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# *SOURCE OF GENETIC INFORMATION FOR SPECIFIC COMPLEMENT-FIXING ANTIGENS IN SV40 VIRUS-INDUCED TUMORS**

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Continuous transmission of noninfectious viral genome in almost all or all tumor cells,[1] without production of detectable quantities of infectious viral DNA,[2, 3] has been demonstrated with SV40 hamster tumors (Eddy strain), which spontaneously[2] or by special methods of induction[1–3] were capable of yielding minute amounts of infectious virus. However, with two other lines of SV40 tumors (the Cincinnati and Melnick H-50 strains) we have been unable with the same methods to induce the production of any detectable amounts of infectious virus, which raised the question whether viral genome necessarily persisted in all virus-induced tumors. The presence of specific complement-fixing (CF) antigens in SV40 hamster tumors[2, 4, 5] (in the Cincinnati and Melnick strains as well as in the Eddy strain) could be due either to a specific, viral-induced mutation, to continuously transmitted viral genome, or both. Huebner *et al.*,[4, 5] who found that their monkey antiviral serum reacted "specifically" with the tumor antigen, interpreted their data as indicating "a direct relationship between the SV40-induced hamster tumor antigen and SV40 viral antigen."[4] Our data,[2] however, indicated that the reaction of monkey antiviral serum with tumor antigen was not specific. Moreover, while Huebner *et al.*[4] at first reported that "serums [from tumor-bearing hamsters] did not react with tissue culture-grown SV40 viral antigen" and more recently[6] that such sera reacted "seldom or not at all with 'viral' antigens," we[2] found that such sera contained CF antibodies for SV40 virus-infected normal cell antigens (INCA). The purpose of this communication is to report data showing that the specific CF antigens present in continuously transplanted SV40 tumor cells are indistinguishable from antigens formed in normal cells during the early stages of infection with SV40 virus, and that these antigens are different from the CF antigens of the virus particles.

*Materials and Methods.—Antigens and CF procedure:* Tumor CF antigens were prepared by homogenizing tumors transplanted in newborn hamsters, and by freezing and thawing (4×) washed, trypsinized tumor cells or tumor cells grown in tissue culture. Antigens from uninfected or SV40 virus-infected BS-C-1 cells[7] were prepared either by freezing and thawing the combined cells and culture fluids or by preparing 10% suspensions of the cells in Eagle's basal medium (BME) in Earle's solution (0.1% $NaHCO_3$) without phenol red. The CF tests were carried out with 0.2-ml quantities of serum, antigen, and complement (1.7–2 exact units) incubated overnight at +4°C, followed by the addition of 0.4 ml of sensitized sheep erythrocytes.

*Absorption of antitumor hamster serum with various antigens:* The BS-C-1 cells, uninfected and infected, used for absorption, were harvested from 32-oz bottles and stored at −60°C as measured quantities of cell pack without the supernatant fluid and without preliminary freezing and thawing. The tumor cells were similarly prepared by trypsinization of tumors or of tumor cells propagated *in vitro* in 32-oz bottles. For absorption 0.2-ml or 0.4-ml quantities of packed cells were suspended in 2 ml of hamster antitumor serum diluted 1:8 in Ca-Mg saline. The suspensions were frozen and thawed 3× using a $CO_2$-alcohol bath (to disrupt the cells) and incubated for 1 hr in a water bath at 37°C. The cell debris was then removed by centrifugation at 1,500 rpm for 10 min and the supernatant liquid was added to additional 0.2-ml or 0.4-ml quantities of packed cells. The process of freezing and thawing was then repeated 3× and the suspensions were incubated overnight at +4°C. Supernatant fluids obtained by centrifugation at 1,500 rpm for 10 min were then centrifuged in stainless steel cups at 37,000 rpm for 2 hr—to remove anticomplementary antigen-antibody complexes. The supernatant fluids, centrifuged again at 2,500 rpm for 10 min when particles were grossly visible, were used for determining the residual CF antibody. When it was necessary to carry out four absorptions, the serum absorbed twice as described above was again absorbed with 0.4-ml quantities of either uninfected or infected BS-C-1-packed cells, incubating the mixtures each time at 37°C for 1 hr and overnight at +4°C. Before use in the CF test, the dose of complement to be used was in each case determined in the presence of the centrifuged supernatant fluids.

