or two plaques were produced when CV-1 monolayers were inoculated with undiluted cell extracts from these lines. Subsequent attempts to isolate SV40 from cell extracts and supernatant fluid of these lines were completely unsuccessful.

The mKS cell lines were also tested for their capacity to produce infectious centers when plated on CV-1 monolayers and overlaid with agar medium. With this method, two of eight lines, mKS-14 and mKS-17, each yielded one plaque on one of five plates inoculated with 10^5 cells. The other six lines tested failed to produce any plaques when 10^5 cells were plated on each of five plates.

In contrast to the foregoing experiments, SV40 was readily recovered from mKS cells by the mixed-culture technique. When mKS cells were permitted to grow in contact with growing CV-1 cells, SV40 was recovered in substantial amounts (200 to 100, 000 PFU per culture) from 15 lines. Lines of mKS cells adapted to grow in medium containing dBU also readily yielded SV40 when grown in mixed culture with CV-1 cells.

Repeated attempts to isolate virus from CV-1 cells used in preparing the mixed cultures were unsuccessful.

Recovery of SV40 from mKS cells serially passaged in SV40 antiviral sera. Previous experiments demonstrated that less than 1% of cells of primary mouse kidney cultures are productively infected with SV40, while the majority of cells are abortively infected (20). To learn whether a host-virus relationship existed in the transformed mouse kidney system in which a very small percentage of cells continually released virus, producing multiple lytic cycles, we cultured the mKS cells in the presence of SV40 antisera (As) to eliminate extracellular virus. mKS-BU 25 cells (passage 41, dBU 24) were passaged at 4- to 7-day intervals in medium containing 25 μg of dBU per ml and 1% anti-SV40 serum. At each passage, 1 million washed and trypsinized mKS-BU 25 cells were mixed with 1 million CV-1 cells in 20 ml of medium lacking both anti-SV40 sera and dBU; 4 to 6 days later, these mixed cultures were harvested, and the sonic-treated cell suspension was assayed for infectious virus. The mKS-BUAs cells have now been cultured for at least 7 months and 45 passages in antiviral sera (Table 2), and SV40 has been recovered from every passage.

Cloning of mKS cells. To further rule out a carrier state in which virus was continually released, the cells were cloned in the presence of

TABLE 2. *Recovery of SV40 from mKS-BU 25 cells after serial passage in media containing 25 μg of dBU per ml and 1% antiviral serum*

No. of passages			Recovery of SV40 (PFU per mixed culture)
Total	in dBU	In antiserum	
41	24	0	8.8×10^3
42	25	1	1.6×10^3
46	29	5	1.6×10^3
51	34	10	3.0×10^3
56	39	15	5.4×10^3
61	44	20	9.8×10^3
66	49	25	3.8×10^3
71	54	30	6.6×10^3
76	59	35	8.4×10^4
81	64	40	6.6×10^3
86	69	45	8.0×10^2

SV40 antiviral serum (Tables 3 and 4). Although all of the clonal isolates (except two which were not tested) displayed complement-fixing T antigen, none of them yielded infectious virus by the direct assay of cell extracts and supernatant fluid; 5 of 6 mKS-A clones and 16 of 16 mKS-B clones yielded SV40 when grown in mixed culture with CV-1 cells (Table 3). Each of these clonal lines was tested several times, and 64% of the trials yielded positive results. mKS-A clone 4 was tested seven times without yielding infectious SV40.

TABLE 3. *Recovery of SV40 from clones[a] of transformed mouse kidney (mKS-A and mKS-B) cells*

Clone		T antigen (CF units per mg of protein)	Recovery of SV40[b] (avg no. of PFU per mixed culture)
mKS-A Cl	1	470	1.4×10^3
	2	310	6.6×10^2
	4	40	Negative
	5	40	3.4×10^2
	6	94	8.2×10^2
	7	240	9.0×10^3
mKS-B Cl	1	+[c]	1.2×10^3
	2	420	8.0×10^2
	3	330	1.2×10^3
	4	140	2.8×10^3
	5	220	2.8×10^2
	6	80	3.4×10^2
	7	180	4.5×10^3
	8	170	7.4×10^2
	9	ND[d]	3.4×10^3
	10	250	3.2×10^3
	11	120	1.4×10^3
	12	290	1.4×10^3
	15	260	1.3×10^3
	16	380	1.0×10^3

[a] mKS-A: cloned at passage 49; tested passages, 51–63. mKS-B: cloned at passage 62; tested passages, 64–76.

[b] Virus could not be recovered from any clonal strains by the direct method.

[c] T antigen present but not quantitated.

[d] Not done.

