DIFFERENTIAL EFFECTS OF INHIBITORS ON THE STEPS LEADING TO THE FORMATION OF SV40 TUMOR AND VIRUS ANTIGENS*

By F. RAPP, Ph.D., J. S. BUTEL, L. A. FELDMAN, Ph.D., T. KITAHARA, M.D., and J. L. MELNICK, Ph.D.

(From the Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas)

Plates 74 and 75

(Received for publication, February 26, 1965)

The demonstration that hamster (1–3) and human (2) cells transformed by SV40 virus synthesize a new antigen under control of the virus genome led to the discovery that the same antigen is also formed during the normal replication cycle of SV40 in green monkey cells (3–5). As the sequence of SV40 replication had been previously studied (6, 7), it was quickly ascertained that this new tumor or T antigen is formed during the latent period and that synthesis of T antigen precedes that of the virus or V antigen found in the SV40 virion (5).

From our earlier investigations of the inhibitory effect of 5-fluorouracil and 5-fluorodeoxyuridine on the synthesis of V antigen and infectious virus (7), we were led into a study of the effects of these and other DNA antagonists and inhibitors on the synthesis of both T and V antigens in an effort to obtain information concerning the nature and role of the T antigen in the synthesis of SV40 virus as well as its formation in cells transformed by the virus but free of infectious virions.

Materials and Methods

Virus.—Stocks of SV40 were prepared in primary African green monkey kidney cells (GMK) growing in 16-ounce bottles. When cytopathic effects (CPE) involved 75 to 100 per cent of the cells, the cultures were disrupted by quick-freezing and thawing. After clarification by centrifugation at 3000 *g* for 5 minutes at 4°C, the virus was dispensed in ampoules which were sealed; the virus was quickly frozen, and stored at −90°C. Titers of the virus stocks were obtained using the plaque assay previously described (8); all stocks employed in this study contained 10^6 to 10^7 plaque-forming units (PFU) per ml. The tumorigenic potential of this virus strain has been reported from this laboratory (9), as has the growth cycle of the virus in GMK cells (7).

Cell Cultures.—Cultures were prepared from African green monkey kidneys using lactal-

* This investigation was supported in part by grants CA-04600, National Cancer Institute, and AI-05382 and 2 T1-AI 74, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

bumin hydrolysate medium (M-H for growth and M-E for maintenance) as described (10). Growth medium contained 2 per cent calf serum; maintenance fluids did not contain serum.

Inhibitors.—The preparation of the inhibitors has been described (11). Stocks of 5-iodo-2′-deoxyuridine (IUDR), 1-β-D-arabinofuranosylcytosine hydrochloride (CA), 5-fluorouracil (FU), 5-fluorodeoxyuridine (FUDR), actinomycin D, mitomycin C, puromycin, and *p*-fluorophenylalanine (FPA) were prepared by dissolving the compounds in either water or Eagle's basal medium at a concentration of 1 mg per ml. The stocks were refrigerated or frozen and protected against exposure to light. Further dilution to give the concentrations desired was made directly into maintenance medium. The inhibitors were added to the cultures after virus adsorption (37°C for 1 hour) had taken place unless otherwise noted.

Immunofluorescent Techniques.—The preparation, fractionation, and fluorescein-labeling of the sera has been described (2, 7). Cells to be examined were grown on 15 mm round coverglasses in Petri dishes, and inoculated with 0.1 ml of undiluted virus suspension in such a way that the fluid did not spill over the edge of the coverglass (12). After adsorption of the virus, the coverglasses were flooded with maintenance medium and incubated at 37°C in 5 per cent CO_2. The cells were harvested at times noted, washed 3 times with tris saline (pH 7.4), air-dried, and fixed for 3 minutes in acetone. T antigen was detected by reacting the cells with serum from hamsters bearing SV40 tumors and anti-hamster globulin prepared from rabbit serum and conjugated with fluorescein isothiocyanate. V antigen was localized with a labeled monkey serum from an African green monkey repeatedly immunized with SV40. The cells were washed, air-dried, and mounted on slides in elvanol (13). The preparations were examined with a Zeiss fluorescence microscope equipped with an Osram HBO-200 mercury arc vapor lamp.

