INCOMPLETE SIMIAN PAPOVAVIRUS SV40

Formation of Non-Infectious Viral Antigen in the Presence of Fluorouracil*

By JOSEPH L. MELNICK, Ph.D., SARA E. STINEBAUGH,‡ and FRED RAPP, Ph.D.

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

Plates 27 to 31

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The successful use of the fluoropyrimidines to gain information about the synthesis of viral DNA (1–3) led us to a study of their effect on the replication of vacuolating virus (SV40). This agent belongs to the papovavirus group[1] (4) which is characterized by 40 to 50 mμ icosahedral particles possessing a shell of 42 capsomeres and a core of DNA (5, 6). As the time sequence of virus production in the one-step growth cycle had already been determined for SV40 (7), experiments could be readily designed with 5-fluorouracil (FU) and 5-fluorodeoxyuridine (FUDR) to determine whether the synthesis of infectious virus might be suppressed while the formation of viral proteins continued. Reissig and Kaplan (1) had shown that with another icosahedral DNA-containing virus (swine herpes virus or pseudorabies) grown in the presence of FU, viral protein was assembled into empty particles having the size and shape of herpes virus but without DNA cores. In view of the well known tumorigenic activity of the papovaviruses, and the recent finding that SV40-induced tumors and transformed cells may contain complement-fixing viral antigen in the absence of infectious virus (8), information on the formation of viral antigens in cells which fail to make infectious virus is highly desirable.

Materials and Methods

Virus.—The same frozen stock of virus that had been used for determining the growth cycle of SV40 (7) was employed. Its titer was $10^{7.5}$ plaque-forming units (PFU) per ml. The detailed

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[1] Papovavirus was officially accepted as the name for the papilloma–polyoma-vacuolating group of viruses by the International Subcommittee on Viral Nomenclature, International Conference on Microbiology, meeting on August 20, 1962, in Montreal, Canada.

properties of the virus and its development in green monkey kidney cells, as followed by acridine orange staining, fluorescent antibody staining, and electron microscopy, are described in the previous report (7). Studies of the tumorigenic and cell-transforming properties of this same strain of virus have also been published (9).

Cell Cultures.—These were prepared from African green monkey cells (*Cercopithecus aethiops sabaeus*) using lactalbumin hydrolysate medium (M-H for growth and M-E for maintenance) as already described (10). No serum was present in the maintenance medium.

Plaque Assay in Bottles.—The method developed for SV40 has been published (11), but instead of a second (neutral red) overlay, a new medium was used which allowed the dye to be incorporated in the original agar at a final concentration of 1:60,000 (12).

Procedure for Measuring Virus Yields in Presence of FU and FUDR.—Cultures containing about 2 to 3 million cells in 1 ounce bottles were incubated 18 to 24 hours with maintenance medium containing the amount of drug which was to be used for the subsequent incubation with virus. The cultures were then drained and inoculated with 0.4 ml of fluid containing $10^{7.5}$ PFU/ml, an input multiplicity of about 10 PFU per cell. After an adsorption period of 5 hours at 37°C, the inoculum was decanted, and the cultures were washed five times with saline. Four ml of maintenance medium containing inhibitor was added to each bottle. Two cultures receiving each drug level were frozen for assay of adsorbed virus. The remaining infected cultures were incubated at 37°C and frozen 72 hours postinoculation for assay of total virus yield. Cultures to be stained with hematoxylin and eosin or with fluorescein-labeled globulin as described below, were pretreated with the drug in the same way.

As a control, the effect of 5-FU on the growth of poliovirus, previously studied in detail by Munyon and Salzman (13), was followed under similar conditions. Green monkey kidney cultures were incubated for 3 days in maintenance medium with 12 μg FU/ml, while control cultures were kept in medium without inhibitor. Cultures were drained and inoculated with 0.1 ml of poliovirus type 1 and incubated at 37°C. After 2 hours, they were washed four times with Hanks' salt solution, and then 4 ml of maintenance medium, with or without inhibitor as indicated, was added to each bottle. Treated and untreated cultures were frozen for titration of adsorbed virus. Others were frozen 8 hours postinoculation for assay of total virus yield.

Cell Counts.—Uninoculated cultures of green monkey kidney cells were held in maintenance medium with various levels of FU or FUDR. Direct counts were made to determine whether the cells multiplied or decreased in number under the experimental conditions employed. It was found that the drug levels used in these experiments did not cause significant reduction in the total number of cells present during the time intervals studied, nor did untreated cultures, already full sheets at the time they were used, multiply further in the absence of serum. Thus differences in cell number did not play a role in influencing virus yields in these experiments.

Hematoxylin and Eosin Staining.—Cells to be stained with hematoxylin and eosin were grown and inoculated on coverslips in Leighton tubes. They were harvested at the intervals indicated below, rinsed in tris buffer, and fixed for 15 minutes in Bouin's picric acid fixative. They were hydrated through decreasing concentrations of ethyl alcohol, stained 8 seconds in 0.5 per cent hematoxylin, rinsed in water, and counterstained 4 seconds with 1 per cent eosin Y. Cells were then dehydrated through increasing concentrations of ethanol, placed in xylol, and mounted on clean slides with permount (Fisher Scientific Company, New York).

Fluorescent Antibody Staining.—Two different methods were used for the preparation of fluorescein-labeled globulin (14). Method 1 was the same as that used in our earlier report (7). The serum globulin fraction from a hyperimmunized rabbit was precipitated with cold, half-saturated ammonium sulfate, conjugated with fluorescein isothiocyanate (15, 16), and then passed through a 3.5 inch column of DEAE cellulose to remove unconjugated isothiocyanate. Aliquots were adsorbed twice with acetone-extracted rhesus monkey liver powder (17).

