ACQUISITION OF ENZYME FUNCTION BY MOUSE KIDNEY CELLS ABORTIVELY INFECTED WITH PAPOVAVIRUS SV40*

By Saul Kit, Del R. Dubbs, Lech J. Piekarski, Ramon A. de Torres,† and Joseph L. Melnick

DIVISION OF BIOCHEMICAL VIROLOGY, DEPARTMENT OF VIROLOGY AND EPIDEMIOLOGY, BAYLOR UNIVERSITY COLLEGE OF MEDICINE, HOUSTON, TEXAS

Communicated by Albert B. Sabin, June 6, 1966

Papovavirus SV40 induces a proliferative response in *mouse kidney* cell cultures accompanied by alterations in cell morphology.[1] The continuous mouse kidney cell lines derived from SV40-infected cultures contain the SV40-specific tumor (T) antigen characteristic of green monkey kidney (GMK) cells acutely infected with SV40 and of transformed human and hamster cell lines.[2–5]

Acute infection of *monkey kidney* cell cultures (GMK or CV-1) by SV40 results in a marked stimulation of DNA synthesis and the induction of thymidine kinase, thymidylate synthetase, dihydrofolate reductase, and DNA polymerase.[6–8] Acute infection of *mouse kidney* cell cultures by another papovavirus, polyoma, also leads to enhancement of DNA biosynthesis and the activities of thymidine kinase, thymidylate synthetase, dihydrofolate reductase, DNA polymerase, deoxycytidylate deaminase, and thymidylate kinase.[8–10] The enzymes mentioned catalyze six of the seven steps in the terminal pathway of thymidine metabolism and DNA synthesis.

In the present study, early events following infection of *mouse kidney* cell cultures by SV40 have been investigated.[11] The data to be presented show that (1) SV40 is adsorbed to mouse kidney cells, but less efficiently than to monkey cells; (2) SV40 undergoes an eclipse phase in mouse kidney cells; (3) new infectious SV40 appears, but less than 1 per cent of the mouse cells are capable of forming infectious centers when plated on noninfected monolayers of monkey cells; (4) SV40 enhances appreciably the activities of dCMP deaminase, thymidine kinase, thymidylate kinase, and DNA polymerase; (5) DNA synthesis is stimulated following infection with SV40; and (6) SV40 induces the formation of T-antigen in mouse cells.

Materials and Methods.—Cells: The propagation of CV-1 cells, an established line of African green monkey kidney cells, has been described.[7] Primary cultures of mouse kidney cells were prepared from 10- to 14-day-old Swiss or Balb C mice.[9] The mouse cells were used 5–12 days after planting and contained 3.5–5.0 $\times$ 10^6 cells per 8-oz prescription bottle.

Virus: Clonal strains (307L and 308S) of SV40 were grown and assayed in CV-1 cells as previously described.[7] CV-1 and mouse kidney cell cultures were inoculated at input multiplicities ranging from about 45 to 620 PFU/cell.

Determination of infectious centers: The percentage of cells capable of forming infectious centers was determined by plating cells from infected cultures on noninfected CV-1 monolayers as previously described.[7] Extracellular virus was removed by washing and treating the cultures with viral antiserum 2 hr after infection and just before plating.

Adsorption of SV40 to CV-1 cells and mouse kidney cells: Four-ounce bottles containing 2–3 $\times$ 10^6 CV-1 or mouse kidney cells were infected, at input multiplicities of approximately 1 PFU for 500 cells, with 0.1-ml aliquots of either chilled or prewarmed SV40. Virus was allowed to adsorb at either 4 or 37°C. At the end of the adsorption period, 5 ml of cold saline-glucose solution was added to each bottle and the supernatant fluid harvested and frozen until assayed on CV-1 monolayers. Four-ounce bottles without cells were treated similarly as controls. The amount of virus adsorbed was determined by subtracting the number of PFU in the supernatant fluid from the cell cultures from the number of PFU recovered from control bottles.