Complement Fixation Tests.—These tests were carried out in wells in linbro disposable plastic trays using a micro-technique described previously (5). Antigen was prepared by harvesting infected cells from 16-ounce bottle cultures into a volume of 1 ml, followed by disruption of the cells by sonication or freezing and thawing. A positive and negative serum was included with each test as were complement, antigen, serum, and cell controls.

RESULTS

Effect of Deoxyribonucleic Acid Antagonists.—None of the antagonists tested inhibited or delayed the synthesis of T antigen. All compounds were tested in concentrations ranging from 0.1 μg per ml to 50 μg per ml added immediately after virus adsorption. Concentrations higher than 50 μg per ml were generally cytotoxic. Pretreatment of the cells with the inhibitors did not result in inhibition of the synthesis of T antigen. Results of representative experiments are summarized in Table I. It is obvious that neither CA, IUDR, nor FU depressed or delayed synthesis of T antigen as measured by complement fixation. The amount of T antigen detectable in the treated cultures 24 and 48 hours post-inoculation was comparable to that of the infected, but drug-free, controls. Similarly, the number of cells positive for T antigen by the immunofluorescence technique was similar in both untreated and treated cultures. FUDR yielded similar results in a separate experiment but the cultures were not examined by complement fixation methods.

The synthesis of V antigen was somewhat depressed in cultures maintained in IUDR or FU but a considerable quantity of this antigen was nevertheless produced (Table I); toxicity of the compounds may have reduced total yield

of V antigen. CA, however, completely inhibited synthesis of V antigen when measured by either complement fixation or immunofluorescence (Table I). The inhibitory effect of CA could be reversed by the addition of 2-deoxycytidine.

Starved cells, maintained for 24 hours in Earle's salt solution prior to inoculation with SV40, and maintained in salt solution containing various inhibitors

TABLE I

Synthesis of SV40 Tumor and Virus Antigens in Monkey Kidney Cells Exposed to DNA Antagonists for 24 to 48 Hours

Inhibitor	Concentration	Post-inoculation	Complement fixation titer*		Immunofluorescence‡	
			T antigen	V antigen	T antigen	V antigen
	μg/ml	*Hrs.*				
CA	10	24	32	<2	25	0
		48	64	<2	50	0
IUDR	50	24	32	4	25	<0.1
		48	64	256	25	25
FUDR	50	24	Not done	Not done	Positive	Not done
		48	" "	" "	Not done	Positive
FU	50	24	32	2	25	Not done
		48	32	128	50	50
None	—	24	16	32	25	5
		48	64	1024	50	75

* Titers represent reciprocal of highest dilution of antigen yielding less than 50 per cent hemolysis, against 1:20 hamster serum (from a tumor-bearing animal) for measuring tumor antigen, and against 1:40 monkey serum (from an SV40-infected animal) for measuring virus antigen.

‡ Numbers represent approximate percentage of cells containing respective antigens. CA, cytosine arabinoside; IUDR, iododeoxyuridine; FUDR, fluorodeoxyuridine; FU, fluorouracil.

following inoculation were also able to synthesize T antigen in the presence of the DNA antagonists (Table II). Neither starved nor well nourished cells formed V antigen in the presence of CA. However, though nourished cells formed V antigen in the presence of FUDR, starved cells were unable to do so (Table II). This was also true for cells maintained in the presence of FPA. Starved control cells infected with SV40 were able to synthesize both T and V antigens.

The distribution and intranuclear location of T antigen was not affected

by the presence of the inhibitors and resembles that in infected cells in the absence of drugs, as shown in Fig. 1. However, though V antigen was produced in cells maintained in the presence of FU and IUDR (Tables I and II), distribution of the antigen in the nucleus was atypical, confirming results described previously with FU (7). Thus, V antigen synthesized in the presence of FU or IUDR was diffusely spread throughout the nucleus (Figs. 2 and 3). Many cells had concentrations of V antigen at the nuclear membrane (Figs. 3 and 4). Particulates of V antigen observed in the nucleus of control infected cultures (Fig. 5) were rarely seen in cells maintained in the presence of the inhibitors tested.

TABLE II

Synthesis of SV40 Tumor and Virus Antigens in Nourished and in Starved Monkey Kidney Cells

Inhibitor	Concentration	Nourished cells		Starved cells	
		T antigen*	V antigen‡	T antigen*	V antigen‡
	μg/ml				
CA	10	+	0	+	0
IUDR	50	+	+	+	+
FUDR	50	+	+	+	0
FU	50	+	+	+	+
FPA	300	+	+	+	0
None	—	+	+	+	+

* Measured by immunofluorescence 24 hours following inoculation.