Method 2 employed as starting material a pool of green monkey serum obtained from animals infected by a single inoculation of the same virus stock as that used in the present experiments (18). Cold ethanol was used to precipitate the globulin by the method of Cohn *et al.* (19). The globulin, at a final concentration of 10 mg/ml was labeled with fluorescein isothiocyanate (16). The preparation was then dialyzed for 5 days against phosphate-buffered saline (pH 7.2) which was changed twice daily to remove unconjugated fluorescein. No color was seen in the saline at the last three changes. After centrifugation at 9000 RPM for 10 minutes, the supernatant fraction, containing the dissolved conjugated globulin, was dispensed in small amounts and stored at −20°C. Prior to staining, an aliquot of conjugated globulin was thawed, adsorbed 1 hour by shaking with 100 mg/ml acetone-extracted mouse liver powder (17), and centrifuged at 12,000 RPM for 45 minutes. The process was repeated with half the amount of liver powder, and the supernatant fraction was stored at 4°C for use within 1 week.

Cells to be stained with the labeled globulin were grown on coverslips in Leighton tubes. When harvested, they were rinsed with tris buffer, air-dried, and fixed in acetone for 10 minutes. They were stored at 4°C before staining. Coverslips to be stained with fluorescein-labeled globulin were removed from the refrigerator, warmed and air-dried, and reacted with a drop of the conjugated globulin (1:2) for 30 minutes at room temperature. They were washed three times in tris buffer and left in the third wash for 10 minutes. They were blotted and mounted in elvanol (20) on clean slides. These preparations were examined with a Zeiss fluorescence microscope with a darkfield condenser using an Osram HBO-200 mercury arc vapor lamp for illumination.

Electron Microscopic Examination of Ultrathin Sections.—Drained monolayers of green monkey kidney cells in 16-ounce bottles containing about 10 million cells were inoculated with 2 ml of SV40 containing $10^{7.5}$ PFU/ml. After 4 hours, the inoculum was decanted, and 20 ml of maintenance medium was added. Cultures to be used for the growth of virus in the presence of FU were pretreated 18 to 24 hours with maintenance medium containing 12 μg/ml of the drug. The same drug-containing medium was added for incubation following the 4 hour adsorption period. After 3 days of incubation at 37°C, the cells were chilled in the bottles and fixed for 10 minutes with 1 per cent buffered OsO_4 at pH 7.8.

Cells were scraped from the bottles with a rubber policeman and dehydrated by passing through 35, 50, 70, and 95 per cent ethanol for 3 minutes each, and then left in 100 per cent ethanol for 5 minutes. They were then treated (*a*) with 0.1 per cent phosphotungstic acid in 100 per cent ethanol for 10 minutes, (*b*) with two changes of propylene oxide for 10 minutes each, and (*c*) with a 1:1 mixture of propylene oxide and *CIBA* 502 araldite resin for 90 to 120 minutes. When necessary, the cells were sedimented by low speed centrifugation (800 RPM) between changes of fluid.

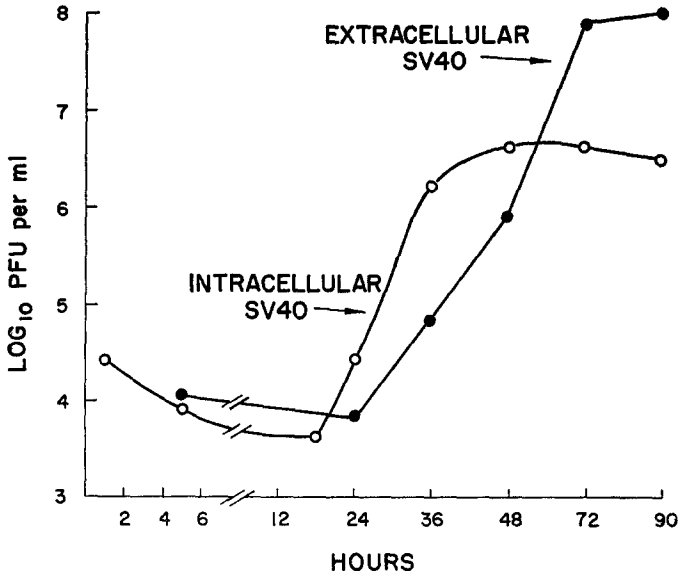
Pellets formed by low speed centrifugation of the cells were then embedded in epoxy resin according to Luft's procedure (21). Ultrathin sections were mounted on copper grids, and examined in the electron microscope.

RESULTS

Effect of 5-Fluouracil and Its Deoxyriboside on Production of Infectious Virus.—From previous studies (7, 22), the one-step growth curve for SV40 in green monkey cells was known to be relatively long. With the present stock of virus at input multiplicities of 3 to 10 PFU/cell, a 20 hour lag period was observed between the initiation of infection and the appearance of intracellular virus (Text-fig. 1). New extracellular virus was detected several hours later; it increased gradually and remained at a lower level than the cell-associated virus until 48 hours postinoculation. At 72 hours, the titer of extracellular virus

reached a plateau at a level much higher than that of the intracellular virus. Microscopic examination of the cultures was carried out periodically. At 50 hours postinoculation, some abnormal rounding of cells was evident but there was no evidence of cytoplasmic vacuolation. Vacuolation and nuclear enlargement were observed at 66 hours in 10 to 20 per cent of the cells, and at 82 hours in approximately 25 per cent of the cells.

As shown in Table I, both FU and FUDR had a marked inhibitory effect on the production of SV40 by infected cells. Assays of infected cultures frozen immediately after adsorption and washing indicated that cells pretreated with



TEXT-FIG. 1. Development of intracellular and extracellular virus in cercopithecus monkey kidney cultures inoculated with SV40.

all drug levels contained amounts of residual virus similar to those in the nontreated cells. Thus the drug did not inhibit virus adsorption to cells, nor was the drug carried over into the assay system in amounts sufficient to suppress plaque formation.

Production of Viral Antigen.—By the direct fluorescent antibody technique, we found that FU levels that suppressed virus multiplication (12 to 25 μg/ml) allowed the synthesis of large amounts of viral antigen. In all experiments, infected cells were harvested for fluorescent antibody studies 40 to 48 hours postinoculation; at this time in the drug-free infected cells virus antigens could be specifically localized in the nucleus (7). As indicated in Table II, which summarizes two experiments with labeled rabbit antiserum, cells treated with

FU also contained intranuclear antigen although fewer cells showed fluorescence equal in brightness to that of the untreated cells. As might be expected (23, 24), FUDR proved to be a more potent inhibitor of virus antigen formation than FU at the same levels.