Growth of SV40 in mouse kidney cells: The procedures used for determining growth rates of

SV40 in monolayer cultures have been described.[7] Extracellular virus was removed at 2 hr post-inoculation by washing the cultures and treating with SV40 antisera.

Determination of SV40 T-antigen and viral-capsid antigen: Cell packs containing 1 vol of cells plus 7 vol of buffer were frozen and thawed, treated for 1 min in a Raytheon sonic oscillator, and centrifuged for 1 hr at 34,800 *g*. The supernatant fluids were used as complement-fixing antigens. A micro CF method, using two full units of complement, was employed.[12] Serum from an SV40-immune green monkey and serum from SV40 tumor-bearing hamsters were used to detect the specific viral and T-antigens, respectively. Sera were used at dilutions which contained 4 antibody units. In each test, 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 were then expressed as CF units per 10^7 cells in order to compare directly the results of different experiments.

Electron microscopy: SV40 particles in the above supernatant fluids were counted by the pseudoreplication and staining methods described by Smith and Melnick.[13]

Incorporation of H^3-thymidine (H^3-TdR) into DNA and radioautography: SV40-infected cultures were inoculated with 0.1 ml of H^3-TdR at various times after virus infection. The cultures were incubated at 37°C for 2 hr and the incorporation of H^3-TdR into DNA and the per cent of cells with labeled nuclei were determined.[7, 9] Control cultures were "mock-infected" and otherwise treated in the same way as virus-infected cultures.

Enzyme assays: The assays used for thymidine kinase, thymidylate kinase,[7, 9] and deoxycytidylate deaminase[14] have been described. For the determinations of thymidylate kinase and deoxycytidylate deaminase, enzyme extracts were prepared in buffers containing 0.1 mM thymidylate or 0.31 mM dCTP, respectively. These nucleotides were required to stabilize and activate thymidylate kinase and deoxycytidylate deaminase.

For the DNA polymerase assay, each tube contained the following substances at the indicated final concentrations in a total volume of 0.2 ml: H^3-dTTP (30×10^6 cpm per μmole), dATP, dCTP, and dGTP, each at a concentration of 0.27 mM; heat-denatured (5 min at 100°C) salmon sperm DNA (500 μg/ml); Mg^{++} ions (8 mM); 2-mercaptoethanol (3.8 mM); Tris-HCl buffer, pH 7.8 (100 mM), and enzyme (200–500 μg protein/tube). The reaction mixture was incubated for 30 min at 38°C and the amount of H^3-dTTP incorporated into DNA was determined.

Results.—Adsorption of SV40 to mouse kidney and CV-1 cells: SV40 was adsorbed by mouse cells at both 4° and 37°C (Fig. 1). The rate of adsorption by mouse kidney cells, however, was considerably slower than by CV-1 cells. Moreover, only about 60 per cent as much virus had been adsorbed in 2 hr by mouse kidney cells as was adsorbed by CV-1 cells.

Growth of SV40 in mouse kidney cell cultures: SV40 entered an eclipse phase in mouse kidney cell cultures which lasted about 24–32 hr (Fig. 2) as compared with 20–24 hr in CV-1 cultures.[7] Thereafter, virus titers increased until about 40 hr and then declined. Even with the high multiplicities used, no cytopathic changes were observed although the virus persisted for at least 7 days.

When infected cultures were trypsinized and individual cells plated on uninfected CV-1 monolayers, less than 1 per cent of mouse cells were capable of initiating infectious-center formation as compared to 59–71 per cent of CV-1 cells (Table 1).

At 36–54 hr the mouse kidney cultures yielded about 1–6 PFU of SV40 per cell, while the CV-1 cultures yielded 100–300 PFU per cell. However, the yield of SV40 per infectious center was 123–700, which was of the same order of magnitude as from CV-1 cells. At this time, the number of SV40 particles found in the supernatant fluids used for analysis of CF antigens was about 10^8 per ml for the mouse cells and 10^{10} per ml for the monkey cells. If one assumes that the virus particles are coming from the cells which plate as infectious centers, then again there was no difference in yield between an infected monkey cell and a competently infected mouse cell. Thus infectious SV40 seemed to be replicated in only a small percentage of the mouse cells and most of the mouse cells were abortively infected.