TABLE 4. *Recovery of SV40 from mKS-BU 25 and mKS-BUAs clones[a]*

Clone		T antigen (CF units per mg of protein)	Recovery of SV40[b] (avg no. of PFU per mixed culture)
mKS-BU 25 Cl	1	83	7.2×10^3
	2	ND	1.1×10^4
	3	140	2.5×10^4
	4	180	6.7×10^4
	5	130	3.6×10^3
	6	150	4.1×10^3
mKS-BU As Cl	1	200	1.0×10^3
	2	570	7.3×10^4
	3	320	4.2×10^3
	4	210	1.1×10^3
	5	130	2.1×10^4
	6	280	1.8×10^4
	7	280	2.8×10^3
	8	750	4.4×10^2
	9	170	9.5×10^2
	10	270	6.0×10^4
	11	140	4.3×10^4
	12	230	1.3×10^4
	13	190	5.3×10^3
	14	140	6.3×10^3
	15	240	8.2×10^3
	16	100	2.9×10^4
	17	260	1.8×10^3
	18	240	2.8×10^3
	19	360	1.8×10^3
	20	190	1.5×10^4

[a] mKS-BU 25: cloned at passage 53, BU 36; tested passages, 55–65. mKS-BUAs clones: cloned at passage 58, BU-41, As-17; tested passages, 62–69.

[b] Virus could not be recovered from any clonal strains by the direct method.

Clones (mKS-BU 25) were also isolated from mKS lines serially passaged in medium containing dBU and from cells grown for 17 consecutive passages in the presence of anti-viral sera (mKS-BUAs). Infectious SV40 was recovered from 6 of 6 mKS-BU 25 and 20 of 20 mKS-BUAs clones by the mixed-culture method (Table 4); 86% of the attempts to recover SV40 by the mixed-culture technique were successful.

Two of the mKS-BUAs clones (2 and 16) were

TABLE 5. *Recovery of SV40 from transformed mouse kidney lines cloned[a] twice in the presence of anti-SV40 serum*

Source	Clone	Recovery of SV40[b] (PFU per mixed culture)
mKS-BU As Cl 2	2-1	5.6×10^4
	2-2	4.4×10^4
	2-3	9.4×10^4
	2-4	4.4×10^4
	2-5	8.6×10^4
	2-6	1.1×10^5
	2-7	7.2×10^3
	2-8	1.7×10^5
	2-9	1.3×10^5
	2-10	9.2×10^4
mKS-BU As Cl 16	16-2	3.2×10^4
	16-3	$>1.0 \times 10^5$
	16-4	1.4×10^5
	16-5	4.8×10^4
	16-6	3.0×10^4
	16-7	5.4×10^4
	16-8	3.6×10^4
	16-9	3.4×10^4
	16-10	8.2×10^4

[a] mKS-BUAs Cl 2: subcloned at passage 73, BU-41, As-17. mKS-BUAs Cl 16: subcloned at passage 74, BU-41, As-17.

[b] Virus could not be recovered from any subclones by the direct method.

selected for recloning in the presence of antiserum. Secondary clones were isolated from each of the two clones, and all yielded SV40 when grown in mixed culture with CV-1 cells (Table 5).

These results rule out the possibility of extracellular virus as a source of infection and suggest that, at least in the SV40-transformed mouse kidney lines which we studied, nearly every transformed cell carried the potential to produce infectious SV40 and, therefore, must have carried the entire SV40 genome in a noninfectious state.

Properties of dBU-resistant mKS cell strains. Previous studies with LM mouse fibroblasts and HeLa cells have shown that development of resistance to dBU was associated with a loss of the capacity of the cell to phosphorylate dBU (15, 19). Therefore, studies were initiated to determine the levels of three enzymes: dT kinase, which catalyzes the first step in the utilization of thymidine and dBU; dTMP kinase, which catalyzes the second step; and DNA polymerase. Although the dTMP kinase and DNA polymerase levels of the dBU-resistant lines were normal, the dT kinase levels were only about 1% of the level of the parental mKS-B line (Table 6). It was interesting to note that, although the mKS-BU 25 and mKS-BU 100 cells were deficient in dT kinase activity, they displayed expected levels of T antigen (Table 6) and yielded SV40 when grown in mixed culture with CV-1 cells (Tables 1 and 3).