*Results.—Absence of specific antitumor CF antibody in sera containing antiviral antibodies:* The results shown in Table 1 indicated (*a*) that postinfection Cercopithecus monkey sera that yielded nonspecific reactions with SV40 tumor homogenate contained no antitumor CF antibody when tissue culture-propagated SV40

TABLE 1

ABSENCE OF SPECIFIC ANTITUMOR CF ANTIBODY IN POSTINFECTION MONKEY SERA POSSESSING CF ANTIBODIES FOR SV40 VIRAL ANTIGEN

| Monkey tested | CF Antibody Titer of Serum with Indicated Antigen: SV40 virus* 2 units | SV40 tumor homogenate "Eddy" strain 4 units | SV40 tumor tissue culture "Melnick" strain 4 units |
|---|---|---|---|
| Cercopithecus 191 | 32 | 16 | <8 |
| " 2310 | 64 | 32 | <8 |
| " 2311 | 128 | 16 | <8 |
| " 2315 | 64 | <8 | |
| " 2486 | 256 + ? | 8 | |
| " 426—pre | <8 | 16 + ? | |
| " " —post | 8 | <8 | |
| " 429—pre | <8 | <8 | |
| " " —post | 32 | 8 | |
| " 436—pre | <8 | 64 + ? | |
| " " —post | <8 | 64 | |
| Rhesus S-67—pre | <8 | 8 | |
| " " —post | 8 | 8 | |
| " S-116—pre | <8 | 8 | |
| " " —post | 16 | 8 | |

* This viral antigen did not react with anti-SV40 tumor hamster sera which had specific CF antibody for SV40 hamster tumor antigens.

*Note:* Cercopithecus sera 191, 2310, 2311, 2315, and 2486 were derived from monkeys experimentally infected with SV40 virus and were kindly supplied by Dr. J. L. Melnick, Baylor University School of Medicine, Houston, Texas. The pre- and postinfection Cercopithecus sera 426, 429, and 436 were kindly supplied by Dr. H. Malherbe, South African Institute for Medical Research, Johannesburg, Republic of South Africa. The preinfection sera of these 3 monkeys contained no neutralizing antibodies for SV40 virus, while the postinfection sera all did; the sera on 426 and 429 were obtained 23 days after infection and on 436 at 38 days. The Rhesus sera were kindly supplied by Dr. J. A. Morris of the National Institutes of Health, Bethesda, Md. The "pre" sera, obtained soon after capture, had no neutralizing antibodies for SV40 virus; infection was naturally acquired by exposure to other monkeys and the "post" sera contained neutralizing antibodies.

tumor cells (Melnick strain) were used, (*b*) that some monkey sera (nos. 2315 and 2486) with high titers of antiviral CF antibody yielded negative or insignificantly low titers of the nonspecific tissue antibody, and (*c*) that monkeys reacting nonspeci-

fically with the Eddy strain tumor homogenate had similar CF titers before and after infection with SV40 virus. From this it is possible to conclude that CF antibodies for the components of the SV40 virus particle do not react with the SV40 tumor antigens.

*Absence of neutralizing antibody in sera of hamsters with transplanted SV40 tumors:* Tests with the very sensitive plaque reduction technique (using undiluted serum versus 10–30 plaque-forming units) on sera of 25 hamsters failed to reveal even minute amounts of neutralizing antibody (Table 2). These results, indicating a lack of relationship between the SV40 tumor antigens and the antigens responsible for the neutralizing antibodies, are different from those obtained with the sera of hamsters carrying transplanted "virus-free" adenovirus tumors, 55 per cent of which were reported to contain neutralizing antibodies.[8]

TABLE 2

ABSENCE OF NEUTRALIZING ANTIBODIES IN SERA FROM SV40 TUMOR-BEARING HAMSTERS IN RELATION TO RECOVERY OF MINUTE AMOUNTS OF VIRUS FROM TUMORS, DURATION OF TUMOR GROWTH, AND PRESENCE OF CF ANTIBODY FOR TUMOR ANTIGENS

| Group | No. of hamsters tested | Time after transplantation blood obtained (days) | Neutralizing antibody in undiluted serum | CF antibody for tumor antigens | Range of CF antibody titers |
|---|---|---|---|---|---|
| Tumors yielded virus | 15 | 37–76 | 0/15 | 8/8 | 16–512 |
| Tumors did not yield virus | 4 | 23–77 | 0/4 | 3/3 | 64–128 |
| Tumors not tested for virus | 6 | 53–64 | 0/6 | 2/2 | 8; 512 |
| Total | 25 | 23–77 | 0/25 | 13/13 | 8–512 |