‡ Measured by immunofluorescence 48 hours following inoculation.

+, Presence of tumor antigen or virus antigen.

0, Absence of tumor antigen or virus antigen.

The suspensions prepared for the complement fixation tests were examined in the electron microscope, after being stained with uranyl acetate (14).[1] Well formed virions were observed in large quantity only in control, infected cultures, but cells treated with IUDR produced virus particles which appeared ragged and often empty.

All DNA antagonists tested inhibited synthesis of infectious virus. Attempts to isolate virus from the fluids of cultures treated with the inhibitors never yielded more than traces of virus. Furthermore, incorporating the inhibitors into the agar overlay resulted in a reduction of plaque formation by SV40. As little as 1 μg per ml of IUDR or CA in such a test resulted in greater than 99 per cent plaque inhibition (Table III).

Effect of Antibiotics and Protein Inhibitors.—Actinomycin D, in concentrations of 1 and 5 μg per ml, suppressed synthesis of both T and V antigens as

[1] Electron microscopy was carried out by Jean P. Brunschwig.

TABLE III

Inhibition of Development of SV40 Plaques by Iododeoxyuridine and Cytosine Arabinoside

Inhibitor	PFU	Inhibition
	per ml	*per cent*
None	4.9×10^7	—
IUDR*	3.0×10^5	99.37
CA*	1.7×10^4	99.97

* Tested at 1 μg per ml.
PFU, plaque-forming units.

TABLE IV

Synthesis of SV40 Tumor and Virus Antigens in Monkey Kidney Cells Exposed to Antibiotics or Protein Inhibitors for 24 to 48 Hours

Compound	Concentration	Complement fixation*		Immunofluorescence‡	
		T antigen	V antigen	T antigen	V antigen
	μg/ml				
Actinomycin D	5	Not done	Not done	0	0
	1	4	<2	0	0
Mitomycin C	50	8	8	<0.1	1
	10	32	64	25	25
	1	32	512	25	25
Puromycin	10	16	128	25	25
	1	32	256	25	25
p-Fluorophenylalanine	500	32	128	25	25
	50	Not done	Not done	25	25
None	—	32	512	25	25

* Titers represent reciprocal of highest dilution of antigen yielding less than 50 per cent hemolysis.
‡ Numbers represent approximate percentage of cells containing respective antigens.
SV40 tumor antigen measured 24 hours postinoculation.
SV40 virus antigen measured 48 hours postinoculation.

measured by complement fixation and immunofluorescence methods (TableIV). Concentrations of 1 and 10 μg per ml of mitomycin C were ineffective, but 50 μg per ml suppressed synthesis of both antigens, probably because mitomycin C at this concentration is extremely toxic to the cells for the period of exposure required. Neither puromycin nor FPA were inhibitory in the concentrations tested (Table IV). Higher concentrations of puromycin could not

be tested because of the toxicity of the compound. Prior starvation did not impair ability of the cells to synthesize T antigen in the presence of FPA (Table II) but synthesis of V antigen was inhibited (Table II).

Distribution of both T and V antigens in the presence of these compounds was generally atypical and often bizarre. T antigen, synthesized in the presence of puromycin, was concentrated around the nucleolus (Fig. 6) and often limited to this area. Parallel fiber-like structures (Fig. 7) were often seen in infected cells maintained in mitomycin C. Cultures maintained in FPA often included cells in which T antigen (Fig. 8) or V antigen (Fig. 9) was concentrated at the nuclear membrane.

The cytotoxicity of the compounds when left in contact with the cells for 24 and 48 hours made interpretation of infectious virus yields difficult; reduced yields would be expected because of destruction of the cells by the antibiotics tested. Comparable studies carried out with the same cell, virus, and inhibitors failed to reveal virus particles in infected cultures maintained in puromycin or actinomycin D whereas particles forming in the presence of FPA were hollow and appeared to be devoid of DNA (15).