Radioautographic experiments were also performed to learn whether ^{3}H-dT was incorporated into DNA by dBU-resistant cells; 2- and 3-day-old cultures of cells were incubated for 6 hr with ^{3}H-dT. Radioautographic slides were prepared, and the percentage of cells with labeled nuclei was determined. About 80% of the mKS-A and mKS-B cells displayed labeled nuclei 48 hr after subculturing, whereas only 32% of mKS-BU 25 and 15% of mKS-BU 100 cells incorporated ^{3}H-dT into DNA (Table 7). Grain counts showed

TABLE 6. *Enzyme activities and T antigen titers of mKS-B, mKS-BU 25, and mKS-BU 100 cells*

Cell line	Passage level	Cells per bottle (millions)	Enzyme activity[a]			T antigen (CF units per mg of protein)
			dT Kinase	dTMP Kinase	DNA Polymerase	
mKS-B	94	22.1	4.0			750
	97	24.3		39.4	0.12	210
mKS-BU 25	74, BU 56	10.3	0.12			400
	76, BU 58	12.4		62.3	0.13	110
	77, BU 59	11.3	0.02		0.18	130
mKS-BU 100	71, BU 48	12.2	0.04			400
	73, BU 50	4.8		42.1	0.11	41
	83, BU 54	14.4	0.05			260

[a] dT kinase: $\mu\mu$moles of deoxyuridine monophosphate (dUMP) formed per μg of protein in 10 min at 38 C; dTMP kinase: $\mu\mu$moles of deoxythymidine diphosphate (dTDP) + deoxythymidine triphosphate (dTTP) formed per μg of protein in 10 min at 38 C; DNA polymerase: $\mu\mu$moles of dTTP incorporated into DNA per μg of protein in 30 min at 38 C.

that 73% of the mKS-B cells were heavily labeled (more than 49 grains per nucleus), while 20% were unlabeled (less than 10 grains per nucleus) and 7% were lightly labeled (Fig. 2). In contrast, only about 7% of the mKS-BU 25 cells and no mKS-BU 100 cells were heavily labeled. Most of the labeled mKS-BU 100 cells had only 10 to 30 grains per nucleus. The specific activity of the DNA was also determined. The mKS-BU 25 and mKS-BU 100 cells incorporated only 6 and 1%, respectively, as much ^{3}H-dT per μg of DNA as did the parental cells (Table 7).

Previous studies in our laboratory have shown that dT kinase can be induced in two dBU-resistant cell strains, LM(TK$^-$) and HeLa BU-100 (15, 19), by infection with vaccinia or herpes simplex viruses. Experiments were performed to learn whether dT kinase and DNA polymerase were induced following infection of mKS-BU 25 and mKS-BU 100 cells with vaccinia and SV40.

TABLE 7. *Incorporation of ^{3}H-thymidine into DNA of mKS-A, mKS-B, mKS-BU 25, and mKS-BU 100 cells*[a]

Cell line	Time after subculturing ^{3}H-dT added	Cells per bottle (millions)	Incorporation (counts per min per μg of DNA)	Percentage of nuclei labeled
	hr			
mKS-A	48	10.9	6,270	82.3
	72	18.7	3,134	61.2
mKS-B	48	11.0	5,920	79.4
	72	22.6	2,489	54.2
mKS-BU 25	48	11.6	376	31.9
	72	20.5	161	25.0
mKS-BU 100	48	8.7	51	15.0
	72	15.4	16	5.9

[a] Cultures were seeded with 2.5 × 10^6 cells in 20 ml of medium. At indicated times, 0.1 ml of ^{3}H-dT (20 μc and 10 μg) was added to each culture; the cultures were then incubated at 37 C for 6 hr.

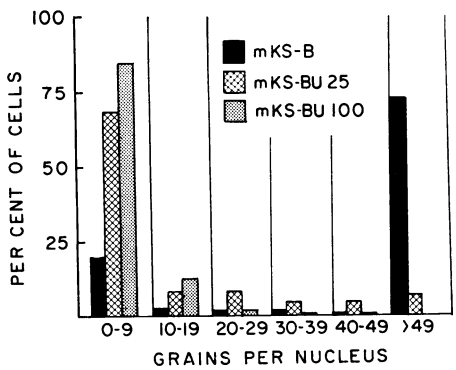

FIG. 2. *Incorporation of ^{3}H-dT into DNA of parental and dBU-resistant mKS cells. Cells were incubated for 6 hr in the presence of ^{3}H-dT (20 μc and 10 μg).*

Although uninfected mKS-BU 25 and mKS-BU 100 cells have extremely low levels of dT kinase, both dT kinase and DNA polymerase are rapidly induced after infection with vaccinia (Table 8). Attempts to induce dT kinase in mKS-BU 25 cells by superinfecting these cells with SV40 (input multiplicity, 240 PFU per cell) were unsuccessful.