Enzymatic activities elicited following SV40-infection of mouse kidney cell cultures: The activities of DNA polymerase, thymidine kinase, thymidylate kinase, and dCMP deaminase increased appreciably following SV40 infection of mouse kidney cell cultures. The kinetics of the increases of the first three of these enzymes are shown in Figures 3 and 4. The enhanced enzymatic activities were first detected about 16–24 hr after infection. The thymidylate kinase activity of infected cultures rose to about two- to threefold higher values than noninfected cultures at 44 hr after infection. The DNA polymerase activity of infected cultures was about 8 times the value of noninfected cultures at 48 hr and remained elevated for at least 72 hr. Thymidine kinase activity in infected cultures was eightfold greater than noninfected cultures at 30 and 40 hr after infection. Subsequently, the thymidine kinase activity declined, but at 72 hr this enzyme activity was still about fourfold higher than the activity found with noninfected mouse kidney cells. Table 2 shows that dCMP deaminase activity also increased two- to fivefold 24–45 hr after infection of mouse kidney cells with SV40. Figure 5 illustrates the thymidine kinase and DNA polymerase activities of SV40-infected CV-1 cells. Activities of both enzymes in noninfected CV-1 cells were appreciably greater than in mouse cells. However, the kinetics of the initial increases were similar in CV-1 and in mouse cultures. DNA polymerase activity of CV-1 cell cultures remained at a high level for at least 72 hr after SV40 infection. Activity of thymidine kinase was about 15 times greater in infected than in noninfected CV-1 cell cultures. From 48 to 72 hr after infection, thymidine kinase activity declined. At 72 hr, the activity of this enzyme was about 10 times higher than in noninfected cell cultures.

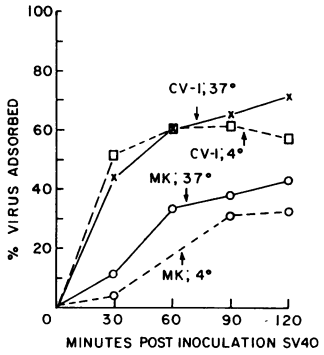

FIG. 1.—Kinetics of SV40 adsorption to mouse kidney (*MK*) and monkey kidney (*CV-1*) cells at 4° and 37°C.

Effect of inhibitors of protein synthesis on the virus-induced enzyme changes: To learn whether the increases in thymidine kinase and thymidylate kinase activities were dependent on *de novo* protein synthesis, mouse kidney cell cultures were treated with puromycin at various times after SV40 infection. Puromycin, 5–10 μg/

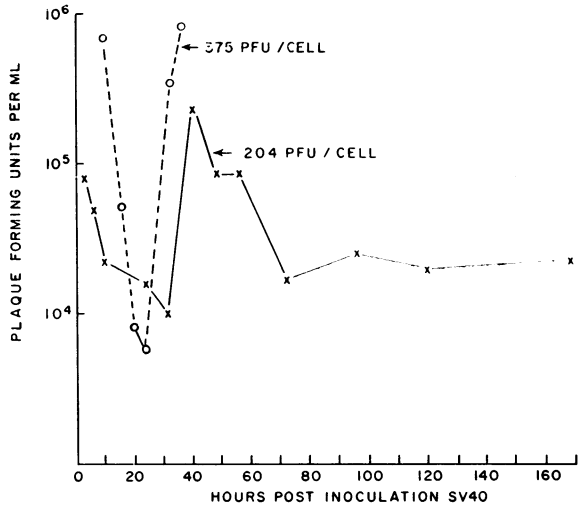

FIG. 2.—Replication of SV40 in mouse kidney cell cultures inoculated with high input multiplicities of SV40. Cultures contained 3–5 × 10^6 cells in 20 ml medium. Cells and supernatant fluid were harvested at the times indicated in the figure, and cells were disrupted by ultrasonic treatment. Virus was assayed on CV-1 monolayers and virus yields are expressed as PFU/ml harvest.