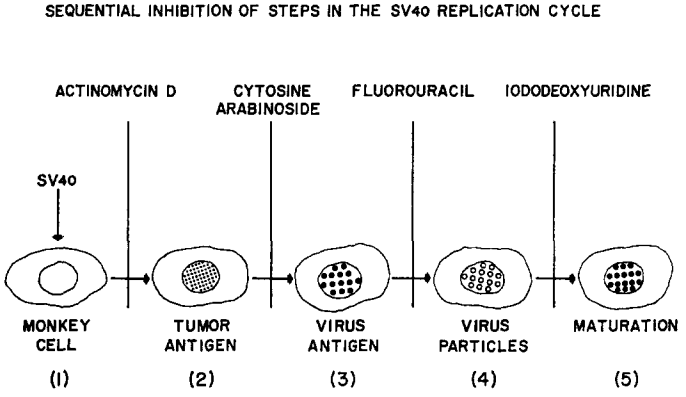
DISCUSSION

The inhibition of the development of DNA-containing viruses by DNA antagonists and by antibiotics represents a useful approach for the study of the molecular events involved in the synthesis of virus components and infectious virions. The compounds used in this study are known to exert different effects on the synthesis of DNA, RNA, and protein. Knowledge of their mode of action coupled with the observations made in this study yields insight into the steps involved in the development of SV40 components and the infectious virion.

The inhibition of the development of infectious SV40 by various DNA antagonists is not surprising since previous studies had shown that FU and FUDR (7) as well as IUDR (16, 17) depress virus synthesis. Analysis of the inhibitory effects of the compounds used and ability to detect early antigens formed during the replicative cycle of SV40 sheds new light, however, on the steps affected in viral replication. Text-fig. 1 diagrammatically summarizes both the sequence of events leading to formation of SV40 in green monkey kidney cells as well as the location of the biochemical block by actinomycin D and by the DNA antagonists.

Actinomycin D is the only compound tested capable of inhibiting synthesis of T antigen, and all the later events of the replicative cycle. Formation of T antigen therefore presumably requires DNA-dependent RNA (18). The synthesis of T antigen (step 2) is not inhibited by CA, FU, IUDR, or FUDR. As these compounds either block development of DNA (19, 20) or allow only faulty DNA synthesis (21), the formation of T antigen does not appear to

require replication of virus DNA. The results with mitomycin C (at 10 μg/ml) would appear to support this assumption since this compound cross-links the strands of DNA and thus inhibits replication (22). Ability of the cells to form T antigen in the presence of puromycin suggests that the antigen is a low molecular weight protein, or even a small polypeptide. This conclusion is suggested by recent studies which demonstrated that the action of puromycin is through its attachment to the incomplete polypeptide chain by substituting for amino acyl sRNA, thus preventing additional peptide bonds from forming (23, 24). The view that T antigen is a low molecular weight compound has previously been hypothesized based on inability to sediment the antigen by high speed centrifugation (1, 4, 5). The concentration of T antigen at the nucleolar mem-



TEXT-FIG. 1. Diagrammatic representation of sequential steps in the synthesis of SV40 and the site of action of each inhibitor.

brane when synthesized in the presence of puromycin suggests that the nucleolus plays a role in the formation of this antigen. Since under the usual conditions of infection T antigen is not concentrated in this region, puromycin may also act by inhibiting an active transport mechanism.

Although both FU and IUDR allow synthesis of T and V antigens, infectious virions do not develop. Previous observations concerning the effect of FU on the synthesis of SV40 had revealed that virus particles did not form in the presence of the inhibitor (7) and these results were verified in the present study. IUDR, however, does allow the formation of particles, although they are non-infectious. This finding is similar to that made by Smith and Dukes (25) for herpes simplex virus; herpes zoster virus antigens are also synthesized in the presence of IUDR (26).

The failure of cells exposed to CA to synthesize V antigen confirms preliminary observations from this laboratory (17, 27). In this respect, the action

of CA is similar to that of FUDR in starved cells. However, failure of FUDR to inhibit synthesis of V antigen in well nourished cells supports the conclusion that CA acts at a different locus. It is possible that the inhibitory effect of CA in this system involves more than one site, a conclusion also supported by the observation that this compound interferes with enzymes not involved in DNA synthesis (20).

The recent finding that some adenovirions can incorporate a portion of SV40 genome (28–30) which is expressed by induction in susceptible cells of the synthesis of SV40 T antigen (but not V antigen) makes available an additional system for study of the synthesis of T antigen. Preliminary experiments in our laboratory suggest that the effects of the compounds described in this study on the synthesis of T antigen induced by the adenovirus-SV40 "hybrids" resemble those seen when T antigen is induced by SV40.

