ON THE ROLE OF THE RESPONSE OF THE CELL MEMBRANE IN DETERMINING VIRUS VIRULENCE

CONTRASTING EFFECTS OF THE PARAINFLUENZA VIRUS SV5 IN TWO CELL TYPES*,‡

BY KATHRYN V. HOLMES AND PURNELL W. CHOPPIN, M.D.

(From The Rockefeller University)

PLATES 33 TO 38

(Received for publication 3 May 1966)

Simian virus 5 (SV5) (1) is a common contaminant of primary cultures of rhesus monkey kidney cells. On the basis of both biological (2–4) and morphological properties (5), it has been shown to be a member of the parainfluenza-mumps-Newcastle disease subgroup of myxoviruses (6). These viruses are 120 to 500 mμ in size with a helical nucleoprotein internal component 180 A in diameter; they replicate in the cytoplasm, possess a hemolysin, and may induce cell fusion.

Viruses antigenically similar to SV5 have been isolated from several other sources (3, 7, 8). One of these viruses, DA virus, was isolated from human blood (3), but has not been shown to be pathogenic for man. DA virus also infects rhesus monkeys, hamsters, and mice without producing overt disease (9).

In primary cultures of rhesus monkey kidney (MK) cells, SV5 multiplies to high titer with little cytopathic effect (4). The infected cells survive and produce large amounts of infective virus for many days. Such a steady state type of virus-cell interaction has been termed moderate (10). SV5 infection of monkey kidney cells does not interfere with superinfection by other viruses, and the yield of superinfecting virus is not significantly reduced (4).

The present communication describes the multiplication of SV5 in a continuous line of baby hamster kidney cells (BHK21-F). It will be shown that, in contrast to monkey kidney cells, SV5-infected BHK21-F cells produce relatively little infective virus and fuse within 12 to 18 hr to form a large syncytium which subsequently disintegrates. At high virus/cell multiplicities, SV5 may cause BHK21-F cells to fuse within 1 hr. The intracellular sites of formation of SV5

* Supported by Research Grant AI-05600 from the National Institute of Allergy and Infectious Diseases and Grant GM-577 from the Institute of General Medical Sciences, United States Public Health Service.

‡ Presented in part at the 49th Annual Meeting of the Federation of American Societies of Experimental Biology, *Fed. Proc.*, 1965, **24**, 571.

antigens and the effect of SV5 infection on cellular ribonucleic acid, deoxyribonucleic acid, and protein synthesis in both MK and BHK21-F cells will be described. The results suggest that whether SV5 infection results in moderate or virulent virus-cell interaction depends on the response of the cell membrane.

Materials and Methods

Virus.—The W3 strain of SV5 (4) was propagated in primary cultures of rhesus monkey kidney cells; at the time of harvest bovine serum albumin was added to a final concentration of 1%. Virus stocks were stored at −60°C. For some experiments virus was concentrated and partially purified by centrifugation. Medium containing released virus was removed from intact SV5-infected monkey kidney cells. Cellular debris was removed by centrifugation at 1100 *g* for 10 min, and virus was sedimented at 35,900 *g* for 1 hr. The pellet was allowed to soften by standing overnight at 4°C in reinforced Eagle's medium (11) with 2% calf serum or in phosphate-buffered saline (PBS) pH 7.2 (12) and then resuspended by pipetting. This procedure usually results in a 40 to 95% recovery of infective virus.

Cell Cultures.—Primary cultures of *rhesus monkey kidney* cells were grown in lactalbumin hydrolysate medium with 2% calf serum as described previously (13). The cell line designated *BHK21-F*, a heteroploid variant of the BHK21 cell line derived from baby hamster kidney by Macpherson and Stoker (14), was kindly supplied by Dr. Sonia Buckley of the Rockefeller Foundation Virus Laboratory, New York, who had previously obtained the cells from the Wistar Institute in Philadelphia. In our laboratory the cells were grown in reinforced Eagle's medium with 10% calf serum and 10% tryptose phosphate broth, and transferred twice weekly, diluting the cells 1:20 at each transfer. These cells are clearly different in appearance and in interactions with virus from *BHK-21 clone 13* cells which had undergone only 12 passages since obtained from Dr. Stoker's laboratory. A continuous line of weanling Syrian hamster kidney cells designated *HKCC* was obtained from Dr. Charles Calisher of Microbiological Associates, Bethesda, Maryland, and propagated by the procedure described for BHK21-F cells.

Virus Assays.—Hemagglutination titrations with chicken erythrocytes at 4°C and *plaque assays* in monkey kidney cells were carried out as previously described (4).

Inoculation of Cells and Virus Growth Curves.—Cells grown to confluent monolayers in 60 mm plastic Petri dishes were washed twice with PBS and inoculated with 0.5 ml of virus suspension. After an adsorption period of 1 hr at 37°C, the cells were washed twice with PBS and growth medium was added. The procedure for growth curves has been described in detail elsewhere (4); total virus yields consisting of both released and cell-associated virus were determined.

Antisera.—Rabbits were immunized with SV5 by the procedure described previously (4). Sera were stored at −25°C and heated at 56°C for 30 min before use.

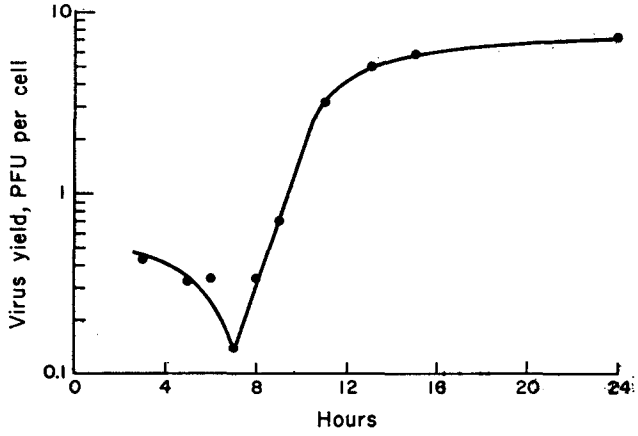
Fluorescent Antibody Staining.—Cells grown on 18 mm square glass cover slips were fixed 10 min in acetone at room temperature, air dried, and stored at −25°C until stained. The indirect staining technique was used with rabbit anti-SV5 serum and fluorescein-labeled duck anti-rabbit gamma globulin which was kindly supplied by Dr. Beatrice C. Seegal and Dr. Konrad C. Hsu of Columbia University College of Physicians and Surgeons, New York. The duck anti-rabbit gamma globulin preparation was adsorbed with rat and mouse liver powder, and the rabbit antiserum and control normal rabbit serum were adsorbed with monkey kidney cell powder or guinea pig liver powder in some experiments. Slides were examined with a Zeiss Ultraphot II using an Osram 200 watt high pressure mercury burner, a BG 12 exciter filter, and an OG 1 barrier filter.

Hematoxylin and Eosin Staining.—Cells grown on glass cover slips were washed in PBS

and fixed in Zenker's solution for 1 hr at room temperature. They were then stained by the procedure of Reissig and coworkers (15) allowing 5 to 6 min in the hematoxylin solution and 3 to 4 min each in the aqueous and alcoholic eosin solutions.

Time-lapse Photomicrography.—BHK21-F cells were grown on round glass cover slips 1 in. in diameter and inoculated with SV5. After a 1 hr adsorption period, the cover slips were washed once and put into Sykes-Moore chambers (16) containing growth medium. The chambers were held at 37°C and observed with phase-contrast optics in a Zeiss inverted microscope to which was attached a Cine-Kodak Special II camera. A × 10 objective was used.

Assays of RNA, DNA, and Protein Synthesis.—The rates of RNA, DNA, and protein synthesis in uninfected and SV5-infected cells grown on cover slips were determined on the basis of incorporation of tritium-labeled uridine, thymidine, or leucine into the respective macromolecules during 30-min pulses. Four to eight cover slips per variable were used in each experiment. The procedure of Bablanian, Eggers, and Tamm (11) was employed. Radioactivity was determined in a Nuclear-Chicago Corporation, Des Plaines, Illinois windowless gas flow counter.



TEXT-FIG. 1. Growth curve of SV5 in monolayers of BHK21-F cells inoculated at a virus multiplicity of 16 PFU/cell.

RESULTS

Growth Curve of SV5 in BHK21-F Cells—The multiplication of SV5 under single cycle conditions was studied in confluent monolayers of BHK21-F cells inoculated at a multiplicity of 16 plaque-forming units (PFU) per cell. Text-Fig. 1 shows such a growth curve. After a latent period of 7 hr, virus increased exponentially with a doubling time of approximately 60 min. A plateau was reached at about 13 hr after inoculation. The total yield of infective virus was approximately 7 PFU/cell and production ceased within 24 hr. In repeated experiments the length of the latent period and the virus yield per cell were similar; the doubling time varied from 45 to 130 min. The explanation for this variation is not clear, but might be related to differences in the rate of cell fusion observed in these experiments.

Although the latent period and the doubling time of SV5 in BHK21-F cells shown in Text-Fig. 1 are similar to the 6 to 7 hr latent period and 50 min doubling time previously found in monkey kidney cells (4), the yield of virus from the two cell types is greatly different. Monkey kidney cells yield up to 1500 PFU per cell in 24 hr and can continue to produce virus for many days, whereas BHK21-F cells yield 3 to 12 PFU per cell and virus production ceases within 24 hr.

Cytopathic Effects of SV5 Multiplication.—

The cytological changes in SV5-infected cells were studied by hematoxylin and eosin staining, phase-contrast microscopy, and time-lapse photomicrography.

Figs. 1 *a* to 1 *f* show the progression of changes in BHK21-F cells inoculated with a multiplicity of 16 PFU/cell.

