viral CF antigens are relatively stable at 56°C/30 min and are completely sedimented with the virus particles, while the INCA and tumor antigens are completely destroyed at 56°C/30 min and remain in the virus-free, centrifuged supernatant fluids. The demonstration that the CF antibodies in the sera of tumor-bearing hamsters can be completely removed by absorption with SV40 virus INCA is the basis for the conclusion that the genetic information for the synthesis of the tumor CF antigens is derived entirely either from provirus or from another incomplete form of the SV40 viral genome continuously transmitted in the tumor cells.

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*SYNTHESIS OF SV40 TUMOR ANTIGEN DURING REPLICATION OF SIMIAN PAPOVAVIRUS (SV40)**

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Hamsters bearing tumors induced either by simian papovavirus 40 (SV40) or by cells tranformed *in vitro* by the virus develop antibodies capable of detecting a new complement-fixing antigen in the transformed cells.[1] Antibodies also develop for a new intranuclear antigen present in all cells transformed by SV40 and detected by the immunofluorescence technique.[2, 3] It appears that the new antigens detected by complement-fixation and by immunofluorescence are similar or identical immunologically.[4] The new tumor antigen is presumably under control of at least a portion of the virus genome, but some of the cell lines producing the antigen do not go on to synthesize infectious SV40.[5] The present experiments, and those reported in the accompanying paper,[6] were designed to determine whether cells sus-

ceptible to SV40 produced the tumor antigens in the course of the replication of the virus.

Materials and Methods.—The cells used in this study were primary green monkey kidney cells (GMK), a stable line of cells derived from the same species (BSC1),[7] hamster embryo fibroblasts (HEF), and embryonic human lung cells (HEL). The cells were grown in 16-oz bottles for complement-fixation tests and on 15-mm round cover glasses in 60-mm plastic Petri dishes for the immunofluorescence tests. Nutrient fluids consisted of Eagle's basal medium, concentrations of calf serum varying from 2 to 10 per cent, 100 units of penicillin, and 100 μg of streptomycin per ml. The Petri dishes were incubated under 5 per cent CO_2; all cultures were kept at 37°C. Following inoculation with approximately 10 plaque-forming units of SV40 per cell, the cultures were reincubated at 37°C and harvested at varying intervals.

For complement-fixation tests, the cells in 16-oz bottles were harvested by scraping with a rubber policeman, the cells disrupted by rapid freezing and thawing or by treatment in a sonic oscillator, the suspensions clarified by centrifugation at 2500 rpm for 10 min, and the supernates used as antigen. Uninfected cells were treated in the same manner to serve as antigen controls. All preparations were tested for anticomplementary activity and both known positive and negative sera were included in all tests. The semimicro complement-fixation tests were carried out in plastic dishes.

Cells growing on cover glasses were harvested and fixed for 3 min in acetone as previously described.[3] The cells were exposed to (*a*) serum from hamsters with or without SV40-induced but virus-free tumors, and antihamster globulin labeled with fluorescein isothiocyanate, or (*b*) labeled anti-SV40 monkey serum previously shown to be highly specific for the detection of SV40 viral antigens.[8] All tests included positive and negative controls for the detection of both SV40 virus antigens and SV40 tumor antigens.

Results.—A large number of experiments were carried out to demonstrate the possible presence of the complement-fixing SV40 tumor antigen in GMK and BSC1 cells following inoculation of the cultures with SV40. Representative experiments, summarized in Table 1, reveal the presence of this antigen in both types of cells. Tumor antigen was detected 12 hr following inoculation of BSC1 cultures at a time when SV40 viral antigens were not present in detectable quantity. The amount of tumor antigen declined with time following inoculation as seen by the decrease in complement-fixing titer of cultures 7 days after inoculation when the antigen from such cultures was reacted with the tumor hamster serum. Neither nontumor hamster serum nor normal monkey serum reacted with any of the antigens tested. The specificity of the sera used was further controlled by reacting them in the tests with SV40 viral antigens and with antigens prepared from the 2X-10 cells; the latter is a line of cells transformed by SV40 and known to contain both the new complement-fixing cellular antigen[4] as well as the new intranuclear cellular antigen.[3] The SV40 viral antigen reacted only with SV40-immune monkey serum, while the 2X-10 antigens reacted only with the tumor hamster serum.