TABLE 1

FORMATION OF INFECTIOUS CENTERS BY SV40-INFECTED CV-1 AND MOUSE KIDNEY CELLS WHEN PLATED ON CV-1 MONOLAYERS

Hours PI when plated on CV-1 monolayers	Cells Forming Infectious Center, %		
		Mouse Kidney	
	CV-1	Expt. 1	Expt. 2
16	71	0.7	
24	61	0.9	0.8
28	59		
40	70		
48	68	0.4	0.2
56	65		
72		0.4	0.1
96		0.4	0.01

Input multiplicities: CV-1, 100 PFU/cell; mouse kidney, 204 and 375 PFU/cell.

TABLE 2

DEOXYCYTIDYLATE DEAMINASE ACTIVITY* OF SV40-INFECTED MOUSE KIDNEY CELL CULTURES

Expt.	Input multiplicities (PFU/cell)	Hr PI	dCMP Deaminase (μμmoles dUMP formed per μg protein in 10 min at 38°C)	
			Noninfected	SV40-infected
		24	2.9	5.9
a	45			
		38	2.5	12.0
		32	3.9	7.9
b	282			
		45	3.1	6.4

* Enzyme extracts prepared in buffer containing 0.31 mM dCTP.

ml (about 1 to 2 × 10^{-5} *M*) suppresses the incorporation of labeled amino acids into the proteins of murine cell cultures and inhibits the induction of thymidine kinase by polyoma virus.[9] However, these concentrations of puromycin are ineffective in monkey kidney cell cultures in inhibiting the incorporation of labeled amino acids into cell proteins or in inhibiting SV40-specific T-antigen synthesis.[15, 16] Twenty- to 40-fold higher concentrations of puromycin (i.e., 2.5–5.0 × 10^{-4} *M*) are required to prevent the SV40-induced increases of thymidine kinase and dihydrofolate reductase in monkey kidney cell cultures.[7, 8] In contrast, the lower concentration of cycloheximide (10^{-5} *M*) suppresses incorporation of labeled amino acids into proteins of either monkey or mouse cultures, and this same low concentration of cycloheximide inhibits SV40-induced enzyme synthesis and T-antigen formation.[7, 15]

Table 3 shows that the addition of puromycin at 2–16 hr prevented the increase in thymidine kinase activity normally observed at 30 hr. From Table 4, it may be seen that the addition of puromycin at 16 hr after SV40 infection also prevented the increase in thymidylate kinase activity which normally occurred at 32 hr. If the

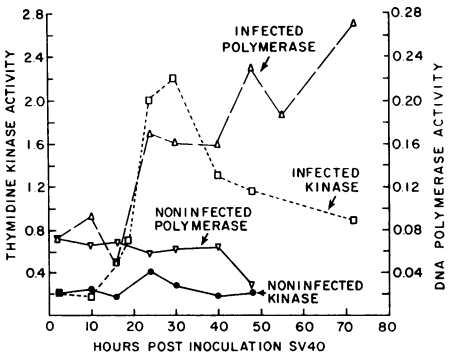

FIG. 3.—Kinetics of thymidine kinase and DNA polymerase formation in confluent monolayer cultures of mouse kidney cells inoculated with SV40 at an input multiplicity of about 170 PFU/cell. Thymidine kinase activity: μμmoles dUMP formed per μg protein in 10 min at 38°C. DNA polymerase activity: μμmoles H^3-TTP incorporated into DNA per μg protein in 30 min at 38°C.