Uninfected BHK21-F cells (Fig. 1 *a*) are spindle-shaped and tend to pile up in some areas; occasional binucleate cells are seen in uninfected monolayers. The first change detectable with the light microscope after infection was the appearance of multinucleate cells beginning at about 6 hr and increasing with time. Figs. 1 *b* and 1 *c* show cells 7.5 and 10 hr after infection. By about 12 hr after infection (Fig. 1 *d*) nearly all of the nuclei were in multinucleate giant cells. The giant cells increased in size and, by 14 to 18 hr after inoculation (Fig. 1 *e*), the entire monolayer appeared to be a syncytium. Often groups of nuclei in the syncytium were arranged in long parallel lines. This is shown in Figs. 1 *d* and 1 *f*, and more clearly in Fig. 2. By 24 to 30 hr after infection (Fig. 1 *f*) the nuclei were usually in tightly packed, round clusters, and large areas of the syncytium had begun to peel from the surface of the Petri dish. Nuclei larger than those found in uninfected cells were commonly found in polykaryocytes, and occasionally extremely large nuclei with many nucleoli were seen (Fig. 3). Small eosinophilic inclusion bodies surrounded by white halos appeared in the cytoplasm about 12 hr after infection and became larger and more numerous with time (Figs. 1 *e* and Fig. 2).

As previously reported (4), SV5 multiplies in primary rhesus monkey kidney cells with minimal cytopathic effects. Figs. 4 *a* and 4 *b* show the lack of morphological changes in monkey kidney cells which were inoculated at a virus multiplicity of 48 PFU/cell and which had been producing large amounts of virus for 2 days with daily changes of the lactalbumin hydrolysate growth medium. It should be pointed out that after several days some cytopathic effects may occur in these cells, particularly if Eagle's minimal essential medium (17) is used instead of growth medium or if the medium is not changed daily. Such changes, when they do occur, consist of formation of some small polykaryocytes and very small eosinophilic cytoplasmic inclusions.

SV5 also causes cell fusion in the standard BHK21 cells and in the HKCC line of Syrian hamster kidney cells; however the fusion of these cells is less rapid and extensive than that of BHK21-F cells. Since SV5 is assembled and released at the cell membrane by budding (18), it is interesting that less virus is pro-

duced in those cells in which membrane alterations leading to cell fusion are most extensive. This inverse relationship between virus yield and the extent of cell fusion is shown in Table I.

Time-lapse Photomicrography of Infected BHK21-F Cells.—Time-lapse studies were carried out to investigate the cytological changes in SV5 infected BHK21-F cells with particular reference to the mechanism of formation of giant cells and to nuclear changes. In these experiments cells were inoculated at a virus multiplicity of 15 to 30 PFU/cell.

Cell Fusion.—Figs. 5 *a* to 5 *i* show frames selected from a time-lapse film of a small area of a monolayer of SV5-infected BHK21-F cells.

TABLE I

Comparison of Virus Yields and Relative Extent of Fusion Caused by SV5 in Different Cell Types

Cells	Virus yield, PFU/cell*	Nuclei in polykaryocytes‡
		%
BHK21-F	7	>99
BHK21	16	80–95
HKCC	345	40–60
Monkey kidney	1440	<1

* Total virus harvested at 24 hr.

‡ Estimated at 24 hr.

In early stages of infection the mobility of the cells appeared normal. The first change observed was the fusion of adjacent cells to form a binucleate cell. Such binucleate cells grew in size (Figs. 5 *a* and 5 *b*) both by fusing with other multinucleate cells and by recruiting single cells. Two cells which were about to fuse appeared to form a small cytoplasmic connection which extended until no borders were visible between the cells (Figs. 5 *c*, 5 *d*, and 5 *e*). The nucleus of the smaller cell moved quickly to the cluster of nuclei in the center of the larger cell. The phase-dense granules of the smaller cell remained close to the nucleus as it migrated into the larger cell. The granules later became dispersed through the extensive cytoplasm of the new, fused cell. The nuclei of a small polykaryocyte which fused with a larger polykaryocyte migrated into the larger cell either singly or in tight formation as if held together.

Cell fusion continued until nearly all cell borders had disappeared (Fig. 5 *f*). The nuclei, which were frequently arranged in long lines, later tended to pack tightly together into rounded clusters in a broad area of thin cytoplasm (Fig. 5 *g*). These large clumps containing hundreds of nuclei moved through the cytoplasm. In Fig. 5 *h*, the three clumps of nuclei in this single syncytium have moved out of the field. During this stage, many large waves rippled vigorously in all directions through the wide expanse of cytoplasm. Ultimately the cytoplasm ripped and the syncytium peeled from the cover slip (Fig. 5 *i*).

The time-lapse movies clearly demonstrate that the syncytia induced by SV5 are formed by cell fusion. These observations are in agreement with previous reports that polykaryocytes induced by parainfluenza and measles viruses result from cell fusion (19–21).

Division of Polykaryocytes.—Time-lapse photomicrography has demonstrated that during the early stages of infection single cells undergo mitosis in an apparently normal manner. As cell fusion progresses, many abnormal divisions of giant cells occur. Cells containing two or more nuclei were frequently observed to round up and attempt to divide into 3 or 4 parts; several of the parts sometimes fused together immediately after division. A particularly striking example of aberrant cell division in a giant cell is illustrated in Figs. 6 *a* to 6 *i*. A cell containing about 16 nuclei of normal size rounded up, constricted in several planes in an apparent unsuccessful attempt to divide into many daughter cells, and finally divided into two cells, each of which contained two or three large nuclei. Thus, SV5-infected cells may divide even after extensive fusion has occurred. Such cell division may be accompanied by a reduction in number of nuclei and an increase in nuclear size. This might be due to confusion of the spindles associated with individual nuclei in the tight clumps of nuclei of a polykaryocyte. One or more of the centrioles might be eclipsed by those of a neighboring nucleus, resulting in larger nuclei with an abnormal number of chromosomes. The many observed instances of abnormal cell division leading to unusually large nuclei provide an explanation for the large nuclei described above in stained preparations. Direct fusion of two nuclei during interphase has not been observed.

Effect of Concentrated Virus.—The contrast between the striking fusion of BHK21-F cells infected with SV5 and the slight changes in monkey kidney cells indicates a marked difference in the reaction of the cell membranes to SV5 multiplication. This was further investigated by examining cells soon after the addition of concentrated virus.

Monolayers of cells were washed with PBS, 0.5 ml of concentrated virus was added, and the cells were held at 37°C. The virus multiplicities were 2000 to 6000 PFU per BHK21-F cell, and 18,000 PFU per monkey kidney cell.

Under optimal conditions fusion of BHK21-F cells can be detected within 20 min and, after 1 hr (Figs. 7 *a* and 7 *b*) most of the nuclei are found in large polykaryocytes. Within 3 hr some areas of the syncytium begin to degenerate and peel from the cover slip. There is some variation in the rate of cell fusion in such experiments. Fusion was most extensive in crowded monolayers and with virus suspended in reinforced Eagle's medium with 2% calf serum. Virus suspended in PBS was significantly less effective. Since the latent period of SV5 in BHK21-F cells is about 7 hr, it is clear that rapid fusion by concentrated virus is due to the inoculum and not to newly synthesized virus. This is also demon-

strated by the finding that virus inactivated by ultraviolet light also induces fusion. Concentrated virus irradiated for 5 min with a General Electric 15 watt germicidal lamp No. G15T8 at a distance of 15 cm caused fusion of cells within 1 hr although infectivity had been reduced by a factor of 10^7.

Cell fusion also occurred within 1 hr after cells had been treated with actinomycin D, 5 μg/ml, or puromycin, 100 μg/ml for 2 hr, before inoculation. These concentrations of actinomycin D and puromycin cause >96% inhibition of cellular RNA and protein synthesis, respectively, within 2 hr. These results indicate that SV5 particles can alter the cell membrane causing rapid cell fusion, and that neither replication of the virus nor synthesis of cellular RNA or protein is required for cell fusion. These findings are in agreement with those reported previously with parainfluenza (22, 23), Newcastle disease (24), measles (25, 26), herpes (27)and vaccinia viruses (28).

In marked contrast to the rapid and extensive fusion of BHK21-F cells, monkey kidney cells showed no effects during 6 hr of exposure to 18,000 PFU/cell. This finding emphasizes the differences in the response of the two cell membranes to SV5.

Development of Viral Antigens in Infected Cells.—

The site and time course of development of SV5 antigen was studied by the indirect method of immunofluorescent staining. Monolayers of BHK21-F and monkey kidney cells were inoculated at multiplicities of 15 and 48 PFU/cell, respectively, in order to obtain infection of all cells approximately simultaneously.

The development of viral antigens in the two cell types was similar. Specific fluorescence was first observed in the perinuclear region at about 3 hr after infection and, by 7 hr, viral antigens were found in the cytoplasm of nearly every cell in the monolayers (Figs. 8 *a* and 9 *a*). Both diffuse fluorescence and small granular fluorescent foci were observed. The fluorescent foci increased in size and number and were found throughout the cytoplasm by 24 hr (Figs. 8 *b* and 9 *b*). Nuclear fluorescence was not observed. Viral antigens were frequently seen in cells in mitosis (Fig. 8 *b*). The development of SV5 antigens in the cytoplasm was similar to that reported for Newcastle disease virus (29, 30). By analogy to studies on separated NDV antigens (30), the granular fluorescent foci would represent the internal component protein of the virus, and the diffuse fine fluorescence, the viral hemagglutinin.

Since essentially all of the BHK21-F cells contained viral antigen by 7 hr when cell fusion was beginning, it is clear that fusion can occur between two cells each of which is producing viral components. In the large syncytia found late in infection (Fig. 9 *b*) the largest foci were usually found near clusters of nuclei and smaller foci were scattered through the wide areas of cytoplasm between these clusters. Occasionally these fluorescent foci were arranged in parallel rows.