No further experiments were performed using FUDR, but additional observations were made to study the distribution of antigen in the FU-treated cells. The labeled globulin used in this and succeeding experiments was made from postinfection monkey serum, which apparently contained more specific and more sensitive antibodies than those in the rabbit antiserum used for the experiments above. This made possible the detection of unmistakable differences in the form and distribution of virus antigens developing in untreated and FU-treated cells.

TABLE I

Inhibition of SV40 Replication by FU and FUDR

Level of drug	Yield of virus at 72 hours compared to control:			
	FU present		FUDR present	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
μg/ml	*per cent*	*per cent*	*per cent*	*per cent*
0	100	100	100	100
6	3	6	1	7
12	2	2	<1	<1
25	1	3	<1	<1
50	<1	1	<1	<1
100	<1	2	<1	<1

Untreated cultures and cultures exposed to 12 μg FU/ml were harvested for staining 40 hours after virus inoculation. In both cases, antigen was seen in the nuclei of 75 to 90 per cent of the cells (Figs. 1 and 7); there was, however, a marked difference in the distribution of viral antigen. In the absence of FU, antigen was particulate throughout the nuclei except for the nucleoli (Figs. 2 to 4). The particles of antigen were coccoid and varied considerably in size; at least a fivefold variation was observed (Figs. 3 and 4). Some cells had antigen concentrated at the nuclear periphery in small masses (Fig. 5). Occasional nuclei were seen with diffuse antigen (Fig. 6) or with one large mass of antigen virtually filling the nucleus. In the FU-treated cultures, on the other hand, fluorescence, while definitely present, was not quite as bright; antigen was seen in the nuclei in diffuse or finely granular form (Figs. 7 to 9). Nucleoli in these cultures also did not react (Fig. 9); in a few cells, antigen was concentrated at the periphery of the nucleus (Fig. 10). The fluorescent

material, even at the nuclear membrane, was diffuse rather than particulate. Only rarely were cells seen with small particles of antigen (Fig. 11), and none contained large particles or a mass filling the entire nucleus.

Results of another experiment in which drug levels were varied are given in Table III. Cells were again harvested for staining 40 hours after inoculation of virus. Results of an experiment conducted under the same conditions to determine the amount of infectious virus produced at 72 hours postinoculation are included in the same table for convenience of comparison. It can be seen

TABLE II

Effect of FU or FUDR on Development of SV40 Virus Antigen as Detected by Immunofluorescence with Labeled Rabbit Antiserum

Level of drug	Fluorouracil (FU)	Fluorodeoxyuridine (FUDR)
μg/ml		
0	(Control: Particulate intranuclear antigen in 65–75 per cent of cells, although a few cells contained a large intranuclear antigenic mass.)	
12	Intranuclear antigen in about 50 per cent of cells. Antigen abnormally distributed in nucleus and at nuclear periphery.	Intranuclear antigen in 1 to 10 per cent of cells. Particulate antigen in nucleus, occasionally at nuclear periphery.
25	Intranuclear antigen in 30 to 50 per cent of cells, distributed abnormally as above.	Very little antigen (in less than 1 per cent of cells). Antigen scattered in nucleus or concentrated at nuclear periphery.
50	Intranuclear antigen in 25 per cent of cells, distributed abnormally as above.	Little or no antigen.
100	Particulate intranuclear antigen seen, but in less than 1 per cent of cells.	Little or no antigen.

that large amounts of virus antigen were produced in the presence of drug levels which completely suppressed the production of infectious virus.

Cultures without virus were treated for 60 hours with levels of FU ranging from 6 to 100 μg/ml and stained with the conjugated virus-immune globulin; no fluorescence was observed in any of the preparations (Fig. 12). Reaction of infected cells with the unlabeled antiserum for 30 minutes prior to fluorescent antibody staining reduced the number of cells reacting from 80 per cent to less than 10 per cent. Cells which were stained were not as bright as those blocked by the unlabeled serum. Reaction with viral antibody probably failed to block completely the reaction with fluorescein-labeled globulin because of the large amount of antigen present. The great reduction in the amount and intensity of staining and failure of uninfected cells to react is sufficient evidence for the specificity of the staining reaction.

Hematoxylin and Eosin Staining.—Infected cultures treated with 12 μg/ml of FU were stained at intervals with hematoxylin and eosin for comparison with untreated infected cells. Uninoculated cultures with and without the drug were also examined; the FU-treated cells appeared normal though some minor nuclear aberrations were noted (Figs. 13 and 14).

In the inoculated cultures, no significant changes were seen until 40 hours after virus had been added, when FU-free cultures showed clumping of nuclear chromatin in almost all cells. Some nuclei had multiple small eosinophilic inclusions and others, although fewer in number, contained one large

TABLE III

Development of Infectious SV40 and of Viral Antigen in FU-Treated Cells

FU	Infectious virus production			Antigen production detected by immunofluorescence with labeled postinfection monkey antiserum	
	Titer of culture for virus at 5 hrs., just after adsorption and washing	Titer of culture at 72 hrs.	CPE at 72 hrs.	Cells containing intranuclear antigen	Distribution of antigen in nucleus
μg/ml	*PFU/ml*	*PFU/ml*		*per cent*	
0	$10^{4.8}$	$10^{6.6}$	Present in 25–50 per cent of cells	75–80	Particulate; a few nuclei contained large masses of antigen
6	$10^{4.8}$	$10^{5.4}$	Absent	75–80	Mainly diffuse; occasional nuclei contained small particles of antigen
12	$10^{4.8}$	$10^{4.8}$	"	75–80	" "
25	$10^{4.9}$	$10^{5.0}$	"	50	" "
50	$10^{4.8}$	$10^{4.5}$	"	50	" "
100	$10^{4.7}$	$10^{4.8}$	"	<10	Only small particles of antigen

eosinophilic body. Some nuclei were enlarged to three to four times the normal size, and darkly staining cells were scattered over most fields. No cytoplasmic vacuolation was observed. At this time, changes in the drug-treated cultures were less pronounced. Eosinophilic inclusions were both smaller and fewer; no nuclei filled with eosinophilic masses were observed.