Properties of SV40 recovered from mKS and dBU-resistant mKS cells. Further evidence that transformed mouse kidney cells carry the entire SV40 genome was obtained by comparing the properties of viruses isolated from mKS cells with the properties of parental SV40. The virus isolates, as well as parental SV40, induced the synthesis of T antigen in both CV-1 cells and primary mouse kidney cells, and were neutralized by SV40 antiviral sera. Moreover, the viruses isolated from mKS cells transformed primary mouse kidney at an efficiency approximately equal to that of parental SV40.

Productive infection of CV-1 cells with parental SV40 has been shown to induce several enzymes which function in DNA biosynthesis: dT kinase, thymidylate synthetase, dihydrofolate reductase, and DNA polymerase (7, 17, 22). When CV-1 cells were infected with virus recovered from mKS-A, mKS-BU 25, or mKS-BU 100, dT kinase was induced (Table 9). SV40 (mKS-BU 100) also induced DNA polymerase activity in CV-1 cells. When virus strains SV40 (mKS-A), SV40 (mKS-BU 25), and SV40 (mKS-BU 100)

TABLE 8. *Induction of dT kinase and DNA polymerase in mKS-BU 25 and mKS-BU 100 cells infected with vaccinia virus*[a]

Cell line	Time after inoculation	dT kinase		DNA polymerase	
		Control	Infected	Control	Infected
	hr				
mKS-BU 25	1	0.00	0.00	0.12	0.16
	3	0.01	0.50	0.17	0.17
	7	0.00	4.36	0.13	0.56
mKS-BU 100	7	0.01	2.08	0.23	0.83

[a] Three-day-old cultures of mKS-BU 25 cells containing 11.3 × 10^6 cells per bottle were inoculated with vaccinia virus at an input multiplicity of 5.3 PFU per cell; 4-day-old cultures of mKS-BU 100 cells containing 13.7 × 10^6 cells were inoculated with virus at an input multiplicity of 6 PFU per cell.

TABLE 9. *Induction of enzyme activity in CV-1 cultures by SV40 recovered from dBU-sensitive and dBU-resistant mKS lines*

Virus	Recovered from mKS passage	Input multiplicity (PFU per cell)	Time after SV40 infection	T antigen (CF units per mg of protein)	Enzyme activity[a]	
					dT kinase	DNA polymerase
			hr			
None				0	1.2	
Parental SV40		138	40	2,100	22.4	
SV40 (mKS-A)	31	85	40	2,000	19.8	
SV40 (mKS-BU 25)	36, BU 19	61	40	1,900	17.1	
None			30	0		0.80
			46	0		0.16
SV40 (mKS-BU 100)	71, BU 49	198	30	430		1.49
			46	340		1.47
None			50	0	1.8	
Parental SV40		127	50	1,200	20.0	
SV40 (mKS-BU 25)	36, BU 19	75	50	2,000	22.3	
SV40 (mKS-BU 100)	71, BU 49	270	50	1,900	22.7	

[a] dT kinase: $\mu\mu$moles of dUMP formed per μg of protein in 10 min at 38 C; DNA polymerase: $\mu\mu$moles of ^{3}H-dTTP incorporated into DNA per μg of protein in 30 min at 38 C.

TABLE 10. *Induction of enzyme activity in primary mouse kidney cultures[a] by SV40 recovered from dBU-sensitive and dBU-resistant mKS lines*

Virus	Recovered from mKS passage	Input multiplicity (PFU per cell)	Time after SV 40 infection	T antigen (CF units per mg of protein)	Enzyme activity[b]			
					dT kinase	dCMP deaminase	dTMP kinase	DNA polymerase
			hr					
None					0.46	4.7		
Parental SV40		360	52		3.31	20.8		
SV40 (mKS-A)	31	237	52		2.44	12.6		
SV40 (mKS-BU 25)	36, BU 19	330	52		3.51	16.2		
None			30	0	0.07		16.3	0.18
			50	0			12.6	
SV40 (mKS-BU 100)	71, BU 49	417	30	140	0.34		20.4	0.45
			50	42			32.7	
None				0			11.8	
Parental SV40		222	39	ND[c]			22.7	
SV40 (mKS-A)	31	181	39	240			16.3	
SV40 (mKS-BU 25)	36, BU 19	508	39	500			20.8	

[a] Seven-day-old primary mouse kidney cultures contained 4×10^6 cells per culture.

[b] dT kinase and dCMP deaminase: $\mu\mu$moles of dUMP formed per μg of protein in 10 min at 38 C; dTMP kinase: $\mu\mu$moles of dTDP + dTTP formed per μg of protein in 10 min at 38 C; DNA polymerase: $\mu\mu$moles of ^{3}H-dTTP incorporated into DNA per μg of protein in 30 min at 38 C.

[c] Not done.

were used to infect primary mouse kidney cells abortively, dT kinase, dCMP deaminase, dTMP kinase, and DNA polymerase were all induced (Table 10).