Experiments carried out to characterize further the SV40 viral and SV40 tumor antigens have shown that the SV40 viral antigen can be sedimented by centrifugation at 30,000 rpm for 2 hr but that the tumor antigen remains in the supernatant

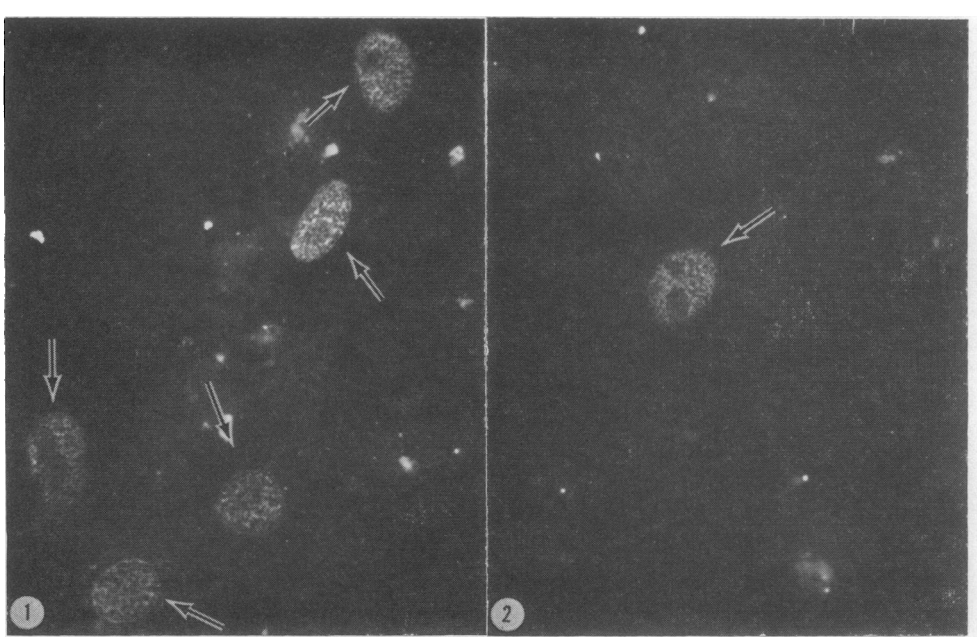
TABLE 1

DETECTION OF ANTIGENS IN MONKEY CELLS INFECTED WITH SV40 BY COMPLEMENT-FIXATION

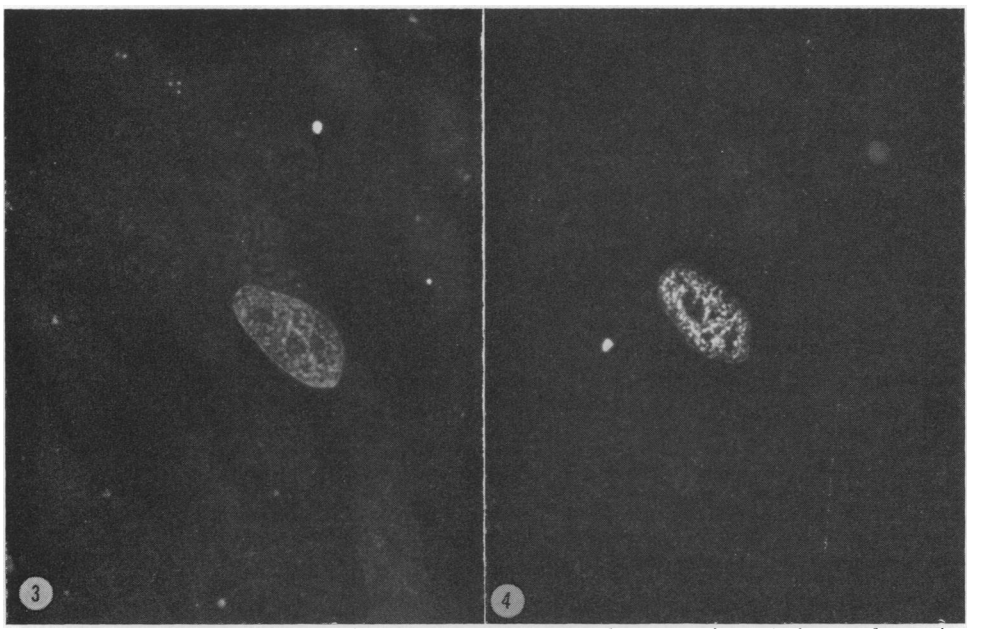
Source of antigen	Days post-inoc.	Complement-Fixation: Antigen Titers SV40-immune monkey serum*	Tumor hamster serum†	Nontumor hamster serum
Infected GMK	3	16‡	8	<2
	7	8	<2	<2
Infected BSCl	0.25	<2	<2	<2
	0.5	<2	4	<2
	1	4	8	<2
	2	32	32	<2
Infected BSCl	2	16	32	<2
	4	32	32	<2
	7	16	<2	<2
2X-10 cells§		<2	32	<2
SV40 virus		32	<2	<2

* Diluted 1:80 to contain 4 antibody units.
† Diluted 1:40 to contain 4 antibody units.
‡ Numbers represent highest dilution of antigen yielding less than 50% hemolysis.
§ A line of hamster cells transformed *in vitro* by SV40 and known to contain SV40-induced cellular antigen in every nucleus.[3]

fluid when treated in the same fashion. Heating the tumor antigen (whether from virus-infected GMK or BSCl cells, or from virus-free 2X-10 cells) for 30 min at 50°C completely destroys its complement-fixing ability, but this amount of heating does not alter the capability of the SV40 viral antigen to fix complement in the presence of specific antibody. Thus the present findings confirm those recently obtained with the SV40 viral and tumor antigens obtained from transformed cells.[1] They also demonstrate that the new antigen which appears early in the infected cell has the same properties as the antigen in the tumor cells.