*Evidence that not all SV40 virus INCA containing viral CF antigens possess antigens reacting with sera of tumor-bearing hamsters:* The first additional antigens we prepared in an attempt to find an explanation for the negative results originally reported by Huebner *et al.*[4] also yielded negative results (see Table 3). The antigens for these tests were prepared from BS-C-1 cells that were infected with low multiplicities of SV40 virus and were harvested at a time when the cytopathic effects were not uniform in all of the tissue culture tubes. The experiment described and summarized in Table 4 showed (*a*) that when the cytopathic effect of the SV40 virus was allowed to go to completion it was possible to obtain potent viral CF antigen which did not react with potent antitumor hamster serum, and (*b*) that antigen reactive with antitumor hamster serum could be obtained from normal cells infected with SV40 virus by harvesting the cells prior to complete destruction by the virus. This experiment also showed that the CF antigens of the SV40 virus particle

TABLE 3

EVIDENCE THAT SV40 TUMOR-BEARING HAMSTERS HAVE CF ANTIBODY FOR ANTIGENS CONTAINED IN SOME SV40 VIRUS-INFECTED CULTURES OF NORMAL CELLS (BS-C-1) BUT NOT IN OTHERS

| Serum tested | CF Antibody Titer of Serum with Preparation A 1 unit | CF Antibody Titer of Serum with Preparation B 2 units |
|---|---|---|
| Cercopithecus postinfection | 64 | 128 |
| Hamster 28–5 | 32 | <8 |
| " 31–4 | 32 | <8 |
| " 374 | 64 | <8 |
| " 38–1 | 32 | <8 |
| " 38–2 | 128 | <8 |

Simultaneous tests with antigens prepared from uninfected BS-C-1 cells always yieldednegative results.

were different from the tumor CF antigens, and suggested that the components in normal infected cells that are similar to the tumor antigens may appear early during the course of infection and then disappear.

TABLE 4

RELATIONSHIP BETWEEN EXTENT OF CYTOPATHIC EFFECT IN BS-C-1 CELLS INFECTED WITH LOW MULTIPLICITY OF SV40 VIRUS AND PRESENCE OF CF ANTIGENS REACTING WITH ANTITUMOR HAMSTER SERUM AND ANTIVIRAL MONKEY SERUM

| | CF Reaction of BS-C-1 Antigen with Antitumor Hamster Serum | | CF Reaction of BS-C-1 Antigen with Antiviral Monkey Serum | |
|---|---|---|---|---|
| Extent of cytopathic effect 14 days after inoculation | Titer of serum | Units of antigen per 0.2 ml of undiluted culture | Titer of serum | Units of antigen per 0.2 ml of undiluted culture |
| Swelling of nuclei and extensive vacuolization; cell sheets intact | 64 | 1 | 128 | 2 |
| Complete or almost complete destruction of cell sheets | <8 | 0 | 128 | 4 |

18-day-old cultures containing at least $10^6$ BS-C-1 cells per tube were infected with $10^{5.5}$ $TCD_{50}$ of SV40 virus in 2 ml of medium (i.e., 1 $TCD_{50}$ or less per 3 cells). After 5 hr in roller drum the inoculum was removed, the cell sheet in each tube was washed with 5 ml of medium, and 2 ml of fresh medium was added. There was no cytopathic effect at 7 days when the medium was changed; at 14 days the cultures that showed complete destruction of the cell sheet were separated from those that still had intact cell sheets. The culture tubes were frozen and thawed, and the antigen was the supernatant fluid after centrifugation at 1500 rpm for 10 min.