These studies indicate that the development of viral antigens in the cyto-

plasm is similar in monkey kidney and BHK21-F cells. Although the BHK21-F cells produce little infective virus, their cytoplasm appears to contain much viral antigen.

Persistent Infection with SV5.—Persistent infection of cells which continue to multiply has been described with several virus-cell systems (31–34). It was reported previously (4) that sparsely seeded cultures of primary monkey kidney cells infected with SV5 grew into confluent monolayers as rapidly as did uninfected controls, suggesting that SV5-infected cells were capable of dividing several times. To investigate further the multiplication of SV5-infected cells, monkey kidney and BHK21-F cells were infected and serially transferred.

Monolayers were inoculated at multiplicities of 15 to 48 PFU/cell. 24 hr or more after infection, the cells were detached from the Petri dish with 0.25% trypsin and 0.05% ethylenediaminetetraacetate, diluted 1:5 or 1:10 in growth medium, and transferred to new Petri dishes. When the cells had again grown to confluent monolayers, the procedure of cell transfer was repeated. At each passage the medium was removed for assay of infective virus, and cells from each passage examined for viral antigen by immunofluorescence.

Infected monkey kidney cells were carried through from 6 to 11 passages in such experiments. The experiments were terminated when multiplication of the cells had slowed drastically or apparently ceased. It should be emphasized that multiplication of uninfected cells carried as controls through the same serial passage procedures also ceased; e.g., in the experiment in which transfer of the infected cells was terminated at the 11th passage, the control cells could be maintained for only 8 passages. Essentially all cells in the infected culture appeared to contain viral antigen in each of the passages. The titer of infective virus produced in 24 hr by primary monkey kidney cultures is approximately 6.0×10^8 PFU/ml; the titer in the medium after the 4th cell passage was 4.9×10^7 PFU/ml; and by the 10th passage, 2.2×10^5 PFU/ml. Although the virus yield per cell in the later passages cannot be precisely calculated since the cells were not counted at each transfer and cell-associated virus was not determined by disruption of cells, it appears clear that less virus is produced by the cells after serial transfer than by the primary cells.

These results indicate that rhesus monkey kidney cells infected with SV5 and producing viral antigens are capable of undergoing many divisions.

Although SV5 infection of BHK21-F cells results in massive cell fusion and cell death, a few scattered cells survive, and such cells have been harvested and passed as described above. These cells grew to confluent monolayers much more slowly than did uninfected cells, and in each passage there was extensive giant cell formation, which increased with the time that the cultures were held. The degree of fusion was not as extensive as in the originally infected BHK21-F cells. As in the case of monkey kidney cells, studies by immunofluorescence showed that $> 99\%$ of the cells contained viral antigen. Infected BHK21-F cells were still multiplying after 28 passages. The yield of virus decreased after the first passage, but then appeared to remain relatively stable at a low level. The titers in the medium harvested at the time the cells became con-

fluent in the first, second, and 27th passages were 1.0×10^7, 5.0×10^5, and 4.0×10^5 PFU/ml respectively.

These results indicate that, although most of the BHK21-F cells are destroyed after SV5 infection, a few infected cells can survive and multiply. Because cell fusion was less extensive in persistently infected BHK21-F cells than in monolayers first exposed to the virus, it is possible that cells less susceptible to fusion have been selected. A line of BHK21-F cells resistant to fusion has not been obtained, however, since there was still extensive giant cell formation even after 28 passages.

Effect of SV5 Infection on Cellular DNA, RNA, and Protein Synthesis.—The ability of infected monkey kidney cells to survive, produce virus for many days, and to divide suggests strongly that SV5 infection does not rapidly shut off cellular RNA, protein, or DNA synthesis. In BHK21-F cells undergoing massive fusion followed by disintegration, eventual cessation of macromolecular synthesis would be expected, but it is also possible that inhibition might occur early in these cells. Rapid inhibition of cellular macromolecular synthesis has been described in several animal virus-cell systems, e.g. NDV in HeLa cells (35), mengovirus in L cells (36), and poliovirus in HeLa or HEL cells (11, 37, 38). To investigate directly the effect of SV5 infection on cellular biosynthesis, the incorporation of thymidine-H^3, uridine-H^3, and leucine-H^3 into DNA, RNA, and protein in SV5-infected monkey kidney and BHK21-F cells was studied.

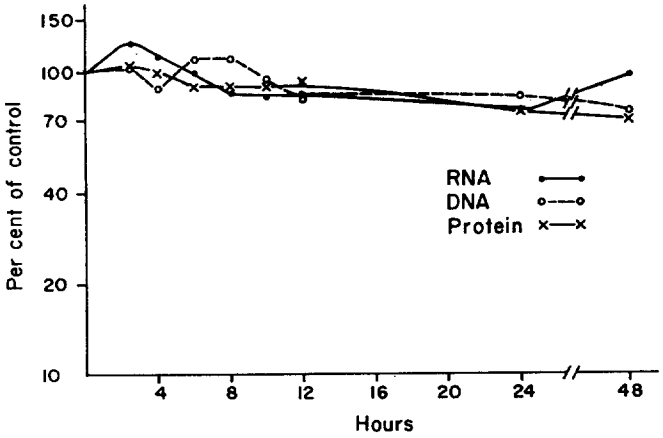
Confluent monolayers were infected at multiplicities of 15 to 40 PFU/cell. At intervals, a 30 min pulse of labeled precursor was given to infected cells and uninfected controls and incorporation into the appropriate macromolecules was determined. For experiments in monkey kidney cells, isotopes were used at a concentration of 2 to 10 μc/ml, and in BHK21-F cells, 2 to 4 μc/ml. At each time point the values obtained in the infected cells were always related to the uninfected controls at that time point. Four to 8 experiments of each type were carried out and the mean values determined.

Text-fig. 2 shows the results of experiments with monkey kidney cells. There was little or no inhibition of cellular biosynthesis during the 48 hr period of the experiments. Text-fig. 3 shows the effect of SV5 infection on BHK21-F cells. Until 12 to 15 hr after infection cellular biosynthesis was not significantly inhibited, but after this time there was a rapid decrease. As described above, by about 12 hr after infection almost all nuclei of BHK21-F cells are found in giant cells, and by 14 to 18 hr the monolayer consists of a giant syncytium which usually degenerates by 24 hr.

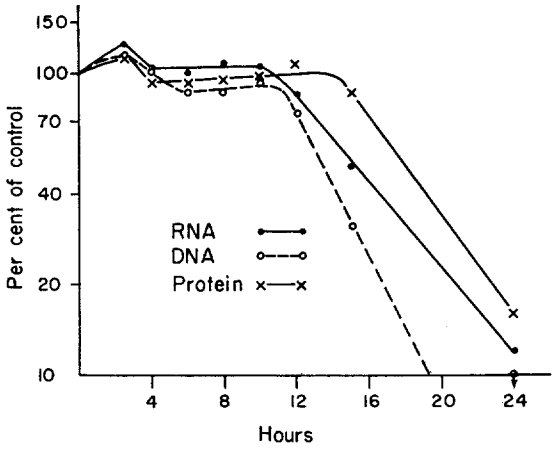
These results indicate that synthesis of cellular RNA, DNA, and protein is not turned off soon after SV5 infection, but decreases only after extensive cell fusion has occurred.

Since SV5 is an RNA virus, the possibility was considered that the amount of viral RNA and protein synthesis occurring in the infected cells might be great enough to mask a significant decrease in the cellular biosynthesis. That

this was not the case was shown in experiments done in the presence of actinomycin D. This compound, which inhibits cellular DNA-dependent RNA synthesis (39, 40), does not inhibit the replication of SV5 except as a late event



TEXT-FIG. 2. Effect of SV5 infection on RNA, DNA, and protein synthesis in primary rhesus monkey kidney cells inoculated at a virus multiplicity of 48 PFU/cell.



TEXT-FIG. 3. Effect of SV5 infection on RNA, DNA, and protein synthesis in BHK21-F cells inoculated at a virus multiplicity of 16 PFU/cell.

secondary to cell damage (41); full yields of SV5 are obtained at 10 hr after infection in the presence of 10 μg/ml of actinomycin. Thus, virus-induced RNA synthesis can be measured by determining RNA synthesis in infected cells treated with actinomycin.

Experiments were carried out as above except that actinomycin D, 5 μg/ml,

was added to the cells 2 hr before infection and was present through the experiment. The rate of viral RNA synthesis was estimated by subtraction of the amount found in uninfected cells from that in infected cells.

TABLE II

Comparison of Rates of Cellular and Virus-Induced RNA Synthesis in SV5-Infected Cells

Cells	Time	Incorporation of uridine-H^3 into RNA, CPM*				
		No actinomycin		Actinomycin, 5 μg/ml		
		Uninfected	Infected	Uninfected	Infected	SV5-induced RNA
	hr					
Monkey kidney	2.5	6,764	7,851	84	85	1
Monkey kidney	8	7,384	6,644	68	111	43
BHK21-F	2.5	12,788	15,352	63	103	40
BHK21-F	8	12,784	11,868	46	131	85

* Cells received 30-min pulse with uridine-H^3; monkey kidney cells, 10 μc/ml, and BHK21-F cells, 3 μc/ml.

TABLE III

Protein Synthesis in Uninfected and SV5-Infected Monkey Kidney and BHK21-F Cells Treated with Actinomycin D

Cells	Time	Incorporation of leucine-H^3 into protein, CPM*			
		No actinomycin		Acinomycin, 5 μg/ml	
		Uninfected	Infected	Uninfected	Infected
	hr				
Monkey kidney	8	1,783	1,594	188	182
BHK21-F	8	13,484	14,569	9,950	10,498

* Cells received 30-min pulse of leucine-H^3, 2 μc/ml.