FIGS. 1 and 2.—Photomicrographs of immunofluorescent green monkey kidney cultures infected 24 hr earlier with SV40. (1) Reacted with anti-SV40 tumor antigen reagents. Arrows point to cells synthesizing intranuclear tumor antigen. ×425. (2) Reacted with anti-SV40 monkey serum. Arrowpoints to cell synthesizing SV40 viral antigen. ×425.



FIGS. 3 and 4.—Photomicrographs of immunofluorescent human embryonic lung cultures infected 72 hr earlier with SV40. (3) Reacted with anti-SV40 tumor antigen reagents. Note single cell with intranuclear tumor antigen. ×425. (4) Reacted with anti-SV40 monkey serum. Single cell synthesizing SV40 viral antigen in the nucleus. ×425.

Immunofluorescent detection of both SV40 tumor and SV40 viral antigens in GMK cells revealed early synthesis of the tumor antigen. Twenty-four hours following the inoculation of the cultures, approximately 50 per cent of the cells were synthesizing the intranuclear tumor antigen (Fig. 1), but only 1 per cent of the cells contained SV40 viral antigen (Fig. 2). With the progression of time, more cells contained viral antigen but then the tumor antigen could no longer be detected in many of the cells. The human HEL cultures synthesized both antigens (Figs. 3 and 4) but only a small proportion of the cells in the culture were able to do so, and it could not be determined whether the same cells were synthesizing both antigens. A rare cell in the hamster HEF cultures synthesized the tumor antigen but viral antigens could not be detected in any of these cells.

Discussion.—The data now accumulating in our laboratory as well as elsewhere[2, 6, 9] that monkey cells synthesize the SV40 tumor antigen during early stages of infection strongly support the hypothesis that information for the synthesis of this antigen in cells transformed by SV40 is directed by the viral genome. Failure to isolate infectious SV40 from transformed cultures[5] may be due to integration of only a portion of the viral genome with that of the cell genome. Such integration is implied by the finding that information for the synthesis of the antigen is transmitted to all daughter cells during mitosis.[3] While the tumor antigen does not appear to be incorporated into the virus capsid, it is unknown at the present time whether it is always extraviral, as it is in the transformed cells, or whether it is part of the internal structure of the virus particle.

Hamsters bearing SV40-induced, virus-free tumors simultaneously develop complement-fixing and immunofluorescent antibodies, suggesting that both antibodies

are formed in response to the same or closely related antigens.[4] The high correlation found in the present study between the complement-fixation and immunofluorescence results in finding SV40 tumor antigen early in virus-infected cells again points to the probability that the two methods are detecting the same or similar antigens.

Summary.—Monkey cells infected with papovavirus SV40 regularly synthesize SV40 tumor antigen during early stages of the replication of the virus. This antigen is synthesized in the nucleus and can be detected by both immunofluorescence and complement-fixation. The antigen also develops in an occasional embryonic human lung and embryonic hamster fibroblast following inoculation of the cultures with SV40. The tumor antigens in infected monkey cells and in human and hamster cells transformed by SV40 are immunologically similar.

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*ELECTRICAL STIMULATION OF THE INTERNODES OF SINGLE FIBERS OF DESHEATHED NERVES**

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This communication completes the presentation of the essential features of the results obtained in experiments on electrical stimulation of single undissected fibers by means of tripolar electrodes.

Technique.—To the description made[1] only a few details need be added.

In order to remove the external connective tissue sheath a circular cut is made in the sheath at the bifurcation of the peroneal trunk, and by holding its margin with fine watchmaker forceps the sheath is pulled off one of the peroneal branches. After this has been done, all the nerve fibers are cut except one large myelinated fiber. In other experiments (Fig. 1, IV, V) immediately after the sheath had been pulled off, it was pulled back on the peroneal branch. Since retraction of its elastic fibers causes a shortening of the sheath, after its replacement the sheath did not cover the entire branch, but it covered considerably more than the segment in which determinations of threshold were made.

The stimulation was effected with tripolar electrodes, one 20-μ-wide cathode at 300 μ from the margins of the two diffuse anodes. The theoretical distribution of the catelectrotonus is given by the curves in Figure 2*a* of reference 1. In actual experiment, however, since the cathode was