*"Tumorlike" and viral CF antigens at different times after infection of normal cells with SV40 virus:* In order to synchronize as much as possible the events occurring in the infected cells, the tests were carried out with antigens derived from cells infected with a high multiplicity of virus, i.e., about 10 $TCD_{50}$ of virus per BS-C-1 cell. The data shown in Table 5 indicate that the "tumorlike" and viral CF antigens first appeared in serologically detectable quantities between 8 and 16 hr after infection. During the first 24–48 hr, when the cells developed nuclear swelling, both types of antigens increased in amount but the "tumorlike" antigens were present in greater concentration than the viral antigens. At 72 hr both types of antigens were present in equal concentration, while at 96 hr there was a two- to fourfold drop in concentration of the "tumorlike" antigens and a comparable increase in concentration of the viral antigens.

TABLE 5

DEVELOPMENT OF CF ANTIGENS REACTING WITH ANTITUMOR HAMSTER SERUM AND WITH ANTIVIRAL MONKEY SERUM IN NORMAL CELLS (BS-C-1) INFECTED WITH SV40 VIRUS

| Time after infection cells harvested (hr) | Changes in cells at time of harvest | No. of CF Antigen Units per 0.2 ml 10% Suspension Frozen and Thawed 4 × and Tested with: Antitumor serum 16 units | Antiviral serum 16 units |
|---|---|---|---|
| Uninfected | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 |
| 16 | 0 | 8 | 4 |
| 24 | 0 | 64 | 32 |
| 48 | Nuclear swelling | 128; 256* | 128; 128 |
| 72 | Small foci of vacuolization | 128; 128 | 128; 128 |
| 96 | Vacuolization of about 90% of cells | 32; 64 | 512; 256 |

* Duplicate figures represent results obtained in different experiments.
*Note:* The "antitumor" hamster serum did not react with the viral antigens (see Table 4), and the antiviral monkey serum did not react with the tumor antigens (see Table 1).

*Properties of SV40 virus INCA compared with tumor and viral antigens:* The data shown in Table 6 indicate (*a*) that while the viral antigens are relatively stable on heating at 56°C for 30 min (about 50% of the activity was lost), the INCA and the tumor antigens lost all of their reactivity; (*b*) that while all of these antigens

TABLE 6

EFFECT OF CENTRIFUGATION AND OF HEAT ON CF ANTIGENS IN SV40 VIRUS-INFECTED NORMAL CELLS AND IN SV40 TUMORS

| Treatment of antigen | Fraction tested | No. of CF Units per 0.2 ml of Indicated Antigen Tested with Indicated Serum: Infected normal cells—10% suspension frozen and thawed 4 × — Antiviral serum 16 units | Infected normal cells—10% suspension frozen and thawed 4 × — Antitumor serum 16 units | Tumor homogenate—20%; supernatant fluid after 500 rpm/10 min — Antitumor serum 16 units |
|---|---|---|---|---|
| None | Whole suspension | 256; 128 | 64 | 32 |
| 2500 rpm[a]—10 min | Supernatant fluid | 64 | 32 | 16 |
| 2500 rpm supernatant fluid 37,000 rpm[b]—2 hr | Supernatant fluid[c] | <4[d] | 32 | 4 |
| | Sediment[e] | 64 | 16 | 16 |
| Original heated 56° C for 30 min | Whole suspension | 64 | 0 | 0[f] |

[a] International centrifuge—model U. [b] Spinco centrifuge, no. 40 rotor. [c] Top third of 9–10 ml. [d] This 2,500 rpm supernatant fluid was diluted 1:4 in Ca-Mg saline before centrifugation at 37,000 rpm. [e] Sediment reconstituted to original volume in BME in Earle's solution. [f] The homogenate used for this test was centrifuged 2500 rpm for 10 min prior to heating.

remain attached to large cell particulates (about 50% being lost by centrifugation at 2,500 rpm for 10 min), the viral antigens were completely removed with the virus particles by centrifugation at 37,000 rpm for 2 hr, while the antigens reacting with antitumor serum remained in varying concentration in the supernatant liquid; and (*c*) that, within the experimental errors of the technique for measuring these CF antigens, most of the INCA and only about 25 per cent of the antigens in the tumor homogenate, contained in the 2,500 rpm centrifuged supernatant fluids, remained in the supernatant liquid after centrifugation at 37,000 rpm for 2 hr.