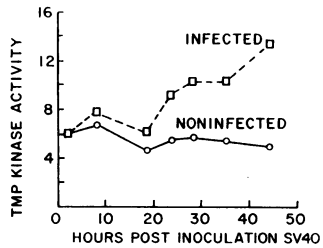

FIG. 4.—Kinetics of thymidylate kinase formation in confluent monolayer cultures of mouse kidney cells inoculated with SV40 at an input multiplicity of 150 PFU/cell. TMP kinase activity: μμmoles TDP + TTP formed per μg protein in 10 min at 38°C.

puromycin-containing medium was removed from the cultures at 32 hr, partial induction of thymidylate kinase activity occurred between 32 and 48 hr. The results thus demonstrate that *de novo* protein synthesis is required for the induction of thymidine kinase and thymidylate kinase. Other experiments have shown that protein synthesis is also needed for induction of DNA polymerase by SV40 in CV-1 cells.

Effect of ara-C treatment on TMP kinase induction. Previous studies have shown that ara-C (1-β-D-arabinofuranosylcytosine) treatment at drug concentrations which suppressed DNA synthesis and virus growth did not inhibit the induction of T-antigen, thymidine kinase, and dihydrofolate reductase in SV40-infected monkey cells.[7-9, 17] The induction of DNA polymerase by SV40 in CV-1 cells was also not inhibited by ara-C treatment. Table 5 shows that the addition of ara-C to mouse kidney cell cultures at 2 hr after SV40 infection had little effect on the increase in TMP kinase activity observed at 30 and 47 hr after infection. Thus, the increase in this enzyme activity can occur in the absence of DNA synthesis.

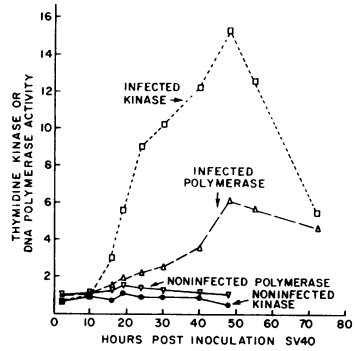

FIG. 5.—Kinetics of thymidine kinase and DNA polymerase formation in confluent monolayer cultures of CV-1 cells inoculated with SV40 at an input multiplicity of 140 PFU/cell. Thymidine kinase activity: μμmoles dUMP formed per μg protein in 10 min at 38°C. DNA polymerase activity: μμmoles H^3-TTP incorporated into DNA per μg protein in 30 min at 38°C.

Formation of T-antigen and viral-capsid antigen in SV40-infected cultures: In confirmation of the results of others,[2-5] SV40-specific tumor (T)-antigens were induced in monkey kidney cells acutely infected with SV40. Figure 6 shows the kinetics of the T-antigen formation in CV-1 cells. An increase in T-antigen formation was detected at 10 hr and attained a maximum at about 30 hr. Thus, T-antigen formation took place at approximately the same time as early enzyme formation. Viral-capsid antigen formation occurred somewhat later than T-antigen formation.

Figure 6 also shows that the kinetics of T-antigen formation in mouse kidney cell cultures was similar to that of T-antigen formation in CV-1 cells. However, the

TABLE 3

EFFECT OF PUROMYCIN (10^{-5} *M*) ON THE INDUCTION OF THYMIDINE KINASE BY SV40-INFECTED MOUSE KIDNEY CELLS

Hr PI puromycin added	Hr PI enzyme assayed	Thymidine Kinase Activity (μμmoles dUMP formed per μg protein in 10 min at 38°C) Noninfected	Infected*
Not added	2	0.34	
Not added	30	0.13	0.98
2	30	0.16	0.17
9	30	0.20	0.13
16	30	0.09	0.18

* SV40 input multiplicity: 87 PFU/cell.

TABLE 4

EFFECT OF PUROMYCIN (10^{-5} *M*) ON THE INDUCTION OF TMP KINASE BY SV40-INFECTED MOUSE KIDNEY CELLS

Treatment of cells	Hr PI enzyme assayed	TMP Kinase Activity* (μμmoles TDP + TTP formed/μg protein in 10 min at 38°C) Noninfected	Infected†
None	2	8.6	
None	16	8.7	10.0
None	32	7.3	17.8
Puromycin, 16–32 hr PI	32	8.6	10.2
Puromycin, 16–32 hr PI	48	8.1	14.7
None	48	10.0	20.7

* Enzyme extracts were prepared in buffer containing 0.1 mM TMP.
† SV40 input multiplicity: 185 PFU/cell.