Induction of host cell DNA synthesis. To learn whether SV40 recovered from transformed mouse kidney cells induced host cell DNA synthesis, CV-1 cells were infected with parental SV40, SV40 (mKS-BU 25), and SV40 (mKS-BU 100), and were pulse-labeled with ^{3}H-dT at 34 to 44 hr after infection. In cultures infected with each of the viruses, the total ^{3}H-dT incorporated into

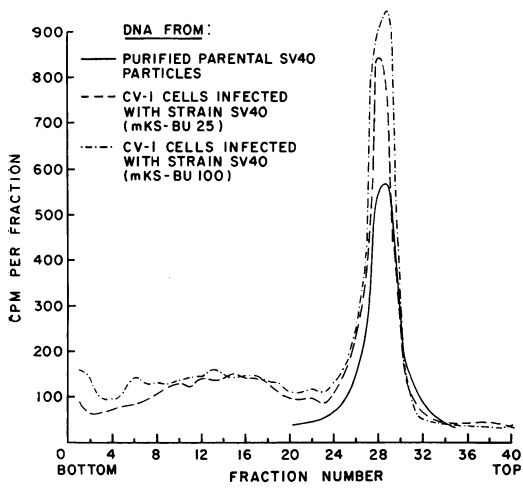

FIG. 3. *Sedimentation velocity (band centrifugation) of ^{3}H-dT-labeled DNA from CV-1 cells infected with SV40 (mKS-BU 25) and SV40 (mKS-BU 100). A marker SV40 DNA (1,500 counts/min), isolated from ^{3}H-dT-labeled and purified parental SV40 particles, was also studied. The DNA preparations were centrifuged for 2.5 hr at 35,000 rev/min and 20 C in the SW 39 rotor of a Spinco model L-2 ultracentrifuge. Bulk solution: 3 ml of CsCl; density, 1.503 g/cm^{-3}. Lamella: 50 μliters of DNA [2.6 μg and 4,760 counts/min from SV40 (mKS-BU 25) and 3.2 μg and 5,400 counts/min from SV40 (mKS-BU 100)]. The solution was overlayed with 1.8 ml of paraffin oil (petrolatum). Eight-drop fractions were collected on 2.5-cm squares of Whatman number 4 paper, washed with 5% trichloroacetic acid and ethyl alcohol, dried for 1 hr at 70 C, and counted in a Packard Tri-Carb liquid scintillation spectrometer.*

DNA was increased 10- to 13-fold, and the net DNA synthesized (estimated by colorimetric methods) was 50% greater than in uninfected cultures. After heat denaturation and nitrocellulose chromatography (24), 37 to 52% of the radioactive DNA from the infected cultures exhibited the properties of single-stranded cellular DNA. In contrast, 97% of the labeled DNA from uninfected cultures had the properties of single-stranded cellular DNA.

Figure 3 illustrates band centrifugation experiments in CsCl density gradients of the tritium-labeled DNA from CV-1 cell cultures infected with SV40 (mKS-BU 25) and SV40 (mKS-BU 100). A marker DNA isolated from purified SV40 parental virus particles was also centrifuged. From 35 to 42% of the radioactive DNA from infected cultures (fractions 1–23) and over 90% of the labeled DNA from uninfected cultures sedimented more rapidly than marker SV40 DNA. From the amount of radioactive DNA sedimenting more rapidly than marker SV40 DNA, an independent estimate can be made of the relative amount of labeled cellular DNA synthesized in the infected cultures. This estimate is in good agreement with that obtained from the nitrocellulose chromatography experiments (24). Since the incorporation of ^{3}H-dT into DNA was increased over 10-fold in the infected cultures, it can be concluded that a substantial increase in the labeling of cellular DNA occurred 34 to 44 hr after SV40 (mKS-BU 25) and SV40 (mKS-BU 100) infections.

About 48 to 65% of the radioactive DNA from infected cultures was resistant to heat denaturation and banded at a position identical with that of radioactive DNA isolated from parental SV40 particles (Fig. 3). It is, therefore, likely that the DNA from SV40 (mKS-BU 25) and SV40 (mKS-BU 100) had approximately the same molecular weight and conformation as that from parental SV40.