*Do tumor cells contain CF antigens that are not present in infected normal cells?:* In order to answer this question, it was necessary to determine whether all of the CF antibody present in the sera of tumor-bearing hamsters could be absorbed with the antigens present in SV40 virus-infected normal cells. The results, summarized in Table 7, indicate (*a*) that absorption with uninfected BS-C-1 cells or with polyoma tumor cells had no effect on the titer of CF antibody for either INCA or tumor antigens; (*b*) that two absorptions with SV40 tumor cells completely removed the

TABLE 7

ABSORPTION OF TUMOR-SPECIFIC CF ANTIBODIES FROM HAMSTER SERUM WITH ANTIGENS IN SV40 VIRUS-INFECTED NORMAL BS-C-1 CELLS AND IN SV40 TUMOR CELLS

| Material used | Absorption of Serum: No. of absorptions | Total no. of units of antigen used per 0.2 ml of 1:8 dilution of serum | CF Titer* of Antitumor Serum Tested with: Infected normal BS-C-1 cells — 8 units | Infected normal BS-C-1 cells — 256 units | Tumor homogenate — 4 units | Tumor homogenate — 16 units |
|---|---|---|---|---|---|---|
| None | 0 | 0 | 128; 256 | | 256 | |
| BS-C-1 uninfected | 4 | 0 | 128 | 128 | 256 | 256 |
| BS-C-1 infected—3-day harvest | 2 | 256 | <8 | 16 | 32 | |
| BS-C-1 infected—3-day harvest | 4 | 1109 | <8 | <8 | 16 | |
| BS-C-1 infected—3-day harvest and 2-day harvest | 2<br>2 | 1963 | <8 | <8 | <8 | |
| SV40 tumor cells (Melnick)—passage 2 in culture | 2 | 128 | 8 | | <8 | 8 |
| Polyoma tumor cells | 2 | None | 128 | | 256 | |

* The CF serum titers in this table are based on complete fixation of the complement used.

antibody for the major component of the tumor antigens (as represented by 4 units of antigen) but still yielded a titer of 8 in the presence of a 4× greater concentration of tumor antigen (i.e., a minor component) and a similar titer with 8 units of INCA; (*c*) that while two absorptions with 3-day INCA removed all the antibody for the major component of INCA (i.e., when 8 units of INCA were used for the test), the absorbed serum still yielded a titer of 32 with the major component of the tumor antigen (i.e., when only 4 units of antigen were used) and a comparable titer of 16 with a minor component of INCA (i.e., when 256 units of INCA were used); (*d*) that while four absorptions with 3-day INCA removed all the antibody for the major and minor components of INCA, the absorbed serum still had a titer of 16 for the major component of the tumor antigen; and (*e*) that 2 absorptions with 3-day INCA followed by two absorptions with 2-day INCA succeeded in removing also the residual antibody for the tumor antigen. The results of these absorption experiments suggest that both the tumor antigens and INCA are not single antigens but consist of mixtures of two or more antigens that are present in different concentrations in each, and that when adequate concentrations of INCA are used, it is possible to absorb all the antibody for the tumor antigens.

As a further test of the justifiable conclusion that the tumor cells do not contain CF antigens that are not present in infected normal cells, a total of 28 sera from hamsters bearing transplanted SV40 tumors of the Cincinnati, Melnick, and Eddy strains were tested with INCA and tumor antigens (see Table 8). It can be seen

TABLE 8

COMPARISON OF CF ANTIBODY TITERS IN SERA OF TUMOR-BEARING HAMSTERS FOR ANTIGENS IN SV40 HAMSTER TUMORS AND IN SV40 VIRUS-INFECTED NORMAL BS-C-1 CELLS