TABLE 5

TMP KINASE ACTIVITY OF SV40-INFECTED MOUSE KIDNEY CELL CULTURES TREATED WITH ARA-C*

Hr PI	TMP Kinase Activity† (μμmoles TDP + TTP formed/μg protein in 10 min at 38°C)			
	Noninfected		SV40-Infected	
	Non-treated	Ara-C treated	Non-treated	Ara-C treated
2	4.4			
30	3.6	3.5	6.6	6.5
47	3.3	3.7	7.3	5.6

* Ara-C (10 μg/ml) was added to the cultures 2 hr PI; SV40 input multiplicity: 220 PFU/cell.
† Enzyme extracts were prepared in buffer containing 0.1 mM TMP.

average CF antigen units per cell in the mouse kidney cultures was about 0.1 that of the CV-1 cell cultures. In contrast, at 40 hr, SV40-capsid antigen titers in mouse kidney cell cultures were less than 1 per cent that in CV-1 cell cultures.

Incorporation of H^3-TdR into DNA of SV40-infected mouse kidney cell cultures: It has previously been shown that the incorporation of H^3-TdR into DNA is appreciably stimulated in CV-1 or GMK cells acutely infected with SV40.[7] Moreover, at 48–50 hr after infection, approximately 80 per cent of the cells were labeled in their nuclei with H^3-TdR, while in noninfected cultures of CV-1 or GMK cells, only about 1–5 per cent of the nuclei were labeled at this time.

Figure 7 presents H^3-TdR pulse-labeling and radioautographic experiments on SV40-infected mouse kidney cell cultures. It may be seen that the incorporation of H^3-TdR into DNA was stimulated 2–3 times from 16 to 48 hr after virus infection (Fig. 7*a*). In noninfected mouse kidney cultures, only 2–5 per cent of the cells exhibited labeled nuclei after a H^3-TdR pulse. After SV40 infection, approximately 20–25 per cent of the nuclei were labeled (Fig. 7*b*, expts. 1, 2). Figure 7*b*, (expt. 2) also shows that incorporation of H^3-TdR into 20 per cent of the nuclei of infected cells continued for at least 96 hr after infection.

Thymidine kinase activity and T-antigen titers of transformed cell lines: Mouse kidney cell cultures transformed by SV40 exhibited high levels of thymidine kinase activity. These activities exceeded by a factor of 33–60 those of secondary mouse kidney cultures which had been planted at the same time as the transformed lines (Table 6). The transformed cell lines all contained the SV40 T-antigen but did not contain detectable SV40-capsid antigen or virus particles.

Discussion.—Upon inoculation of monkey cell cultures with SV40, approximately 60–80 per cent of the cells become productively infected. In contrast, less than 1 per cent of mouse cells inoculated with SV40 at high input multiplicities become capable of initiating infectious center formation. Consistent with these findings is the marked reduction of SV40 capsid-antigen formation in SV40-infected mouse cultures, compared to that in infected monkey cells. Cytopathic effects were not seen after infection of mouse cells in spite of the high multiplicity, but 14–21 days after infection, colonies of "transformed" cells were noticeable. Some of these "transformed" cells have been subcultured for seven passages; they contain high levels of T-antigen but no detectable viral antigen or viral particles.

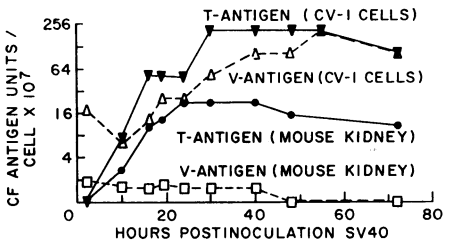

FIG. 6.—Kinetics of T-antigen and viral-capsid antigen formation in mouse kidney and CV-1 cell cultures inoculated with SV40. The data presented in this figure were obtained from the same cultures used for the enzyme experiments of Figs. 3 and 5.