As shown in Table II, in both monkey kidney and BHK21-F cells the level of virus-induced RNA synthesis is extremely low compared to cellular synthesis, less than 1% at 8 hr after infection, when viral RNA synthesis was maximal. It is clear that the amount of virus-induced RNA synthesis in infected cells is not sufficient to replace a significant amount of the cellular RNA synthesis.

The rate of viral protein synthesis in actinomycin-treated cells could not be determined directly because, as shown in Table III, even 8 hr after infection there is still a significant amount of cellular protein synthesis, particularly in BHK21-F cells. However, in monkey kidney cells, viral protein synthesis could

be no more than about 10% of normal cellular synthesis, even if all the counts in the infected actinomycin-treated cells are assumed to be due to viral protein synthesis. In addition, since viral RNA synthesis is <1% of cellular RNA synthesis, it appears unlikely that viral protein synthesis would be great enough to mask a significant decrease in cellular protein synthesis.

As shown in Text-figs. 2 and 3, there was a slight increase in cellular biosynthesis between about 2 and 4 hr after infection. Although this increase was small it was a consistent finding in 4 experiments. It was apparent in BHK21-F cells with regard to RNA, DNA, and protein synthesis, and in monkey kidney cells it was clearly seen in the case of RNA synthesis. Although the explanation for this initial stimulation in cellular biosynthesis is unknown, the results in Table II indicate that the early increase in RNA synthesis could not be accounted for by viral RNA synthesis.

DISCUSSION

There is a striking contrast between the low virus yield, extensive fusion, and disintegration of SV5-infected BHK21-F cells, and the high virus yield, little cell damage, and long survival of SV5-infected rhesus monkey kidney cells. The present studies suggest that the ability of the virus to alter cell membranes may explain the differences in the interactions of SV5 with the two cell types. That the membranes of the two cells respond differently to the virus is clearly demonstrated not only by syncytium formation by BHK21-F cells during virus multiplication, but also by the rapid fusion of these cells, in contrast to monkey kidney cells, after inoculation with concentrated virus. That the death of BHK21-F cells is secondary to alterations of cell membranes which result in cell fusion is suggested by the finding that cellular DNA, RNA, and protein synthesis are not inhibited until after there has been massive cell fusion. Disintegration occurs through the tearing and detachment of large areas of the syncytium from the monolayer. The ability of SV5-infected BHK21-F cells, including some polykaryocytes, to divide, and the fact that infected BHK21-F cells can be carried through many serial passages also indicate that SV5-infection per se does not rapidly shut off vital cellular biosynthesis or inevitably result in cell death. Since viral RNA synthesis in BHK21-F cells, as well as in monkey kidney cells, amounts to a small fraction of cellular RNA synthesis, cell death would not be expected to occur because of diversion of precursors to virus production or the preempting of the protein-synthesizing system of the cell. All of the available evidence is compatible with the hypothesis that whether SV5 behaves as a moderate virus or as a virulent virus causing cell death depends on the response of the cell membrane to the virus.

It is also possible that the cell membrane may play an important role in determining the large difference in the amount of infective virus produced by the two cell types. Since SV5 matures by budding from the cell surface, continued

production of virus depends on a functioning cell membrane. If the cell membrane has been sufficiently injured as a result of the virus-induced alterations which lead to cell fusion, then a lower yield of complete virus particles might be expected. Furthermore, as cells fuse, the total cell surface area decreases. The loss of some of the available surface area for budding might also decrease the virus yield. On the other hand, virus production can continue at high levels for days in monkey kidney cells which show little morphological change. Further support for the suggestion that lower yields of virus are determined at least in part by the condition of the cell membrane is provided by the following findings: (*a*) there is an inverse relationship between the degree of cell fusion and the yield of infective virus; (*b*) the kinetics of virus multiplication are similar during the early stages of growth in monkey kidney and BHK21-F cells; and (*c*) in spite of the low yield of virus, the cytoplasm of BHK21-F cells appears to contain much viral antigen. In addition, it has recently been demonstrated by electron microscopy (18) that large aggregates of the helical internal component of SV5 accumulate in the cytoplasm of infected BHK21-F cells. This might be explained if the altered membrane of BHK21-F cells were unable to incorporate the internal component into SV5 virions. In contrast, such aggregates were rare in monkey kidney cells in which there appears to be a balance between synthesis of internal component and its extrusion from the cell in mature virus particles. In the light of these findings, it is interesting to consider whether in other myxovirus-cell systems in which the yield of infective virus is low but the cells appear to contain much antigen, e.g. Newcastle disease virus in HeLa cells (42), the low yield of virus could also be due to inability of the cell membrane to participate efficiently in the process of virus elaboration.

Many viruses induce giant cell formation, including the members of the parainfluenza group of myxoviruses, herpes viruses, and pox viruses (43–45). Some of the most rapid examples of cell fusion have been illustrated in cells grown in suspension cultures (46, 47). The effect of concentrated SV5 on monolayers of BHK21-F cells represents an example of extremely rapid and extensive cell fusion. Similar results have been obtained when BHK21-F cells were inoculated with Visna virus (48) which causes a demyelinating disease of sheep. The observation that SV5 fuses BHK21-F cells but not primary cultures of rhesus monkey kidney cells agrees with previous reports that giant cell formation induced by Newcastle disease, measles, and parainfluenza types 1 and 3 viruses is generally more extensive in continuous cell lines than in primary cell cultures from a variety of species (24, 26, 49, 50). The reason why the cell membranes of continuous cell lines are more likely to be altered by the virus in such a manner that cell fusion results, and whether this property is in any way related to the properties of these cells which enable them to be grown in continuous culture remain to be elucidated.

The present time-lapse photomicrography studies have demonstrated clearly

that the giant cells induced by SV5 form by cell fusion. Amitotic nuclear division has not been observed. Time-lapse studies of cells infected with parainfluenza virus 3 (19, 20), measles virus (21), and herpes B virus (51, 52), and autoradiographic studies of measles-infected amnion cells (53) have indicated that giant cells are formed by fusion. Herpes simplex virus also induces cell fusion, but the course of giant cell formation may differ from that found with SV5. It has been reported (43, 54) that cell fusion induced by herpes virus involves one infected and one uninfected cell, or cells infected at different times. The present studies have demonstrated that fusion can occur between single cells infected with SV5 approximately simultaneously under conditions in which all cells produce virus antigens. It is also possible for a cell of normal morphology to fuse with a polykaryocyte, and for two polykaryocytes to fuse together. Thus contact between uninfected and infected cell surfaces is not necessary for SV5-induced cell fusion.

The fate of the polykaryocyte is of considerable interest. It is clear from the present studies that some giant cells do divide. Since fusion proceeds rapidly to the formation of a huge syncytium which usually tears and degenerates by 24 to 30 hr, the question of the frequency of division or the long term survival of polykaryocytes containing a moderate number of nuclei cannot be evaluated. Perhaps under conditions in which fusion was limited, e.g., by isolating the cells, some giant cells might survive and give rise to viable progeny. It has been found that hybrids of two types of cells fused by ultraviolet-irradiated parainfluenza virus type 1 may survive for as long as 15 days (55).

In giant cells produced by measles virus (26, 56), nuclear abnormalities have been described including aggregation of chromatin, disappearance of nuclear membranes, bridges between nuclei, aberrant cleavage of nuclei, and, late in infection, a fused mass of nuclei. The present finding of very large nuclei in polykaryocytes induced by SV5 suggests some form of nuclear fusion. The time-lapse studies demonstrated that formation of large nuclei from the material of several smaller nuclei apparently occurs during cell division. Indeed, this may be the only mechanism of formation of large nuclei, since fusion of interphase nuclei has not been observed. It would be of great interest to learn whether giant nuclei might survive for a time under more favorable conditions, for instance, if they were not rapidly incorporated into an enormous syncytium which disintegrates.

A striking feature of the syncytia formed by BHK21-F cells is the arrangement of nuclei into lines. Although the explanation for this is not clear, it would seem likely that the syncytial cytoplasm is predisposed to this arrangement. It is of interest that linear arrangement of nuclei in giant cells is more common with the elongated, fibroblastic BHK21-F cells than with the epithelioid HKCC cells. In BHK21-F cells the first small polykaryocytes are elongated, spindle-shaped cells in which the nuclei are arranged in short lines. As cell fusion progresses the syncytial cells become flattened at the periphery with a central clump of nuclei. One possible factor in the arrangement of the nuclei is that tension on the plasma membrane might exert a force on an entering

nucleus directing it toward the center of the syncytium to join the other nuclei, much as balls under a sheet collect in the center when tension is applied at the edges. This type of force could give rise to rounded clusters of nuclei or to lines of nuclei. If tension were unequal in different directions then the linear arrangement of nuclei could be accentuated.

The fact that the members of the parainfluenza-mumps-Newcastle disease group of myxoviruses cause giant cell formation both during the infectious cycle and rapidly after inoculation of concentrated active or u.v. inactivated virus, has led to the conclusion that a component of the virus particle acts on the cell membrane, causing cell fusion (50, 57–59). The possibility has also been considered that this cell-fusing activity might be related to the ability of these viruses to cause hemolysis (59, 60) although some evidence has been presented to suggest that these activities may not be identical (24). Both the hemolytic and cell-fusing activities are lost after treatments which disrupt the integrity of phospholipids in the viral envelope, but the two activities may be inactivated differentially by other agents such as heat or trypsin (24, 47). The hemolytic activity of parainfluenza 1 virus has been suggested to be due to a lysophosphatide (61), and more recently to an esterase (62). The mechanisms of the cell-fusing and hemolytic reactions are not completely clear at this time, and a precise explanation will require isolation and characterization of the active component(s) of the virus.