SUMMARY

The effect of DNA antagonists and various antibiotics on steps in the synthesis of SV40 virus in green monkey kidney cells was investigated. Both the early forming tumor (T) antigen, as well as the later synthesized virus (V) antigen, were synthesized in the presence of fluorouracil and iododeoxyuridine. Cytosine arabinoside (and fluorodeoxyuridine in starved cells) prevented synthesis of V antigen but not T antigen. The synthesis of T antigen therefore does not require synthesis of virus DNA. Virus particles formed only in the presence of the iododeoxyuridine and they were non-infectious.

Actinomycin D inhibited synthesis of both tumor and virus antigens, suggesting that the synthesis of these antigens involves DNA-dependent RNA. Puromycin allowed synthesis of the T antigen which remained localized at the nucleolar membrane. This finding with puromycin suggests that the T antigen is a protein of low molecular weight. Virus antigen forming in the presence of mitomycin C, *p*-fluorophenylalanine, iododeoxyuridine, or fluorouracil was distributed atypically. These inhibitors caused the V antigen to be diffusely spread throughout the nucleus, or to be concentrated at the nuclear membrane.

BIBLIOGRAPHY

1. Black, P. H., Rowe, W. P., Turner, H. C., and Huebner, R. J., A specific complement-fixing antigen present in SV40 tumor and transformed cells, *Proc. Nat. Acad. Sc.*, 1963, **50**, 1148.
2. Rapp, F., Butel, J. S., and Melnick, J. L., Virus-induced intranuclear antigen in cells transformed by papovavirus SV40, *Proc. Soc. Exp. Biol. and Med.*, **116**, 1131.
3. Pope, J. H., and Rowe, W. P., Detection of specific antigen in SV40-transformed cells by immunofluorescence, *J. Exp. Med.*, 1964, **120**, 121.
4. Sabin, A. B., and Koch, M. A., Source of genetic information for specific complement-fixing antigens in SV40 virus-induced tumors, *Proc. Nat. Acad. Sc.*, 1964, **52**, 1131.

5. Rapp, F., Kitahara, T., Butel, J. S., and Melnick, J. L., Synthesis of SV40 tumor antigen during replication of simian papovavirus (SV40), *Proc. Nat. Acad. Sc.*, 1964, **52,** 1138.
6. Mayor, H. D., Stinebaugh, S. E., Jamison, R. M., Jordan, L. E., and Melnick, J. L., Immunofluorescent, cytochemical, and microcytological studies on the growth of the simian vacuolating virus (SV40) in tissue culture, *Exp. and Mol. Path.*, 1962, **1,** 397.
7. Melnick, J. L., Stinebaugh, S. E., and Rapp, F., Incomplete simian papovavirus SV40. Formation of non-infectious viral antigen in the presence of fluorouracil, *J. Exp. Med.*, 1964, **119,** 313.
8. Stinebaugh, S., and Melnick, J. L., Plaque formation by vacuolating virus, SV40, *Virology*, 1962, **16,** 348.
9. Ashkenazi, A., and Melnick, J. L., Tumorigenicity of simian papovavirus SV40 and of virus-transformed cells, *J. Nat. Cancer Inst.*, 1963, **30,** 1227.
10. Melnick, J. L., Wenner, H. A., and Rosen, L., The enteroviruses, *in* Diagnostic Procedures for Viral and Rickettsial Diseases, (E. H. Lennette and N. J. Schmidt, editors), New York, American Public Health Association, 3rd edition, 1964, 194.
11. Rapp, F., Inhibition by metabolic analogues of plaque formation by herpes zoster and herpes simplex viruses, *J. Immunol.*, 1964, **93,** 643.
12. Rapp, F., Localization of antinuclear factors from lupus erythematosus sera in tissue culture, *J. Immunol.*, 1962, **88,** 732.
13. Rodriguez, J., and Deinhardt, F., Preparation of a semi-permanent mounting medium for fluorescent antibody studies, *Virology*, 1960, **12,** 316.
14. Smith, K. O., and Melnick, J. L., A method for staining virus particles and identifying their nucleic acid type in the electron microscope, *Virology*, 1962, **17,** 480.
15. Mayor, H. D., Jamison, R. M., Jordan, L. E., and McGregor, S., The influence of p-fluorophenylalanine, puromycin, and actinomycin on the development of simian papovavirus (SV40), *Exp. and Mol. Path.*, in press.
16. Haas, R., and Maass, G., Die Wirkung von 5-Jod-2′-desoxyuridine auf die Vermehrung von SV-40 in Gewebekulturen, *Arch. Ges. Virusforsch.*, 1964, **14,** 567.
17. Rapp, F., Melnick, J. L., and Kitahara, T., Tumor and virus antigens of simian virus 40: Differential inhibition of synthesis by cytosine arabinoside, *Science*, 1965, **147,** 625.
18. Goldberg, I. H., and Reich, E., Actinomycin inhibition of RNA synthesis directed by DNA, *Fed. Proc.*, 1964, **23,** 958.
19. Chu, M. Y., and Fischer, G. A., A proposed mechanism of action of 1-β-D-arabinofuranosylcytosine as an inhibitor of the growth of leukemic cells, *Biochem. Pharmacol.*, 1962, **11,** 423.
20. Cardeilhac, P. T., and Cohen, S. S., Some metabolic properties of nucleotides of 1-β-D-arabinofuranosylcytosine, *Cancer Research*, 1964, **24,** 1595.
21. Prusoff, W. H., Bakhle, Y. S., and McCrea, J. F., Incorporation of 5-iodo-2′-deoxyuridine into the deoxyribonucleic acid of vaccinia virus, *Nature*, 1963, **199,** 1310.
22. Iyer, V. N., and Szybalski, W., Mitomycins and porfiromycin: Chemical mechanism of activation and cross-linking of DNA, *Science*, 1964, **145,** 55.