The results summarized in Table 11 indicate that SV40 strains recovered from mKS cells express at least six functions characteristic of parental SV40 in CV-1 cells and also six characteristic SV40 functions in abortively infected mouse kidney cells. The observations concerning the sedimentation rates of DNA from SV40

TABLE 11. *Summary of functions expressed in CV-1 and primary mouse kidney cells by SV40 strains recovered from mKS cells*

Viral function	Parental SV40	SV40 (mKS-A)	SV40 (mKS-BU 25)	SV40 (mKS-BU 100)
Synthesis of T antigen (CF) in CV-1 and mouse kidney	+	+	+	+
Synthesis of V antigen (neutralization) in CV-1	+	+	+	+
Synthesis of viral DNA in CV-1	+	+	+	+
Induction in CV-1 cultures of				
dT kinase	+	+	+	+
DNA polymerase	+	ND[a]	ND	+
Induction in mouse kidney cultures of				
dT kinase	+	+	+	+
dCMP deaminase	+	+	+	ND
dTMP kinase	+	+	+	+
DNA polymerase	+	ND	ND	+
Induction of host cell DNA synthesis in CV-1 cells	+	ND	+	+
Transformation of mouse kidney	+	+	+	ND

[a] Not done.

strains and the phenotypic characteristics of these strains provide strong additional evidence that essentially the *entire* complement of SV40 genetic information is maintained in the transformed cell.

DISCUSSION

The induction of synthesis of SV40 in transformed cells by association with susceptible cells has previously been reported (10, 31, 33). Utilizing this technique, it has been possible to show that nearly every cell in the transformed mouse kidney lines which we studied carried the potential to synthesize infectious virus. These results are in contrast to those reported for certain SV40-transformed hamster kidney strains (1, 8, 26, 32, 33), which have not yielded infectious virus by this technique. At this time, SV40 has not been recovered from mKS-A, clone 4. Failure to recover SV40 from that clonal line, as well as from other strains cited above, suggests that in these instances either a defective SV40 genome is maintained in the transformed cell or that conditions for inducing virus synthesis have not yet been achieved.

The mechanism by which copropagation of transformed cells with susceptible cells induces SV40 synthesis has not been completely elucidated. Experiments indicate that viability of the transformed cell and direct contact with the susceptible cell are essential (9). Perhaps, interspecific hybridization (6) of mKS and CV-1 cells occurs, thereby establishing an intracellular environment favorable for the expression of those SV40 functions blocked in mKS cells. Some evidence supporting this possibility has been obtained by Gerber (9), who has shown that there is an earlier appearance of SV40 when mixed cultures of transformed and sensitive cells are treated with ultraviolet-irradiated Sendai virus, a treatment known to enhance cell fusion.

In general, virus has been easier to recover from mKS lines which have been passaged in medium containing dBU. Larger yields of virus were usually obtained and a higher percentage of trials were successful. The reason for this empirical finding has not been established.

Initially, mKS cells were propagated in medium containing dBU to inhibit possible replication of SV40 in occasional cells (17). However, even after 69 passages in the presence of dBU, SV40 could readily be recovered from mKS cells. Moreover, cloning experiments demonstrated that virtually every cell in the mKS-BUAs lines carried the SV40 genome. It has also been shown that SV40-induced hamster tumor cells serially passaged in and resistant to 10 μg of arabinofuranosylcytosine (ara-C) per ml are virogenic, although the same concentration of ara-C completely inhibits SV40 replication in an established strain of rhesus monkey kidney cells (9).

After prolonged passage in the presence of increasing concentrations of the analogue, the mKS cells gradually became resistant to dBU, and dT kinase activity was lost. Some of the mKS (BU) cell lines exhibited less than 1% of the dT kinase activity of parental mKS cells. Nevertheless, all dBU-resistant mKS cell lines had normal levels of SV40 T antigen, thus providing further evidence that dT kinase and T antigen are dissimilar (21).

The fact that dBU-resistant mKS cells were deficient in dT kinase activity was not unexpected. It is interesting, however, that SV40 strains recovered from either dBU-resistant or dBU-sensitive mKS cell lines retained their capacity to induce dT kinase in CV-1 and in primary mouse kidney cultures. One motivation for propagating mKS cells in the presence of dBU was the expectation that mutant SV40 strains might be obtained, some of which would lack dT kinase-inducing activity. Nevertheless, all of the SV40 strains studied to date retained an undamaged gene for inducing this enzyme.

The hypothesis can be advanced that the SV40 function under discussion is a regulatory gene which normally derepresses host cell dT kinase. This hypothesis would be consistent with the finding that dT kinase activity is enhanced in mouse kidney and in CV-1 cell cultures by SV40 infection and that dT kinase activity is elevated in mKS cell lines. This function would be inoperative in mKS-BU 100 cells, since these latter cells presumably are mutants selected by prolonged growth in dBU for defective cellular dT kinase cistrons (15, 19). Thus, derepression of the defective dT kinase cistron would not be expected to restore dT kinase activity to mKS-BU 100 cells. However, infection by vaccinia virus, which replicates in murine cells and induces a new virus-specific enzyme (14), would restore dT kinase activity to dBU-resistant mKS cells.