SUMMARY

The simian myxovirus SV5 multiplies in a continuous line of baby hamster kidney (BHK21-F) cells causing extensive cell fusion, followed by cell death. After inoculation of 15 PFU/cell, the latent period was 7 hr, the doubling time approximately 60 min, and the yield 7 PFU per cell. Giant cell formation began about 6 hr after infection and rapidly progressed to the formation by 14 to 18 hr of a single syncytium which disintegrated by 24 to 36 hr. In contrast, SV5 multiplies in primary rhesus monkey kidney cells for long periods of time producing high yields of virus with little cytopathic effect.

High multiplicities of SV5 induced cell fusion in BHK21-F cells within 1 hr in the absence of virus multiplication but had no visible effect on monkey kidney cells.

Time-lapse photomicrography has demonstrated that giant cells form by fusion of infected cells, and that some polykaryocytes divide. During aberrant division of polykaryocytes giant nuclei are formed from the nuclear material of several parent nuclei.

The cytoplasmic development of viral antigens as demonstrated by immunofluorescence is similar in BHK21-F and monkey kidney cells. Synthesis of cellular DNA, RNA, and protein in monkey kidney cells is not shut off by SV5-infection, and in BHK21-F cells synthesis of these macromolecules is not in-

hibited until after extensive cell fusion has occurred 12 to 15 hr after infection. Persistently infected BHK21-F and monkey kidney cells have been serially carried through 11 and 28 cell passages, respectively.

The results suggest that whether SV5 acts as a moderate virus, as in monkey kidney cells, or a virulent virus, as in BHK21-F cells, depends on the response of the cell membrane to the virus.

The authors wish to thank Miss Cathleen O'Connell for excellent technical assistance.

BIBLIOGRAPHY

1. Hull, R. N., Minner, J. R., and Smith, J. W., New viral agents recovered from tissue cultures of monkey kidney cells. I. Origin and properties of cytopathogenic agents S.V.$_1$, S.V.$_2$, S.V.$_4$, S.V.$_5$, S.V.$_6$, S.V.$_{11}$, S.V.$_{12}$, and S.V.$_{15}$, *Am J. Hyg.*, 1956, **63,** 204.
2. Chanock, R. M., Johnson, K. M., Cook, M. K., Wong, D. C., and Vargosko, A., The hemadsorption technique, with special reference to the problem of naturally occurring simian para-influenza virus, *Am. Rev. Respirat. Dis.*, 1961, **83,** (2), 125.
3. Hsiung, G. D., Isacson, P., and McCollum, R. W., Studies of a myxovirus isolated from human blood. I. Isolation and properties, *J. Immunol.*, 1962, **88,** 284.
4. Choppin, P. W., Multiplication of a myxovirus (SV5) with minimal cytopathic effects and without interference, *Virology*, 1964, **23,** 224.
5. Choppin, P. W., and Stoeckenius, W., The morphology of SV5 virus, *Virology*, 1964, **23,** 195.
6. Waterson, A. P., Two kinds of myxovirus, *Nature*, 1962, **193,** 1163.
7. Schultz, E. W., and Habel, K., SA virus—a new member of the myxovirus group, *J. Immunol.*, 1959, **82,** 274.
8. Krim, M., Wong, S. C., and Kilbourne, E. D., Isolation of a virus from a clone of a human cell line in continuous cultivation, *Fed. Proc.*, 1961, **20,** 442.
9. Chang, P. W., and Hsiung, G. D., Experimental infection of parainfluenza virus type 5 in mice, hamsters and monkeys, *J. Immunol.*, 1965, **95,** 591.
10. Dulbecco, R., Characteristics of virus-cell complexes, *Am. J. Med.*, 1965, **38,** 669.
11. Bablanian, R., Eggers, H. J., and Tamm, I., Studies on the mechanism of poliovirus-induced cell damage. I. The relation between poliovirus-induced metabolic and morphological alterations in cultured cells, *Virology*, 1965, **26,** 100.
12. Dulbecco, R., and Vogt, M., Plaque formation and isolation of pure lines with poliomyelitis viruses, *J. Exp. Med.*, 1954, **99,** 167.
13. Choppin, P. W., and Philipson, L., The inactivation of enterovirus infectivity by the sulfhydryl reagent p-chloromercuribenzoate, *J. Exp. Med.*, 1961, **113,** 713.
14. Macpherson, I., and Stoker, M., Polyoma transformation of hamster cell clones—an investigation of genetic factors affecting cell competence, *Virology*, 1962, **16,** 147.
15. Reissig, M., Howes, D. W., and Melnick, J. L., Sequence of morphological changes in epithelial cell cultures infected with poliovirus, *J. Exp. Med.*, 1956, **104,** 289.
16. Sykes, J. A., and Moore, E. B., A simple tissue culture chamber, *Texas Rept. Biol. and Med.*, 1960, **18,** 288.

17. Eagle, H., Amino acid metabolism in mammalian cell cultures, *Science*, 1959, **130**, 432.
18. Compans, R. W., Holmes, K. V., Dales, S., and Choppin, P. W., An electron microscopic study of moderate and virulent virus-cell interactions of the parainfluenza virus SV5, *Virology*, in press.
19. Marston, R. Q., Cytopathogenic effects of hemadsorption virus type 1, *Proc. Soc. Exp. Biol. and Med.*, 1958, **98**, 853.
20. Lépine, P., Chany, C., Droz, B., and Robbe-Fossat, F., Cytopathogenic effect of two newly recognized myxovirus strains: mechanism of syncytial formation, *Ann. New York Acad. Sc.*, 1959, **81**, 62.
21. Aoyama, Y., Changes of cultured cells infected with measles virus, *Japan. J. Exp. Med.*, 1959, **29**, 535.
22. Okada, Y., The fusion of Ehrlich's tumor cells caused by HVJ virus *in vitro*, *Biken's J.*, 1958, **1**, 103.
23. Okada, Y., Yamada, K., and Tadokoro, J., Effect of antiserum on the cell fusion reaction caused by HVJ, *Virology*, 1964, **22**, 397.
24. Kohn, A., Polykaryocytosis induced by Newcastle disease virus in monolayers of animal cells, *Virology*, 1965, **26**, 228.
25. Toyoshima, K., Hata, S., and Miki, T., Virological studies on measles virus. IV. The effect of active and inactivated measles virus on cultured cells, *Biken's J.*, 1960, **3**, 281.
26. Cascardo, M. R., and Karzon, D. T., Measles virus giant cell inducing factor (fusion factor), *Virology*, 1965, **26**, 311.
27. Nii, S., and Kamahora, J., Cytopathic changes induced by herpes simplex virus, *Biken's J.*, 1961, **4**, 255.
28. Kaku, H., and Kamahora, J., A study on the giant cell formation of L cells infected with UV inactivated vaccinia virus, *Biken J.*, 1964, **7**, 37.
29. Wheelock, E. F., and Tamm, I., Mitosis and division in HeLa cells infected with influenza or Newcastle disease virus, *Virology*, 1959, **8**, 532.
30. Reda, I. M., Rott, R., and Schäfer, W., Fluorescent antibody studies with NDV-infected cell systems, *Virology*, 1964, **22**, 422.
31. Rustigian, R., A carrier state in HeLa cells with measles virus (Edmonston strain) apparently associated with noninfectious virus. A preliminary report, *Virology*, 1962, **16**, 101.
32. Walker, D. L., and Hinze, H. C., A carrier state of mumps virus in human conjunctiva cells. I. General characteristics, *J. Exp. Med.*, 1962, **116**, 739.
33. Fernandes, M. V., Wiktor, T. J., and Koprowski, H., Mechanism of the cytopathic effect of rabies virus in tissue culture, *Virology*, 1963, **21**, 128.
34. Ishida, N., Homma, M., Osato, T., Hinuma, Y., and Miyamoto, T., Persistent infection in HeLa cells with hemadsorption virus type 2, *Virology*, 1964, **24**, 670.
35. Wheelock, E. F., and Tamm, I., Biochemical basis for alterations in structure and function of HeLa cells infected with Newcastle disease virus, *J. Exp. Med.*, 1961, **114**, 617.
36. Franklin, R. M., and Baltimore, D., Patterns of macromolecular synthesis in normal and virus-infected mammalian cells, *Cold Spring Harbor Symp. Quant. Biol.*, 1962, **27**, 175.