By 72 hours after inoculation, fewer cells remained in the FU-free cultures, but their nuclei appeared the same as earlier, pyknotic with clumped chromatin (Figs. 15 and 16). The cell sheets in the FU-treated cultures were intact, and nuclei again resembled those seen at 40 hours postinoculation (Figs. 17 and 18). Occasional nuclei, however, contained small eosinophilic inclusion bodies (Fig. 18). While most of the cells remaining in FU-free cultures showed ex-

tensive vacuolation (Figs. 15 and 16), vacuolated cells were rarely seen in the FU-treated cultures. In the FU-free cultures, no correlation could be observed between appearance of inclusions in the nucleus and of vacuoles in the cytoplasm. They sometimes appeared in the same cell, but often cells showed either vacuolation or clumping of chromatin.

Effect of FU on Poliovirus.—It has been reported (13, 25, 26) that the growth of the RNA-containing poliovirus is not inhibited by the levels of FU used here. As a control for the present experiments, cells were pretreated with 12 μg/ml of FU for 3 days, then infected with poliovirus type 1, and harvested after 8 hours of incubation with the drug. Such FU-treated cultures yielded the same amount of virus as the untreated cultures (see Table IV). It is obvious, therefore, that FU specifically inhibits a DNA-containing papovavirus at a level which is without effect on the replication of an RNA-containing virus.

Electron Microscopic Observations.—Several experiments were carried out. In all, about 250 control cells and about the same number of FU-treated cells

TABLE IV

Development of Infectious Poliovirus in FU-Treated Cells

FU	Titer of culture for virus at 2 hrs., just after adsorption	Titer of culture at 8 hrs.
μg/ml	*PFU/ml*	*PFU/ml*
0	$10^{4.4}$	$10^{6.6}$
12	$10^{4.4}$	$10^{6.3}$

were examined with the electron microscope.[2] Cells that had been exposed 3 days earlier to SV40 in the absence of FU revealed, as already reported (7, 27, 28), a large number of virus particles in the nuclei of almost all cells (Fig. 19). Nucleoli were often disrupted and in many cells the chromatin was concentrated just inside the nuclear membrane. Particles were sometimes seen lining cytoplasmic vacuoles and sometimes formed chains enclosed by membranes.

FU-treated cultures were also examined after 3 days of incubation. Typical electron micrographs are shown in Figs. 20 and 21. Very few well formed virus particles were seen, and those seen were never in concentrated nests typical of the normal drug-free infection. Nucleolar disruption and migration of chromatin to the nuclear periphery were sometimes seen, but the changes were not as intense as those produced in infected cells in the absence of FU.

DISCUSSION

The levels of 5-fluorodeoxyuridine (FUDR) used in these studies cause a marked inhibition of the production of SV40 antigen as well as infectious virus,

[2] We wish to acknowledge the able assistance of Dr. Shawky Hassan, United Arab Republic Fellow in Virology, who carried out some of the electron microscopic observations.

similar to that found in studies with other DNA viruses (2, 3, 25, 29, 30). However, the incorporation of 5-fluorouracil (FU) into the culture medium of SV40-infected cells allows the synthesis of viral protein in the absence of a net increase in the amount of infectious virus. It is difficult to explain, on the basis of the available evidence, the differences in the production of viral antigen in the presence of FU and FUDR. At a concentration of 12 to 25 μg/ml, FU permitted the synthesis of viral antigen by most cells; the same level of FUDR almost completely suppressed the formation of this protein. FU has been found to be incorporated into the RNA of poliovirus (13) and of tobacco mosaic virus (31). Bussard *et al.* (32) reported that beta galactosidase produced by *Escherichia coli* in the presence of FU was antigenically normal but totally inactive. They suggested that the drug acted on the system responsible for transferring structural information from the gene, namely the RNA. A similar mechanism may apply in the SV40-infected cells. If FU were incorporated into RNA, limited DNA synthesis might continue, but the protein produced might be functionally ineffective although antigenically nearly normal. If one assumes that synthesis of the protein of a DNA-containing virus requires at least one prior replication of the infecting virus DNA, FUDR might prevent the formation of virus protein by completely suppressing synthesis of new DNA.

The distribution of virus antigen in FU-treated cells at 40 to 48 hours resembles that of a much earlier stage in untreated cultures (7). In the absence of the drug, cells exhibit diffuse antigen in the nucleus 20 to 24 hours after infection, at which time new intracellular virus is just beginning to appear. Then, as virus assembly proceeds, the antigen appears as large particulates. When virus synthesis is blocked by FU, the infection appears to be arrested at the early stage characterized by the appearance of the diffuse antigen.

There are interesting similarities and differences between the present experiments and those of Reissig and Kaplan (1) on FU-treated cells infected with another DNA virus exhibiting icosahedral symmetry, swine herpes virus. They found that the virus-induced inclusions in FU-treated cells remained smaller than those in control cultures and never became Feulgen-positive. We made similar observations on our SV40-infected cells treated with fluorouracil. Definite indications of nuclear involvement were present, but the nuclear inclusions remained small and the cytoplasmic vacuoles seen normally in the last stages of virus replication were virtually absent in FU-treated cells.

However, Reissig and Kaplan (1) found that although their infected cells treated with FU produced as many virus particles as untreated cells, most of the particles from drug-treated cultures were non-infectious, presumably because they lacked DNA cores. Our results indicate that a different situation exists with SV40. In the electron microscope very few virus particles were seen in thin sections of SV40-infected cells tested with FU. Since considerable amounts of virus antigen can be located in the nuclei of the vast majority of cells by the fluorescent antibody technique, the absence of empty virus particles

is not due to lack of protein synthesis. The electron microscopic results suggest that the large amounts of SV40 antigen produced are not assembled into the protein capsid in the presence of fluorouracil.

At least four laboratories have reported that SV40 induces the malignant transformation of cell cultures prepared from embryonic or newborn hamsters. In two laboratories (33, 34) the transformation was associated with the continuous appearance of virus in the cultures. In the other two laboratories (9, 35), infective virus could not be found in the transformed cultures. However, Shein *et al.* (35) observed a faint circumnucleolar fluorescence in transformed cells stained with SV40 antibody by the indirect fluorescent antibody method, and suggested that this might result from the presence of an SV40-specific antigen not associated with infectious virus. Huebner *et al.* have recently found that cells transformed by SV40 but free of the virus contain a specific viral complement-fixing antigen, similar to the findings in tumor cells resulting from adenovirus infection of hamsters (8). However no evidence was obtained to show that the viral CF antigen in the tumor was related to the viral antigen detected in the present study (36).