Primary monolayer cultures of mouse kidney cells show contact inhibition and

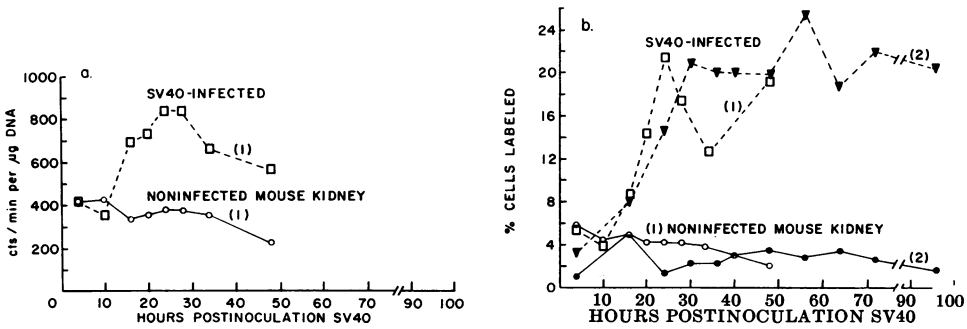

FIG. 7.—Radioautographic and biochemical studies of the incorporation of H^3-TdR into DNA of SV40-infected mouse kidney cell cultures. Cultures were infected at input multiplicities of 230 and 620 PFU/cell for expts. 1 and 2, respectively, and were pulse-labeled for 2 hr with H^3-TdR.

attain a population density of approximately 3–5 million cells/55 sq cm in 5–7 days. The cell population then gradually declines and very little cell growth occurs even after subculture. The transformed cultures, however, grew to populations of 10–15 million cells/55 sq cm and were subcultured regularly at 3–4-day intervals.

Although infection of mouse kidney cells with SV40 was abortive, formation of T-antigen and of four enzymes of thymidine metabolism was induced. In association with the enzyme inductions, DNA synthesis was initiated in about 20 per cent of the cells. This enhanced DNA biosynthesis persisted for at least 4 days. The present experiments do not establish whether the newly synthesized DNA is only viral or both viral and mouse cell DNA. T-antigen and enzyme induction and the stimulated DNA biosynthesis are very similar in kinetics, and occur in mouse cells at the same time as in acutely infected CV-1 cultures in which most cells are SV40 producers. Thus the enzymes presumably are elicited in the mouse cells infected by SV40. The very small percentage (less than 1%) of cells which yields infectious virus undoubtedly constitutes part of this population, but these early events probably occur in cells abortively infected by SV40 as well. It is not known whether the cells ultimately transformed by SV40 are among those cells exhibiting early

TABLE 6

THYMIDINE KINASE ACTIVITY AND SV40 T-ANTIGEN TITERS OF SV40-TRANSFORMED MOUSE KIDNEY CELL CULTURES

Cells	Days after SV40 infection* when 1st passage was made	Passage number†	Total days in culture	Thymidine kinase (μμmoles dUMP formed/μg protein in 10 min at 38°C)	CF Antigen Units/Cell × 10^7 T-antigen	Capsid antigen
Noninfected mouse kidney‡		2	52	0.1		
Transformed	1	5	53	3.3	5.4	0
	4	4	53	3.6	8.1	0
	11	4	53	3.4	7.7	0
	14	4	53	3.6	10.2	0
	17	4	53	5.1	7.0	0
	22	7	62	6.0	6.5	0
	30	6	62	4.2	7.3	0

* Confluent 5-day-old monolayer cultures were inoculated with SV40 at an input multiplicity of about 150 PFU/cell.

† Transformed cells were harvested for enzyme and antigen assays at 3 days after the passage shown in the table.

‡ Replicate primary cultures of noninfected mouse kidney cells were subcultured at 6, 9, 16, 19, and 22 days, respectively. Cultures were fed 2–3 times weekly. These sparse monolayers were harvested 52 days after planting, pooled, and assayed for thymidine kinase activity.

enzyme induction and enhanced DNA biosynthesis. The transformed cell lines do exhibit high levels of thymidine kinase activity and T-antigen titers comparable to those of SV40-transformed hamster cells (H-50).