37. Darnell, J. E., Jr., Early events in poliovirus infection, *Cold Spring Harbor Symp. Quant. Biol.*, 1962, **27,** 149.
38. Holland, J. J., Depression of host-controlled RNA synthesis in human cells during poliovirus infection, *Proc. Nat. Acad. Sc. U.S.*, 1963, **49,** 23.
39. Reich, E., Franklin, R. M., Shatkin, A. J., and Tatum, E. L., Effect of actinomycin D on cellular nucleic acid synthesis and virus production, *Science*, 1961, **134,** 556.
40. Goldberg, I. H., and Rabinowitz, M., Actinomycin D inhibition of deoxyribonucleic acid-dependent synthesis of ribonucleic acid, *Science*, 1962, **136,** 315.
41. Choppin, P. W., Effect of actinomycin D and halogenated deoxyuridines on the replication of simian virus 5, *Proc. Soc. Exp. Biol. and Med.*, 1965, **120,** 699.
42. Wheelock, E. F., and Tamm, I., Effect of multiplicity of infection on Newcastle disease virus-HeLa cell interaction, *J. Exp. Med.*, 1961, **113,** 317.
43. Roizman, B., Polykaryocytosis, *Cold Spring Harbor Symp. Quant. Biol.*, 1962, **27,** 327.
44. Roizman, B., Polykaryocytosis induced by viruses, *Proc. Nat. Acad. Sc.*, 1962, **48,** 228.
45. Warren, J., Jensen, K., and Mason, R., The syncytial viruses, *Ann. New York Acad. Sc.*, 1962, **101,** 520.
46. Okada, Y., Analysis of giant polynuclear cell formation caused by HVJ virus from Ehrlich's ascites tumor cells. I. Microscopic observation of giant polynuclear cell formation, *Exp. Cell. Research*, 1962, **26,** 98.
47. Okada, Y., and Tadokoro, J., Analysis of giant polynuclear cell formation caused by HVJ virus from Ehrlich's ascites tumor cells. II. Quantitative analysis of giant polynuclear cell formation, *Exp. Cell Research*, 1962, **26,** 108.
48. Harter, D. H., and Choppin, P. W., Cell-fusing activity of a virus which causes a demyelinating disease, *J. Clin. Inv.*, 1966, **45,** 1020.
49. Love, R., and Suskind, R. G., Cytopathology of parainfluenza, type 3, virus infection in HeLa and monkey kidney cells *in vitro*, *Exp. Cell Research*, 1961, **24,** 521.
50. Okada, Y., and Tadokoro, J., The distribution of cell fusion capacity among several cell strains or cells caused by HVJ, *Exp. Cell Research*, 1963, **32,** 417.
51. Falke, D., and Richter, I. E., Mikrokinematographische Studien über die Entstehung von Riesenzellen durch Herpes-B-Virus in Zellkulturen. I. Mitteilung: Vorgänge an den Zellgrenzen und Granulabewegungen, *Arch. Ges. Virusforsch.*, 1961, **11,** 73.
52. Falke, D., and Richter, I. E., Mikrokinematographische Studien über die Entstehung von Riesenzellen durch Herpes-B-Virus in Zellkulturen. II. Mitteilung: Morphologisches Verhalten und Bewegungen der Kerne, *Arch. Ges. Virusforsch.*, 1961, **11,** 86.
53. Atherton, J. G., Chaparas, S. D., Cremer, M., and Gordon, I., Mechanism of polykaryocytosis associated with noncytopathic infection by measles virus, *J. Bact.*, 1965, **90,** 213.
54. Bungay, C., and Watkins, J. F., Observations on polykaryocytosis in HeLa cells infected with herpes simplex virus, *Brit. J. Exp. Path.*, 1964, **45,** 48.

55. Harris, H., Behaviour of differentiated nuclei in heterokaryons of animal cells from different species, *Nature*, 1965, **206,** 583.
56. Roizman, B., and Schluederberg, A. E., Virus infection of cells in mitosis. III. Cytology of mitotic and amitotic HEp-2 cells infected with measles virus, *J. Nat. Cancer Inst.*, 1962, **28,** 35.
57. Okada, Y., Suzuki, T., and Hosaka, Y., Interaction between influenza virus and Ehrlich's tumor cells. III. Fusion phenomenon of Ehrlich's tumor cells by the action of HVJ Z strain, *Med. J. Osaka Univ.*, 1957, **7,** 709.
58. Zhdanov, V. M., and Bukrinskaya, A. G., Studies on the initial stage of virus-cell interaction, *Acta Virol. (Prague)*, 1962, **6,** 105.
59. Ho Yun-de, Investigations into syncytium formation in cultures of stable cell lines infected with parainfluenza 1 virus. II. Morphology of syncytium formation in tissue cultures and a hypothesis concerning its mechanism, *Acta Virol. (Prague)*, 1962, **6,** 202.
60. Henle, G., Deinhardt, F., and Girardi, A., Cytolytic effects of mumps virus in tissue cultures of epithelial cells, *Proc. Soc. Exp. Biol. and Med.*, 1954, **87,** 386.
61. Rebel, G., Fontanges, R., and Colobert, L., Nature lipidique des substances responsables de l'activité hémolytique de *Myxovirus parainfluenzae* 1 (virus Sendai), *Ann. Inst. Pasteur*, 1962, **102,** 137.
62. Neurath, A. R., A component splitting diisopropylfluorophosphate in Sendai and Newcastle disease virus preparations; its possible identity with haemolysin, *Acta Virol. (Prague)*, 1965, **9,** 25.

EXPLANATION OF PLATES

PLATE 33

FIGS. 1 *a* to 1 *f*. Cytopathic effects in BHK21-F cells infected with SV5. Cells were inoculated at a multiplicity of 16 PFU/cell and at intervals were fixed and stained with hemotoxylin and eosin. × 184.

FIG. 1 *a*. Uninfected cells are spindle shaped and overlap in some areas.

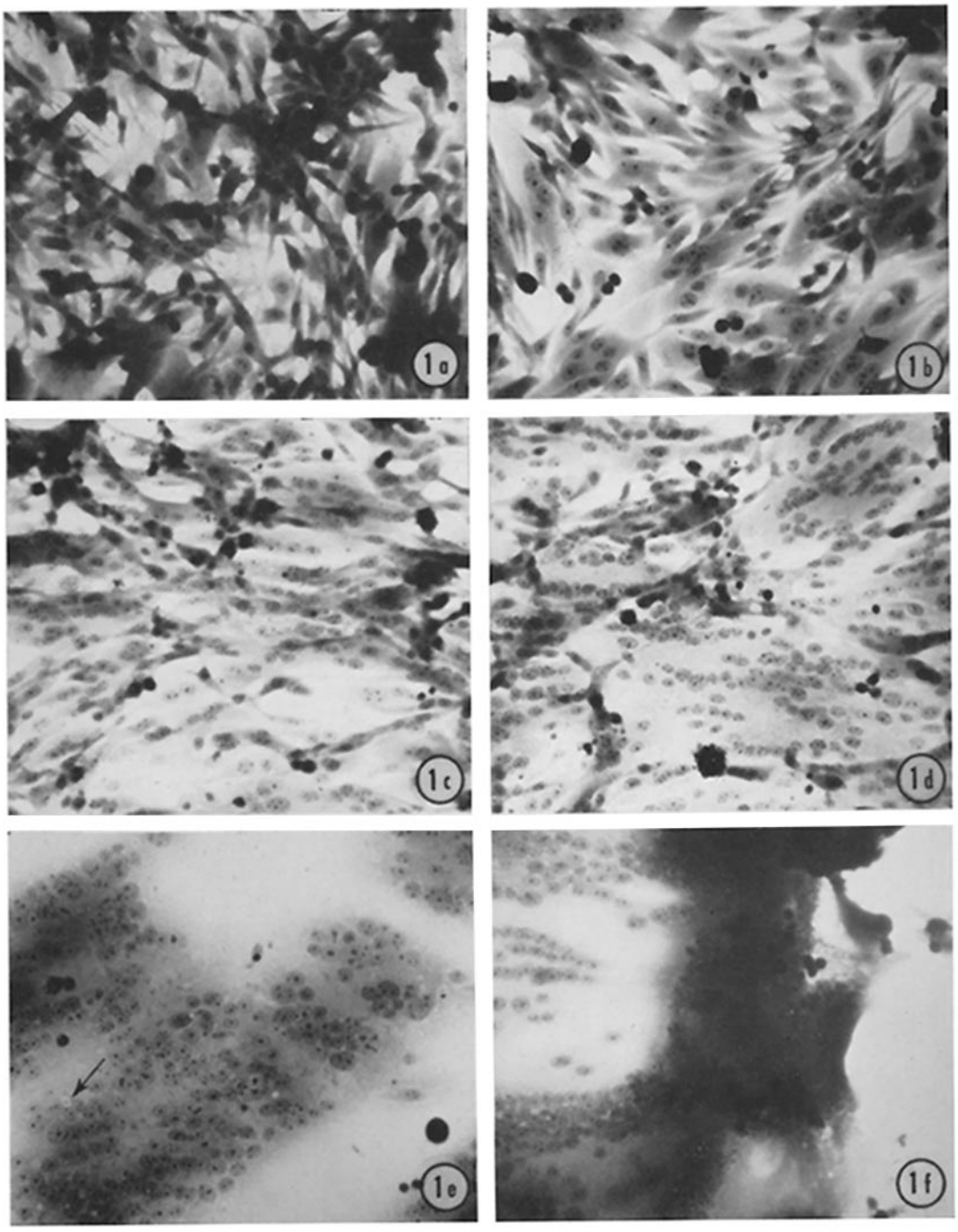
FIG. 1 *b*. 7.5 hr after infection. Small polykaryocytes are scattered in the monolayer.

FIG. 1 *c*. 10 hr after infection. Polykaryocytes have increased in size.

FIG. 1 *d*. 12 hr after infection. Very large polykaryocytes contain nuclei arranged in lines.

FIG. 1 *e*. 18 hr after infection. All nuclei appear to be within a single large syncytium. Eosinophilic cytoplasmic inclusions are present (arrow).

FIG. 1 *f*. 24 hr after infection. The syncytium has detached from the cover slip on the right and is peeling off.



(Holmes and Choppin: Effects of SV5 in two cell types)

PLATE 34

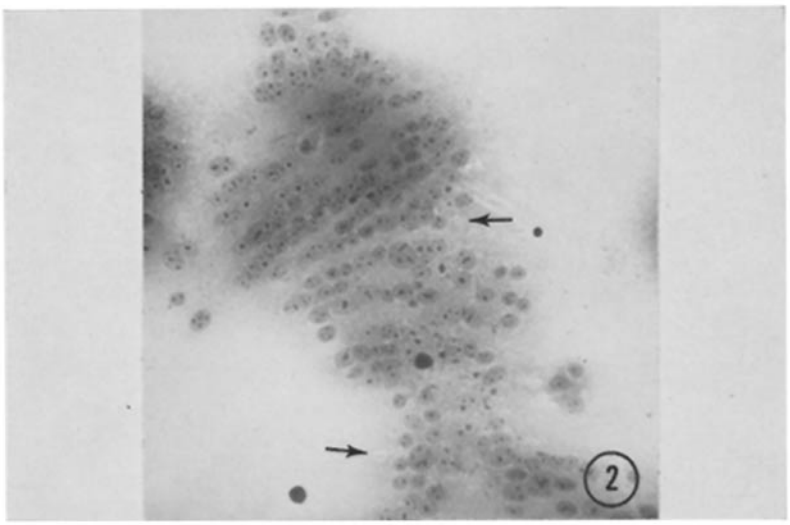
FIG. 2. Syncytium formed from BHK21-F cells 18 hr after inoculation with SV5. Nuclei are arranged in long parallel lines. Cytoplasmic inclusions surrounded by white halos are indicated by arrows. × 184.