There is other evidence to show that a specific non-infectious antigen, in some way related to SV40, is present in the hamster tumor cells induced by this virus (37–40). Adult hamsters immunized with live SV40 become resistant to SV40 tumor cells, even when virus-free transformed cells are used for challenge. Injection of the murine papovavirus (polyoma) confers no such immunity, although suggestive crossings against SV40 were obtained with papovaviruses of rabbit and man (40). The present experiments indicate a possible probe towards the production of such non-infectious viral protein antigens even before they are assembled into viral capsids.

SUMMARY

A study was made of the effects of 5-fluorouracil (FU) and 5-fluorodeoxyuridine (FUDR) on the replication of the simian papovavirus SV40 in cercopithecus monkey kidney cells and on the production of virus antigen by these cells. Both drugs markedly suppressed the production of new infectious virus by SV40-infected cells. Synthesis of viral protein was also markedly suppressed by FUDR, but not by FU.

In the presence of FU, infected cells produced large amounts of viral protein which were detected by the fluorescent antibody technique. The antigen was not distributed in a particulate fashion as in untreated cells. Diffuse virus antigen was observed in the nuclei of FU-treated cells, resembling the distribution of antigen near the end of the eclipse period in untreated, infected cultures. This stage of antigen production presumably preceded viral assembly.

Virus particles with or without cores were rarely seen with the electron microscope in infected FU-treated cells, although large numbers of SV40

particles were readily visualized in untreated, infected cells. It appears that at least one antigenic protein of this papovavirus is synthesized abundantly in FU-treated cells, but is not assembled into virus shells in the presence of the inhibitor.

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EXPLANATION OF PLATES

PLATE 27

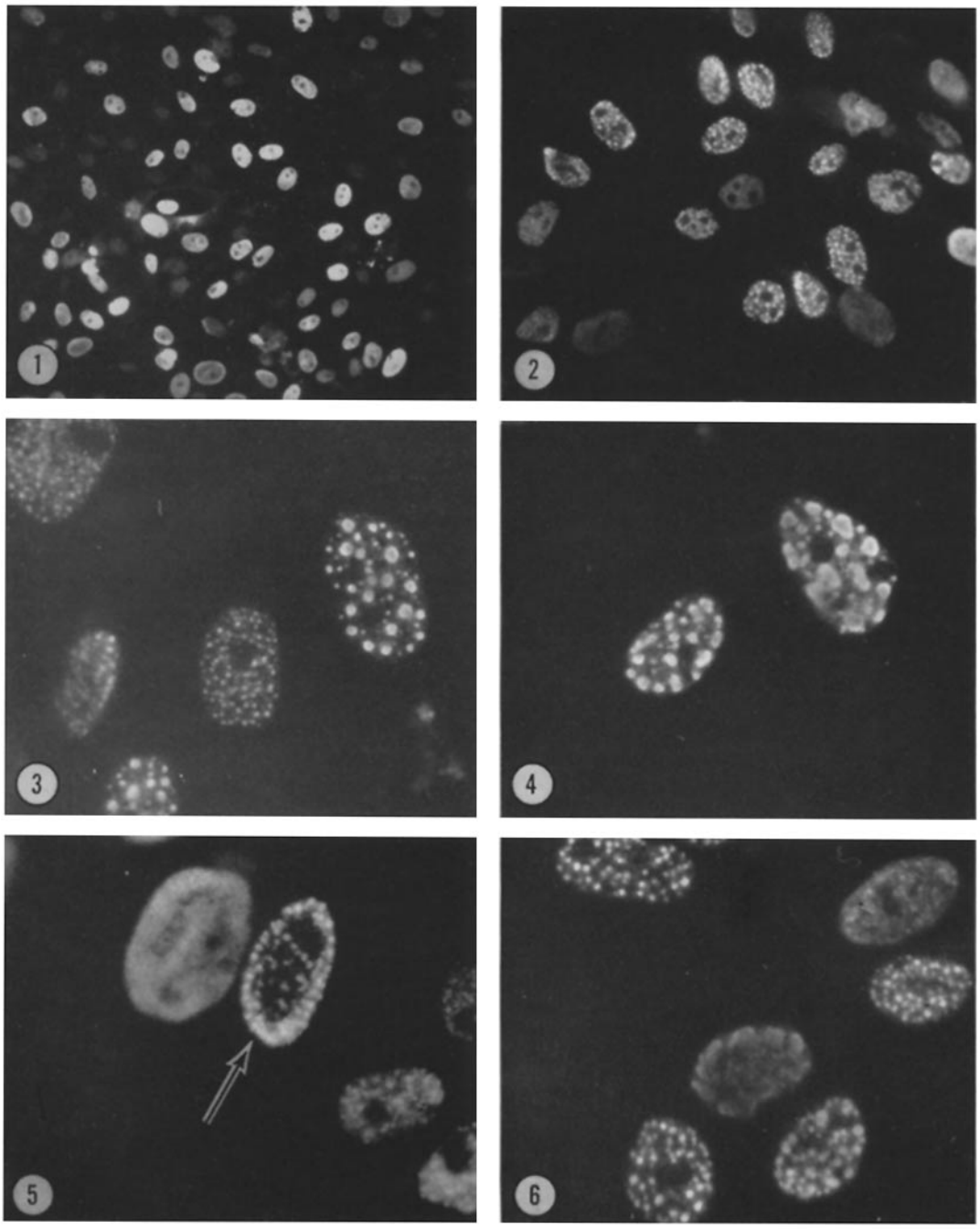
FIGS. 1 to 6. Immunofluorescence photomicrographs of green monkey kidney cells 40 hours following inoculation with SV40. Fluorouracil not present.

FIG. 1. Virus antigens localized in the nuclei of 75 to 90 per cent of the cells. × 180.