The postulate that SV40 derepresses a host cell dT kinase does not account for the finding that the Michaelis constant of the dT kinase partially purified from SV40-infected CV-1 cells is altered, although the Michaelis constant of the enzyme from SV40-infected mouse kidney cultures or from SV40-transformed mKS cells is similar to that of the enzyme from uninfected mouse or CV-1 cells. These latter findings might signify the existence of a second SV40 function controlling the formation of a new SV40-specific

dT kinase in CV-1 cells (13, 17, 21). To explain the failure to express the second dT kinase function in mKS-BU 100 cells, it may be assumed that expression of an SV40 gene controlling the formation of a new dT kinase is inhibited in primary mouse kidney and in mKS cell lines. It has been shown that twisted-circular SV40 DNA and SV40 capsid proteins are not made in primary mouse kidney or mKS cells, although T antigen is formed in both of these cell types and cellular DNA synthesis is induced by SV40 in mouse kidney cultures. The suggestion made here is that the block in expression of SV40 genes in mKS cells is prior to a structural gene for an SV40-specific dT kinase. In contrast, all the SV40 genes under discussion are expressed in CV-1 cells, in which SV40 replicates.

An implication of our findings is that viral genomes persisting in transformed cells are not necessarily defective. On the contrary, genes for capsid protein formation or other viral functions may be intact but not expressed in the transformed cell. Further evidence that mKS cells carry the entire SV40 genome was obtained by investigating the properties of the virus recovered from mKS lines. Not only was the SV40 from mKS cells able to replicate in CV-1 cells, but it also expressed several other viral functions characteristic of the parental virus, such as induction of T antigen, dT kinase, DNA polymerase, and host cell DNA synthesis. Moreover, the virus was neutralized by anti-SV40 sera, was capable of inducing dT kinase, dCMP deaminase, dTMP kinase, and DNA polymerase in mouse kidney cultures, and could transform the mouse kidney cells. Finally, band centrifugation and nitrocellulose chromatography experiments have shown that the molecular size and conformation of the DNA obtained from the SV40 strains recovered from mKS cells were indistinguishable from that of parental SV40 DNA. At this time, tumor induction in newborn hamsters has not been studied with the virus recovered from mKS cells, nor have transplantation antigens been studied. However, the facts already established suggest that the SV40 genome is maintained *in toto* in the transformed mouse kidney cells.

ACKNOWLEDGMENTS

This investigation was supported by grants from the American Cancer Society (E 291), the National Science Foundation (GB 5917), the Robert A. Welch Foundation (Q-163), and by Public Health Service grants CA-06656, 1-K6-AI-2352, and 5-K3-Ca 25,797.

We thank Judith Rotbein, Carolyn Smith, Sun Ock Yim, and Marjorie Johnson for able technical assistance.

LITERATURE CITED

1. BLACK, P. H. 1966. An analysis of SV40-induced transformation of hamster kidney tissue *in vitro*. III. Persistence of SV40 viral genome in clones of transformed hamster cells. J. Natl. Cancer Inst. **37**:487–493.
2. BLACK, P. H., W. P. ROWE, AND H. L. COOPER. 1963. An analysis of SV40-induced transformation of hamster kidney tissue *in vitro*. II. Studies of three clones derived from a continuous line of transformed cells. Proc. Natl. Acad. Sci. U.S. **50**:847–854.
3. BLACK, P. H., W. P. ROWE, H. C. TURNER, AND R. J. HUEBNER. 1963. A specific complement-fixing antigen present in SV40 tumor and transformed cells. Proc. Natl. Acad. Sci. U.S. **50**: 1148–1156.
4. CAMPBELL, D. H., J. S. GARVEY, N. E. CREMER, AND D. H. DUSSDORF. 1963. Methods in immunology, p. 246–247. W. A. Benjamin, Inc., New York.
5. DUBBS, D. R., AND S. KIT. 1964. Isolation and properties of vaccinia mutants deficient in thymidine kinase-inducing activity. Virology **22**:214–225.
6. EPHRUSSI, B., AND M. C. WEISS. 1965. Interspecific hybridization of somatic cells. Proc. Natl. Acad. Sci. U.S. **53**:1040–1042.
7. 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.
8. GERBER, P. 1964. Virogenic hamster tumor cells: induction of virus synthesis. Science **145**:833.
9. GERBER, P. 1966. Studies on the transfer of subviral infectivity from SV40-induced hamster tumor cells to indicator cells. Virology **28**:501–509.
10. GERBER, P., AND R. L. KIRCHSTEIN. 1962. SV40-induced ependymomas in newborn hamsters. I. Virus-tumor relationships. Virology **18**:582–588.
11. HOGGAN, M. D., W. P. ROWE, P. H. BLACK, AND R. J. HUEBNER. 1965. Production of "tumor-specific" antigens by oncogenic viruses during acute cytolytic infection. Proc. Natl. Acad. Sci. U.S. **53**:12–19.
12. JENSEN, F. C., A. J. GIRARDI, R. V. GILDEN, AND H. KOPROWSKI. 1964. Infection of human and simian tissue cultures with Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S. **52**:53–59.
13. KIT, S. 1967. Induction of enzymes of DNA metabolism by simian virus 40. *In* Y. Ito [ed.], Subviral carcinogenesis.
14. KIT, S., AND D. R. DUBBS. 1965. Properties of deoxythymidine kinase partially purified from noninfected and virus-infected mouse fibroblast cells. Virology **26**:16–27.
15. KIT, S., D. R. DUBBS, AND P. M. FREARSON. 1966. HeLa cells resistant to bromodeoxyuridine and deficient in thymidine kinsase activity. Intern. J. Cancer **1**:19–30.
16. 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.