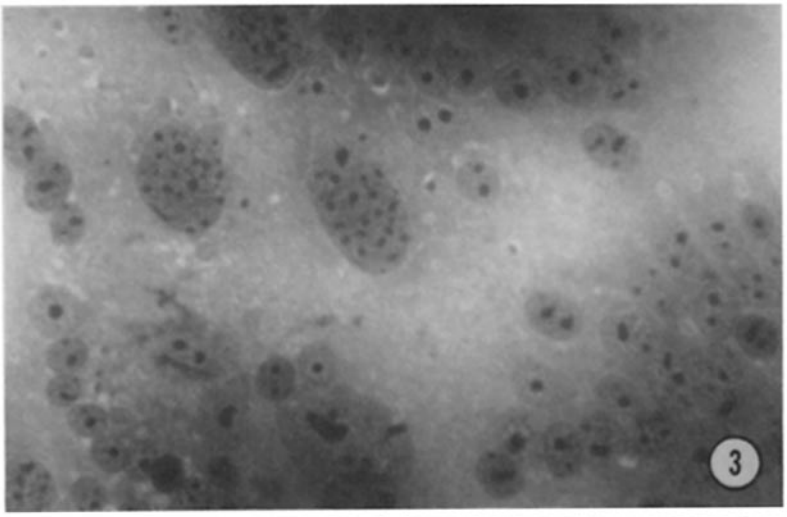
FIG. 3. Large nuclei containing many nucleoli in BHK21-F syncytium 18 hours after infection. A number of nuclei of normal size are also present. × 460.

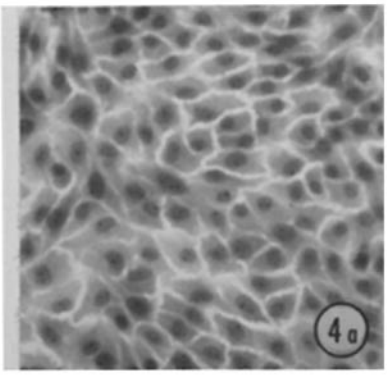
FIGS. 4 *a* and 4 *b*. Primary rhesus monkey kidney cells stained with hematoxylin and eosin. × 184.

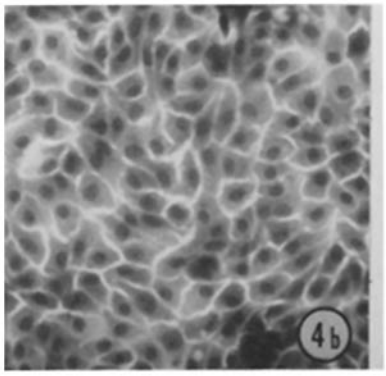
FIG. 4 *a*. Uninfected cells.

FIG. 4 *b*. Cells 2 days after infection with SV5, showing little cytopathic effect. The inoculum contained 48 PFU/cell.





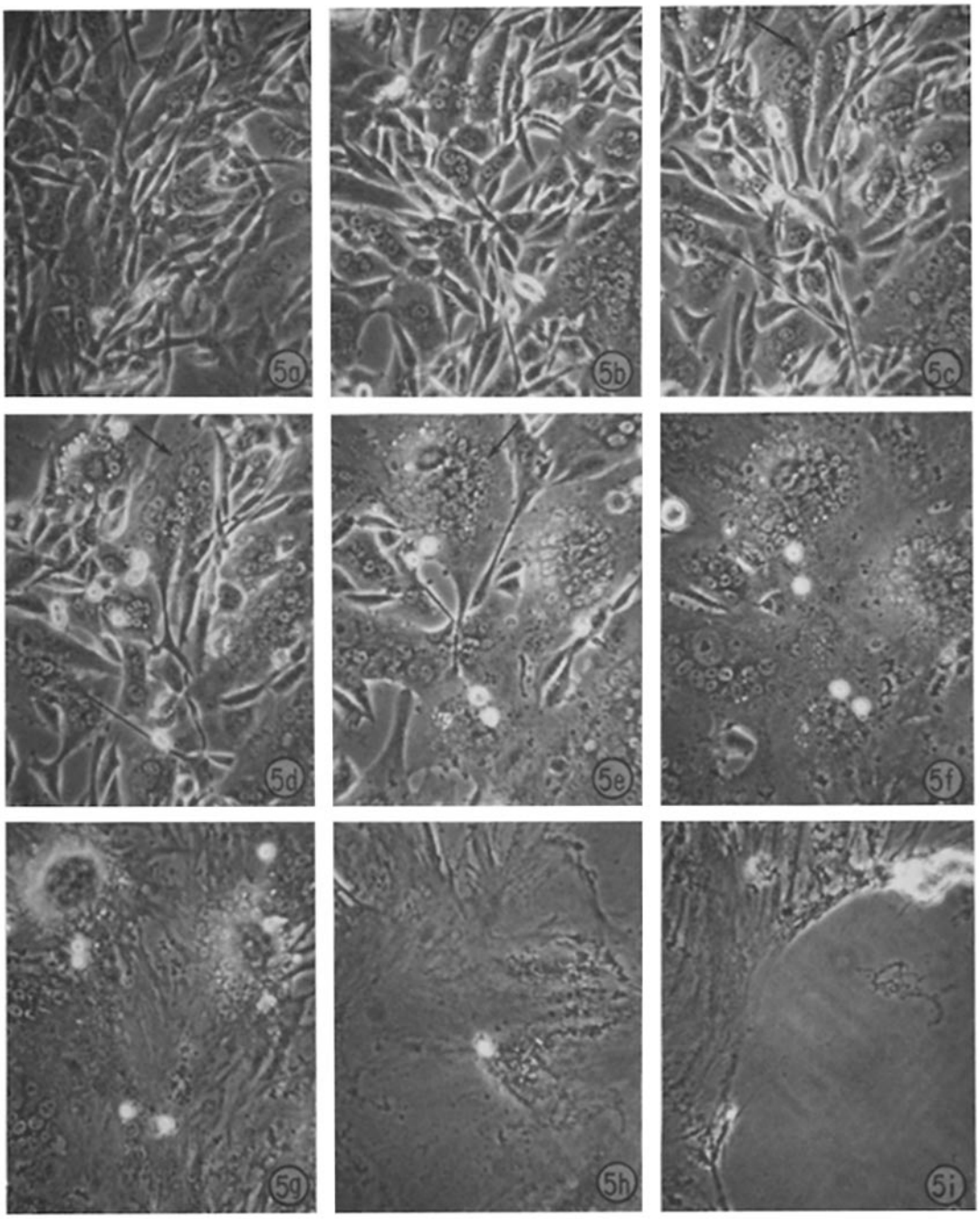




(Holmes and Choppin: Effects of SV5 in two cell types)

PLATE 35

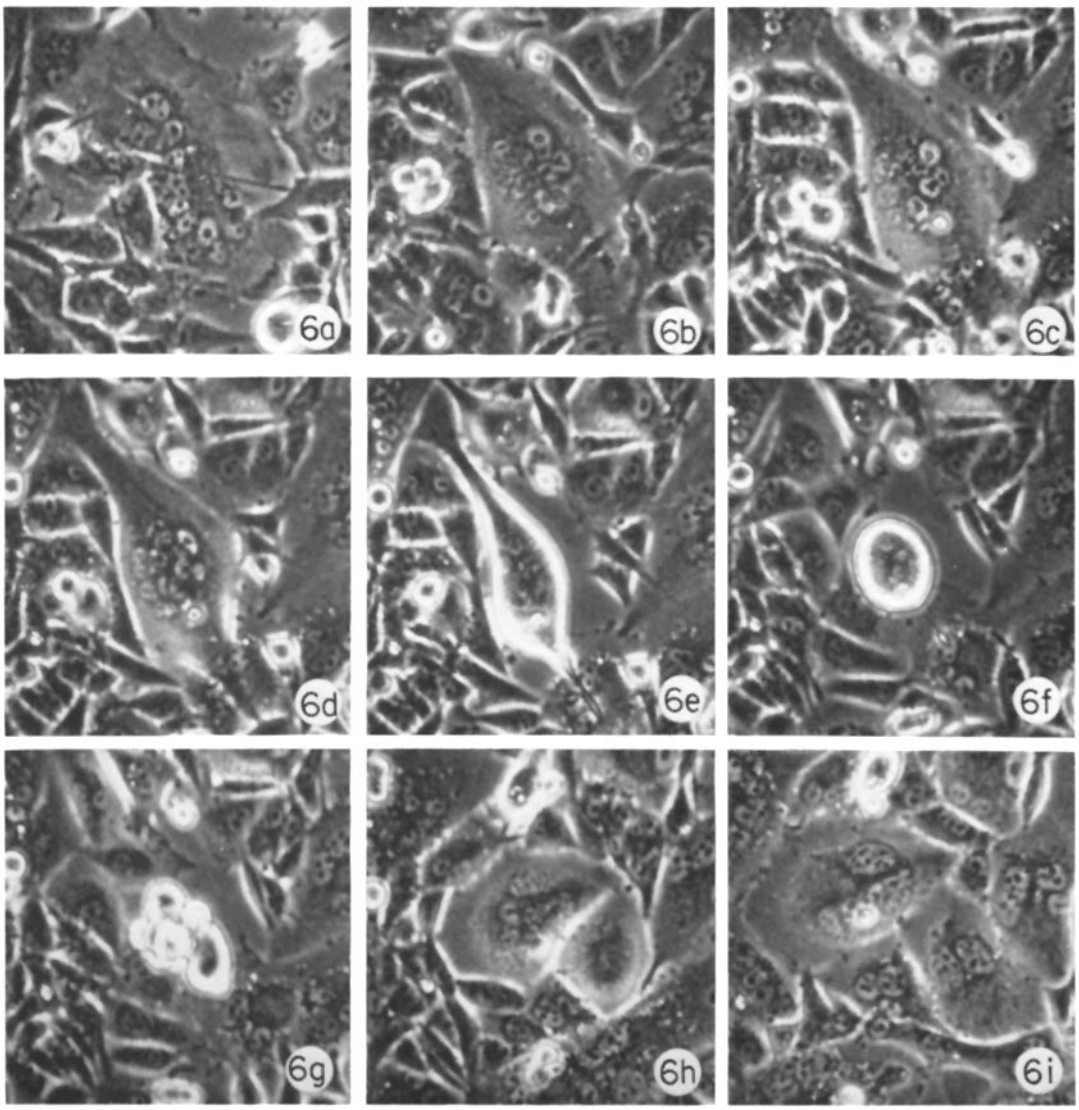
FIGS. 5 *a* to 5 *i*. Sequential time-lapse, phase-contrast photomicrographs of SV5-infected BHK21-F cells. Small polykaryocytes are seen in Fig. 5 *a* which subsequently increase in size. In Figs. 5 *c*, 5 *d*, and 5 *e* two giant cells are shown in the process of fusing (arrows). In Fig. 5 *d* cells have fused at the top (arrow); a boundary is still visible at the bottom. Figs. 5 *e*, 5 *f*, and 5 *g* show continuing cell fusion to form a single syncytium. Two clumps of nuclei within this syncytium become rounded up (Fig. 5 *g*) and migrate out of the field (Fig. 5 *h*). At this stage very active waves ripple through the cytoplasm which then rips and peels from the substrate (Fig. 5 *i*). × 208.