FIG. 2. Particulate virus antigen in the nuclei. Note dark, non-reacting nucleoli. × 720.

FIGS. 3 and 4. Intranuclear particulate virus antigen varying in size and shape. Note dark, non-reacting nucleoli. × 1800.

FIGS. 5 and 6. One cell shows particulate virus antigen concentrated at the nuclear membrane. Some show intranuclear virus antigen distributed diffusely throughout the nucleus. × 1800.



(Melnick *et al.*: Simian papovavirus SV40)

PLATE 28

FIGS. 7 to 11. Immunofluorescence photomicrographs of green monkey kidney cells 40 hours following inoculation with SV40 in the presence of fluorouracil at a concentration of 12 μg/ml.

FIG. 7. Virus antigen localized in the nuclei of 75 to 90 per cent of the cells. × 180.

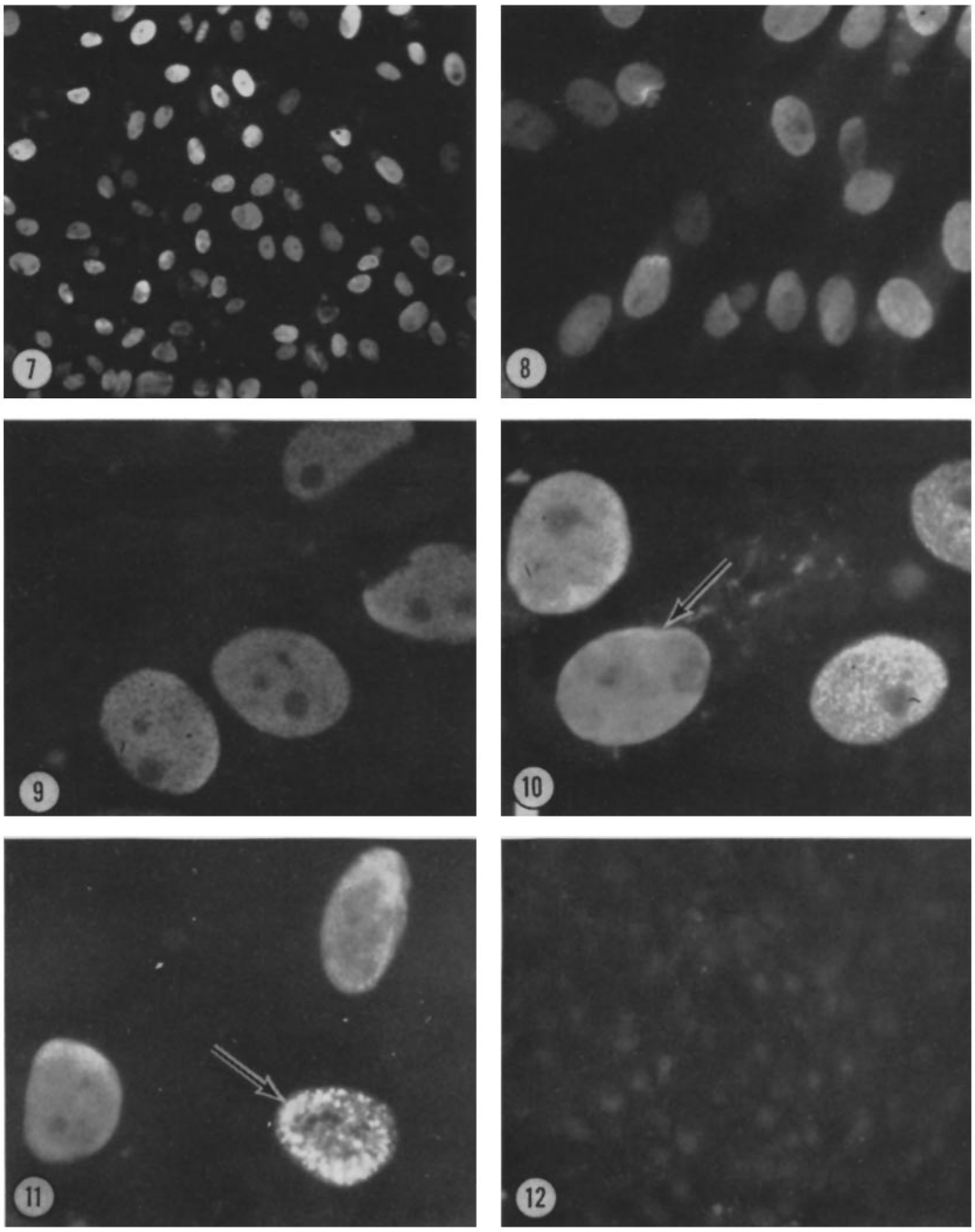
FIG. 8. Intranuclear virus antigen distributed as diffuse or finely granular material. × 720.

FIG. 9. Diffuse virus antigen; note dark, non-reacting nuclei. × 1800.

FIG. 10. Diffuse virus antigen concentrated at nuclear membrane of cell in lower left. × 1800.

FIG. 11. A rare cell with small particles of virus antigen. × 1800.

FIG. 12. Immunofluorescence photomicrograph of uninfected green monkey kidney cells exposed to FU for 40 hours; reacted with anti-SV40 fluorescein-labeled serum. × 180.



(Melnick *et al.*: Simian papovavirus SV40)