23. Nathans, D., Inhibition of protein synthesis by puromycin, *Fed. Proc.*, 1964, **23,** 984.
24. Darken, M. E., Puromycin inhibition of protein synthesis, *Pharmacol. Rev.*, 1964, **16,** 223.
25. Smith, K. O., and Dukes, C. D., Effects of 5-iodo-2-desoxyuridine (IDU) on herpesvirus synthesis and survival in infected cells, *J. Immunol.*, 1964, **92,** 550.
26. Rapp, F., and Vanderslice, D., Spread of zoster virus in human embryonic lung cells and the inhibitory effect of iododeoxyuridine, *Virology*, 1964, **22,** 321.
27. Melnick, J. L., and Rapp, F., The use of antiviral compounds in analyzing the sequential steps in the replication of SV40 papovavirus, *Ann. New York Acad. Sc.*, 1965, in press.
28. Huebner, R. J., Chanock, R. M., Rubin, B. A., and Casey, M. J., Induction by adenovirus type 7 of tumors in hamsters having the antigenic characteristics of SV40 virus, *Proc. Nat. Acad. Sc.*, 1964, **52,** 1333.
29. Rowe, W. P., and Baum, S. G., Evidence for a possible genetic hybrid between adenovirus type 7 and SV40 viruses, *Proc. Nat. Acad. Sc.*, 1964, **52,** 1340.
30. Rapp, F., Melnick, J. L., Butel, J. S., and Kitahara, T., The incorporation of SV40 genetic material into adenovirus 7 as measured by intranuclear synthesis of SV40 tumor antigen, *Proc. Nat. Acad. Sc.*, 1964, **52,** 1348.