17. 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.
18. 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-^{3}H uptake into DNA of L-M cells and rabbit cells in primary culture. Virology **19**:13–22.
19. KIT, S., D. R. DUBBS, L. J. PIEKARSKI, AND T. C. HSU. 1963. Deletion of thymidine kinase activity from L cells resistant to bromodeoxyuridine. Exptl. Cell Res. **31**:297–312.
20. 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 papovavirus SV40. Proc. Natl. Acad. Sci. U.S. **56**:463–470.
21. KIT, S., J. L. MELNICK, M. ANKEN, D. R. DUBBS, R. A. DE TORRES, AND T. KITAHARA. 1967. Nonidentity of some simian virus 40-induced enzymes with tumor antigen. J. Virol. **1**:684–692.
22. KIT, S., L. J. PIEKARSKI, AND D. R. DUBBS. 1967. DNA polymerase induced by simian virus 40. J. Gen. Virol. **1**:163–175.
23. KIT, S., R. A. DE TORRES, AND D. R. DUBBS. 1967. Induction of deoxycytidylate (dCMP) deaminase activity in SV40-infected mouse kidney cultures. Proc. Am. Assoc. Cancer Res. **8**:37.
24. KIT, S., R. A. DE TORRES, D. R. DUBBS, AND M. L. SALVI. 1967. Induction of cellular deoxyribonucleic acid synthesis by simian virus 40. J. Virol. **1**:738–746.
25. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. H. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
26. MELNICK, J. L., K. S. KHERA, AND F. RAPP. 1964. Papovavirus SV40: failure to isolate infectious virus from transformed hamster cells synthesizing SV40-induced antigens. Virology **23**:430–432.
27. PUCK, T. T., P. I. MARCUS, AND S. J. CIECIURA. 1956. Clonal growth of mammalian cells in vitro. Growth characteristics of colonies from single HeLa cells with and without a "feeder" layer. J. Exptl. Med. **103**:273–284.
28. RAPP, F., J. S. BUTEL, AND J. L. MELNICK. 1964. Virus-induced intra-nuclear antigen in cells transformed by papovavirus SV40. Proc. Soc. Exptl. Biol. Med. **116**:1131–1135.
29. RAPP, F., T. KITAHARA, J. S. BUTEL, AND J. L. MELNICK. 1964. Synthesis of SV40 tumor antigen during replication of simian papovavirus (SV40). Proc. Natl. Acad. Sci. U.S. **52**:1138–1142.
30. SABIN, A. B., AND M. A. KOCH. 1963. Evidence of continuous transmission of noninfectious SV40 viral genome in most or all SV40 hamster tumor cells. Proc. Natl. Acad. Sci. U.S. **49**:304–311.
31. SABIN, A. B., AND M. A. KOCH. 1963. Behavior of noninfectious SV40 viral genome in hamster tumor cells: induction of synthesis of infectious virus. Proc. Natl. Acad. Sci. U.S. **50**:407–417.
32. SABIN, A. B., AND M. A. KOCH. 1964. Source of genetic information for specific complement fixing antigens in SV40 virus-induced tumors. Proc. Natl. Acad. Sci. U.S. **52**:1131–1138.
33. TOURNIER, P., R. CASSINGENA, R. WICKER, J. COPPEY, AND H. SUAREZ. 1967. Etude du méchanisme de l'induction chez des cellules de hamster syrien transformées par le virus SV40. I. Propertiés d'une lignée cellulaire clonale. Intern. J. Cancer **2**:117–132.