PLATE 29

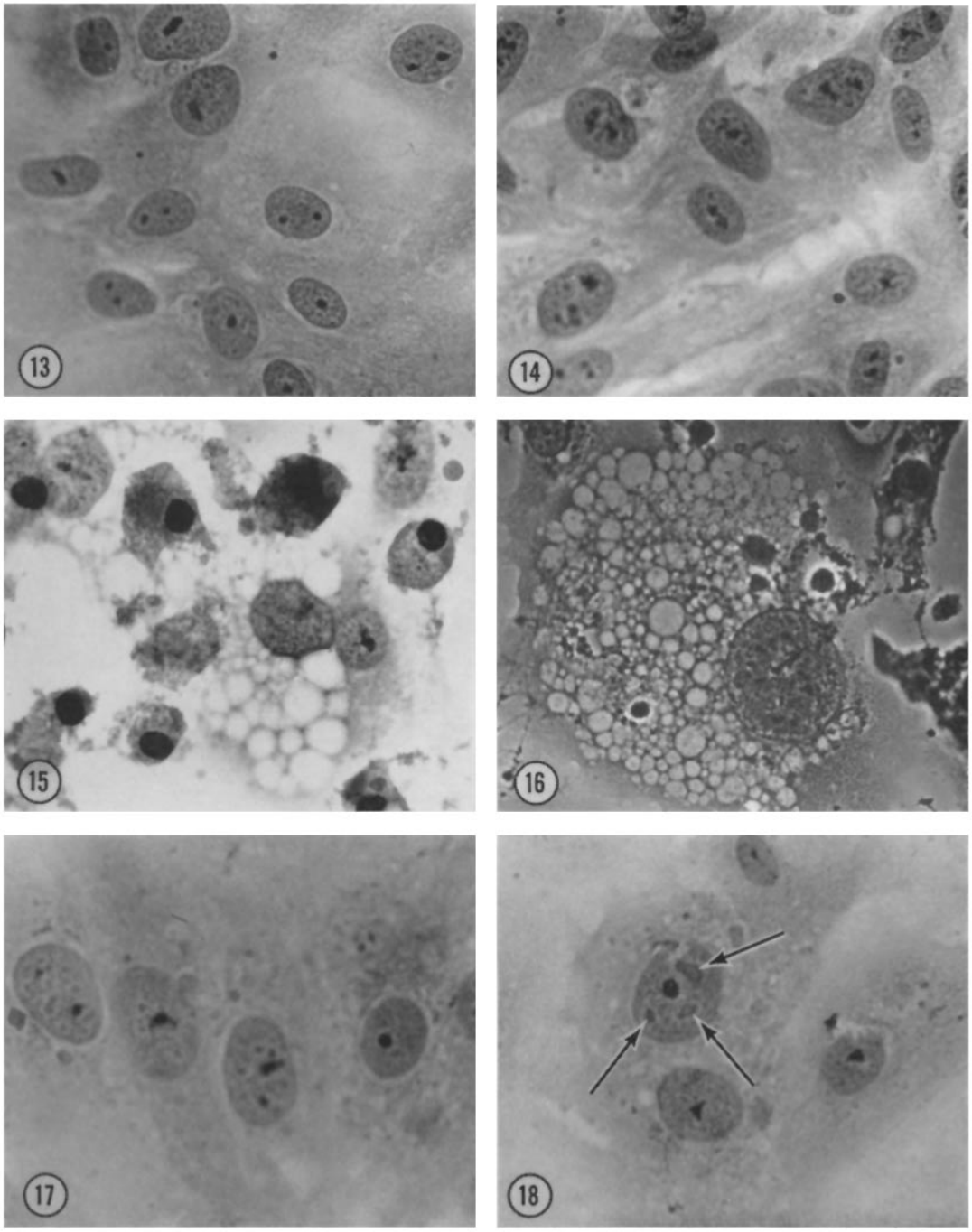
FIGS. 13 to 18. Photomicrographs of green monkey kidney cells stained with hematoxylin and eosin.

FIG. 13. Normal cells in the absence of virus or drug. × 600.

FIG. 14. Uninfected cells treated with 12 μg FU/ml. × 600.

FIGS. 15 and 16. Cells inoculated 72 hours previously with SV40. No FU. Note pyknotic nuclei, clumping of chromatin, vacuolation, and destruction of cell sheet. Fig. 15, × 600. Fig. 16, × 750.

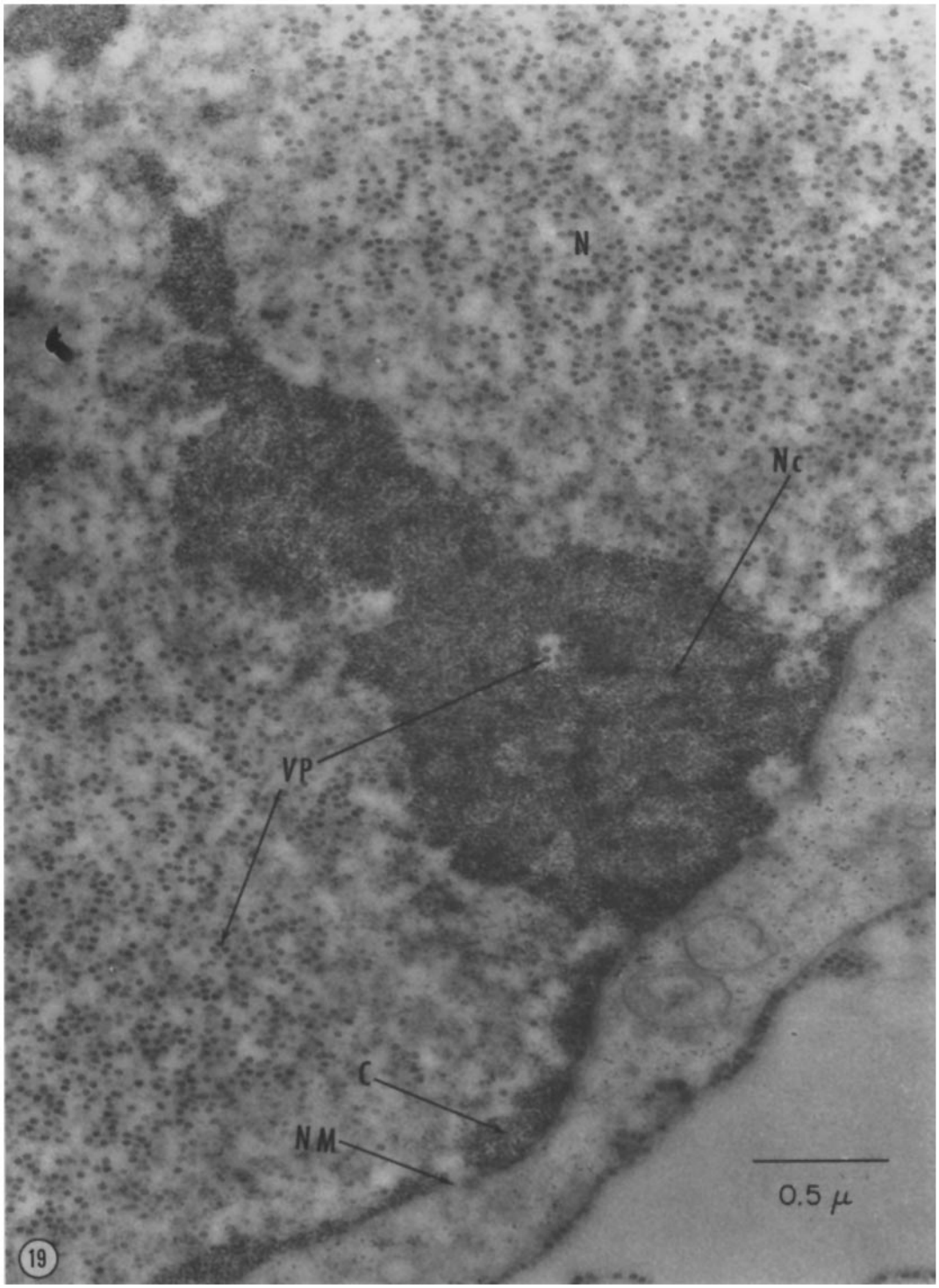
FIGS. 17 and 18. FU-treated cells inoculated 72 hours previously with SV40. Small intranuclear bodies are evident in some cells. Cell sheet is intact. × 600.