EXPLANATION OF PLATES

PLATE 74

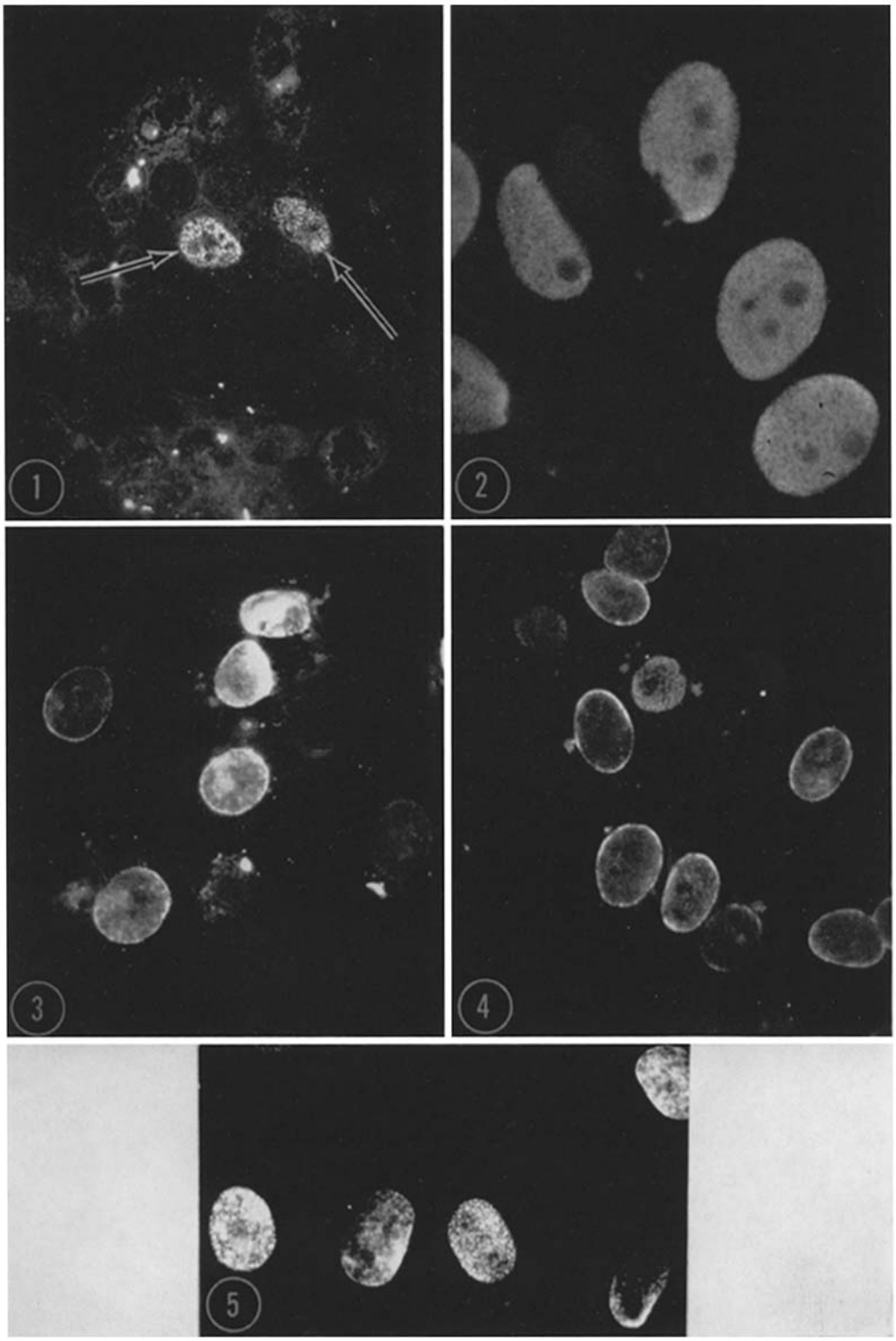
FIGS. 1 to 5. Immunofluorescence photomicrographs of green monkey kidney cells following inoculation with SV40.

FIG. 1. Cultures maintained in the absence of drugs. Reacted 24 hours following inoculation with anti-T antigen reagents. Arrows point to cells containing intranuclear T antigen. × 360.

FIG. 2. Cultures maintained in 10 μg of fluorouracil per ml. Reacted 48 hours following inoculation with anti-V antigen reagents. V antigen distributed diffusely throughout nucleus. × 1440.

FIGS. 3 and 4. Cultures maintained in 50 and 10 μg of iododeoxyuridine per ml respectively. Reacted 48 hours following inoculation with anti-V antigen reagents. Especially note concentration of V antigen at nuclear periphery. × 360.

FIG. 5. No drugs. Reacted 48 hours following inoculation with anti-V antigen reagents. V antigen distributed in particulates in the nuclei. × 360.