(Holmes and Choppin: Effects of SV5 in two cell types)

PLATE 36

FIGS 6 *a* to 6 *i*. Sequential time-lapse, phase-contrast photomicrographs of SV5-infected BHK21-F cells. A polykaryocyte containing about 16 nuclei of average size (Fig. 6 *a*) rounds up (Figs 6 *b* to 6 *f*), constricts in several planes (Fig. 6 *g*), and then divides (Fig. 6 *h*) into two cells which flatten out, and together contain fewer but larger nuclei than the original giant cell. The lower daughter cell contains two large nuclei, and the upper cell appears to contain one small and two large nuclei (Fig. 6 *i*). × 277.



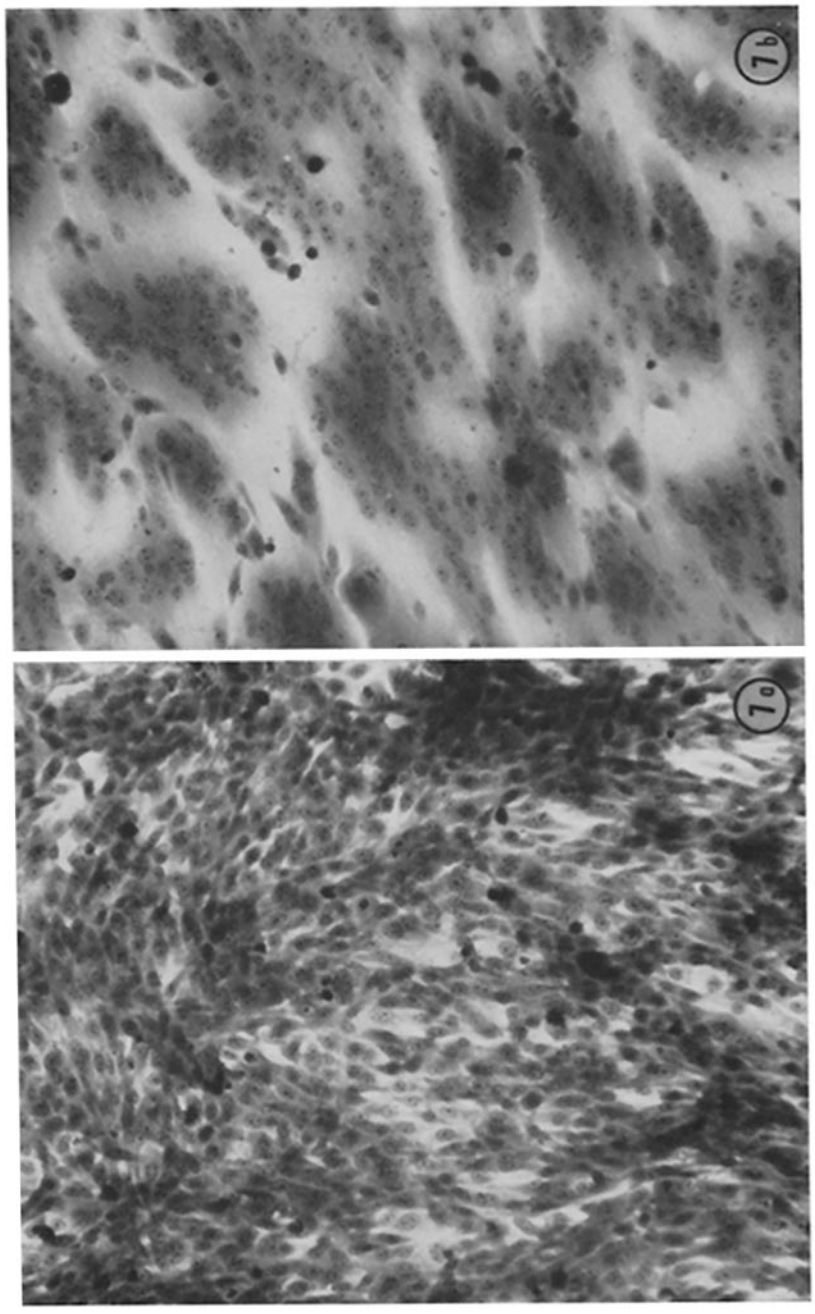
(Holmes and Choppin: Effects of SV5 in two cell types)

PLATE 37

FIGS. 7 *a* and 7 *b*. BHK21-F cell monolayers. × 184.

FIG. 7 *a*. Control.

FIG. 7 *b*. 1 hr after the addition of concentrated SV5 at a multiplicity of 2000 PFU/cell, extensive cell fusion has occurred.



(Holmes and Choppin: Effects of SV5 in two cell types)

PLATE 38

FIGS. 8 *a* and 8 *b*. Rhesus monkey kidney cells inoculated with SV5 virus at a multiplicity of 48 PFU/cell and stained for viral antigen by the indirect immunofluorescence technique. × 320.

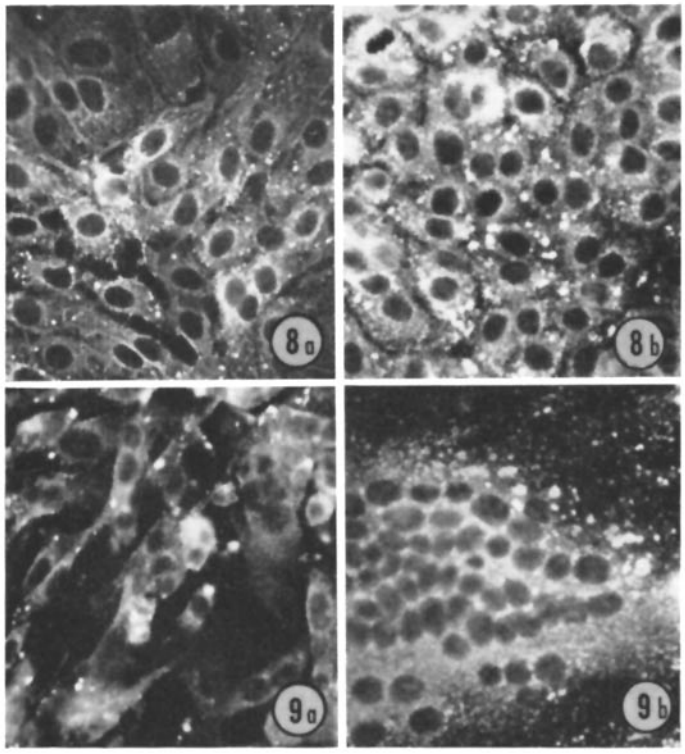
FIG. 8 *a*. 8 hr after infection, all cells contain viral antigen in the cytoplasm, particularly in the perinuclear area. Both diffuse fluorescence and coarse granular fluorescent foci are present.

FIG. 8 *b*. 24 hr after infection, the cells appear to contain more and larger foci of antigen. There is no nuclear fluorescence. A cell in mitosis containing viral antigen is shown in the upper left corner.

FIGS. 9 *a* and 9 *b*. BHK21-F cells inoculated with SV5 at a multiplicity of 16 PFU/cell and stained for viral antigens by the indirect immunofluorescence technique. × 320.

FIG. 9 *a*. 8 hr after infection, all cells contain viral antigen. In the cytoplasm are both diffuse fluorescence and granular foci of fluorescent material. Cell fusion has already begun and small polykaryocytes are visible.

FIG. 9 *b*. 24 hr after infection, the entire field is occupied by one giant cell. Both small and large fluorescent foci are concentrated near the nuclei in the cytoplasm.



(Holmes and Choppin: Effects of SV5 in two cell types)