| SV40 Tumor Transplanted | | | | CF Titer of Serum with | |
|---|---|---|---|---|---|
| Strain | Passage no. | Hamster no. | Days after transplantation | Tumor homogenate 4 units | Infected BS-C-1 cells 8 units |
| Cincinnati | 1 | 4 | 79 | 128 | 64 |
| | | 7 | 95 | 32 | 16 |
| | | 9 | 87 | 16 | 16 |
| | 2 | 1 | 167 | 512 | 512 |
| | | 5 | 83 | 16 | 16 |
| | 2a | 1 | 59 | 128 | 256 |
| | 3 | 2 | 90 | 128 | 256 |
| | | 3 | 54 | <8 | <8 |
| Melnick | 1 | 1956 | 28 | <8 | <8 |
| | | 1954 | 40 | 128 | 256 |
| | 2 | 1961 | 59 | 64 | 64 |
| | 3 | 2 | 36 | 16 | 32 |
| | | 5 | 45 | <8 | <8 |
| Eddy* | 43 | 415 | 21 | 32 | (16)† |
| | | 425 | " | 16 | <8 |
| | | 423 | 28 | 32 | (16) |
| | | 430 | " | 16; 32 | <8 |
| | | 433 | " | 16 | <8 |
| | | 409 | 35 | 256 | 64 (16) |
| | | 417 | " | 256 | 64 (16) |
| | | 421 | " | 128 | 64 (16) |
| | | 432 | " | 256 | 64 (32) |
| | | 443 | " | 256 | 64 (16) |
| | | 419 | 42 | 256 | 64 (16) |
| | | 426 | " | 256 | 64 (32) |
| | | 436 | " | 128; 256 | 64 (64) |
| | | 437 | " | 256 | (32) |
| | | 444 | " | 256 | (128) |

* Sera obtained from all the hamsters in this group prior to transplantation of the tumors contained no CF antibody for these antigens.

† Titers in parentheses were obtained with 2 units of the indicated antigen.

that all the sera from the hamsters bearing the Cincinnati and Melnick strain tumors (obtained at long intervals after transplantation because they grow more slowly than those of the Eddy strain) yielded practically identical titers with both types of antigen. The significant differences in titer found with many of the sera from hamsters bearing SV40 tumors of the Eddy strain, particularly when only 2 units of INCA were used, may be a reflection of the difference in the amount of the different antigenic components in the tumors and the infected normal cells.

*Discussion.*—Pope and Rowe[9] and Rapp *et al.*[10] using the fluorescent antibody technique with antitumor hamster and antiviral monkey sera, recently demonstrated that *all* tumor cells contain the tumor CF antigen (significantly only within the nucleus) but not viral antigen. This is in line with our own previous demonstration that tumor cells in tissue culture exhibited no fluorescence with antiviral serum[1] and with the present work indicating that the tumor CF antigens are different from the CF antigens of the viral particle. Also in agreement with our original report,[2] Pope and Rowe[9] showed that normal cells at 38 and 53 hr after infection with SV40 virus, exhibited specific nuclear fluorescence with antitumor hamster serum, indicating that at least one component of the tumor CF antigen was synthesized in infected normal cells. Moreover, after our work was completed, we learned that Rapp *et al.*[11] had independently obtained results entirely comparable to our own, indicating that "tumorlike" CF antigen appeared very early after infection of normal cells with SV40 virus and disappeared later when the cells had been completely destroyed by the virus but still contained abundant viral antigen. The data reported in the present communication showing that the CF antibody in the serum of tumor-bearing hamsters can be completely removed by absorption with infected normal cell antigens permits the conclusion that the genetic information for the synthesis of the SV40 tumor CF antigens is derived entirely from a portion of the viral genome. Since there is no evidence for synthesis of any of the antigens comprising the virus particle in the SV40 tumors, the pattern is different from the adenovirus (type 12)-induced hamster tumors because the latter appear to contain at least one component of the virus particle (the C antigen) which, however, constitutes a very minor fraction of the tumor CF antigens.[8] Since the inoculation of large doses of SV40 virus in adult hamsters, which gives rise to the specific transplantation resistance phenomenon,[12–14] fails to stimulate CF antibodies for the tumor and infected cell antigens,[2] it can be assumed that SV40 tumor cells must contain at least another antigen that may still be either related to the virus particle or a consequence of a specific viral induced mutation.

*Summary.*—Postinfection monkey sera, containing neutralizing and complement-fixing (CF) anti-SV40 viral antibodies, failed to react with SV40 tumor CF antigens. The sera of tumor-bearing hamsters, containing CF antibodies for tumor antigens, failed to react with SV40 virus-infected BS-C-1 cultures, containing only viral antigen, and lacked even traces of viral neutralizing antibodies. However, soon after infection of normal cells with SV40 virus and during the period of maximal viral synthesis CF antigens are synthesized which are not demonstrable in the viral particles but are indistinguishable from the specific CF antigens present in continuously transplanted SV40 tumors. The "tumorlike," infected normal cell antigens (INCA) disappear as the cytopathic effect progresses to completion and the CF antigens of the viral particles reach their maximum concentration. The

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-