The properties of the enzymes elicited in SV40-infected mouse kidney are presently being studied. A comparison of the properties of these enzymes with the enzymes formed after acute SV40 infection of CV-1 cells and with the enzymes of transformed cell lines may shed light on the significance of early enzyme synthesis in SV40-infected murine cultures.

Summary.—Early after SV40 infection of confluent primary cultures of mouse kidney cells, increases were observed in the activities of thymidine kinase, thymidylate kinase, deoxycytidylate deaminase, and DNA polymerase. H^3-TdR "pulse"-labeling experiments demonstrated that DNA biosynthesis was initiated in approximately 20 per cent of the cells. High levels of SV40-specific complement-fixing T-antigen were synthesized in the infected mouse cultures in contrast to the curtailed formation of viral-capsid antigen. Although mouse kidney cells adsorbed SV40 and the virus underwent a normal eclipse in these cells, less than 1 per cent of the cells were virus yielders, as determined by the ability to form infectious centers on monkey kidney cells. This observation of complete infection in only 1 per cent of the cells is consistent with the finding of about 1 per cent of the number of virus particles in mouse cell cultures when compared to the increase found in monkey kidney cells treated under the same conditions. Transformation was observed at 14–21 days in the SV40-infected cultures. The morphological transformation was associated with the appearance of T-antigen, but not viral-capsid antigen, and also the development of high levels of thymidine kinase.

* Aided by grants from the American Cancer Society (E 291), the National Science Foundation (GB 3126), and by U.S. Public Health Service grants (CA-04600, CA-06656, AI-05382, 1-K6-AI-2352, 1-K3-CA-25, 797).

† Postdoctoral fellow of the Consejo Nacional de Investigaciones Cientificas y Tecnicas(Argentina).

[1] Black, P. H., and W. P. Rowe, *Proc. Soc. Exptl. Biol. Med.*, **114**, 721 (1963).

[2] Sabin, A. B., and M. A. Koch, these PROCEEDINGS, **52**, 1131 (1964).

[3] Rapp, F., T. Kitahara, J. S. Butel, and J. L. Melnick, these PROCEEDINGS, **52**, 1138 (1964).

[4] Black, P. H., W. P. Rowe, H. C. Turner, and R. J. Huebner, these PROCEEDINGS, **50**, 1148 (1963).

[5] Hoggan, M. D., W. P. Rowe, P. H. Black, and R. J. Huebner, these PROCEEDINGS, **53**, 12 (1965).

[6] Kit, S., D. R. Dubbs, R. A. de Torres, and J. L. Melnick, *Virology*, **27**, 453 (1965).

[7] Kit, S., D. R. Dubbs, P. M. Frearson, and J. L. Melnick, *Virology*, **29**, 69 (1966).

[8] Frearson, P. M., S. Kit, and D. R. Dubbs, *Cancer Res.*, **25**, 737 (1965); *Cancer Res.*, in press.

[9] Kit, S., D. R. Dubbs, and P. M. Frearson, *Cancer Res.*, **26**, 638 (1966).

[10] Dulbecco, R., L. H. Hartwell, and M. Vogt, these PROCEEDINGS, **53**, 403 (1965).

[11] Dubbs, D. R., S. Kit, and R. A. de Torres, *Bacteriol. Proc.* (1966), p. 111.

[12] Melnick, J. L., I. Wimberly, and T. Kitahara, in preparation.

[13] Smith, K. O., and J. L. Melnick, *Virology*, **17**, 460 (1962).

[14] Kit, S., R. A. de Torres, and D. R. Dubbs, *Cancer Res.*, in press.

[15] Gilden, R. V., and R. I. Carp, *J. Bacteriol.*, **91**, 1295 (1966).

[16] Melnick, J. L., and F. Rapp, *Ann. N. Y. Acad. Sci.*, **130**, 291 (1965).

[17] Rapp, F., J. L. Melnick, and T. Kitahara, *Science*, **147**, 625 (1965).