(Rapp *et al.*: SV40 tumor and virus antigens)

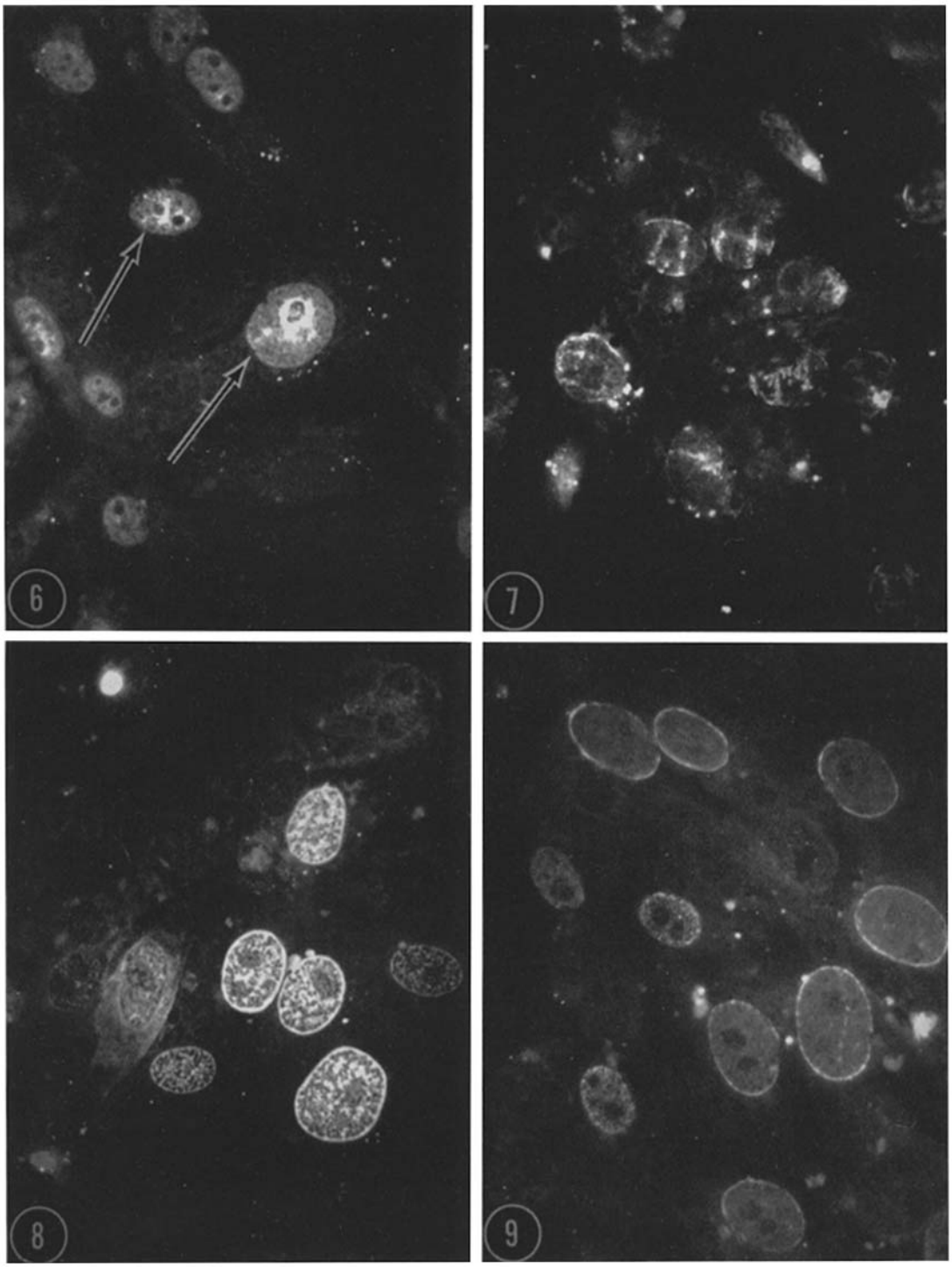
PLATE 75

FIGS. 6 to 9. Immunofluorescence photomicrographs of green monkey kidney cells following inoculation with SV40.

FIG. 6. Culture maintained in 1 μg of puromycin per ml. Reacted with anti-T antigen reagents 24 hours following inoculation. T antigen concentrated around nucleoli. $\times$ 380.

FIG. 7. Culture maintained in 1 μg of mitomycin C per ml. Reacted with anti-T antigen reagents 24 hours following inoculation. Note parallel fiber-like intranuclear structures reacting with anti-T reagents. $\times$ 380.

FIGS. 8 and 9. Cultures maintained in 500 μg of *p*-fluorophenylalanine per ml. $\times$ 380. Fig. 8. Reacted with anti-T antigen reagents. Fig. 9. Reacted with anti-V antigen reagents. Note concentration of antigens at nuclear membrane.



(Rapp *et al.*: SV40 tumor and virus antigens)