(Melnick *et al.*: Simian papovavirus SV40)

PLATE 30

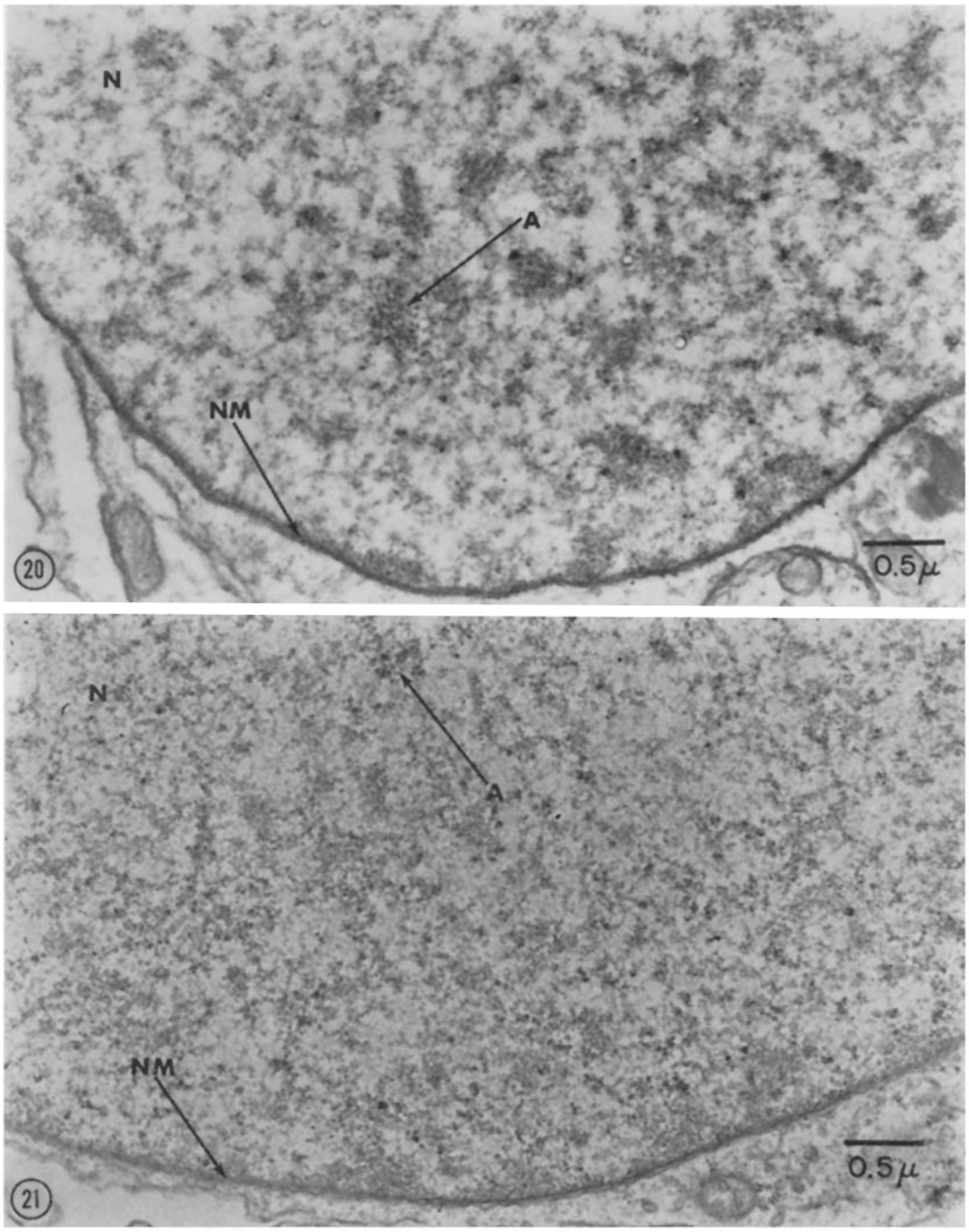
FIG. 19. Electron micrograph of green monkey kidney cells 72 hours after inoculation with SV40. No FU. Numerous virus particles (*VP*) are seen in the nucleus (*N*) and within the nucleolus (*Nc*). Nuclear chromatin (*C*) can be seen just inside the nuclear membrane (*NM*). Particles have mean diameter of 42 mμ. $\times$ 42,000.



(Melnick *et al.*: Simian papovavirus SV40)

PLATE 31

FIGS. 20 and 21. Electron micrographs of two FU-treated cells 72 hours after inoculation with SV40. Chromatin is distributed throughout the nucleus (*N*). Some areas (*A*) may be seen in which particles ranging from 10 to 40 mμ in size are clustered, perhaps in an abortive attempt at virus replication. × 23,500.



(Melnick *et al.*: Simian papovavirus SV40)