*SPECIFIC COMPLEMENT-FIXING TUMOR ANTIGENS IN HUMAN CELLS MORPHOLOGICALLY TRANSFORMED BY SV40 VIRUS**

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It has been reported that the complement-fixing (CF) antigen contained in SV40 virus-induced hamster tumors[1, 2] is also present in hamster, mouse, rabbit, and pig kidney cells that have undergone morphologic transformation following infection with SV40 virus.[3] The purpose of the present study was to determine (*a*) whether SV40 virus-transformed human embryonic kidney cells,[4] which no longer yielded infectious SV40 virus except for traces demonstrable by association of large numbers of viable human transformed cells with susceptible African monkey kidney cells, also contained the *specific* SV40 tumor CF antigen, and, if so, (*b*) how the concentration of the antigen in the human cells compared with that in cells grown in tissue culture from continuously transplanted SV40 hamster tumors.

Materials and Methods.—The 10 per cent suspensions of the washed, trypsinized cells derived either from tissue cultures of the transformed human embryonic kidney cells (passages 63 and 70) or from tissue cultures of transformed, hamster kidney cells were prepared in the Boston laboratory. All the other antigens and sera were prepared in the Cincinnati laboratory where the CF tests were carried out by procedures described elsewhere.[2, 5] Since one of us (A. B. S.) found that many but not all hamsters, after transplantation of the Eddy strain of SV40 tumor, develop cross-reacting CF antibodies, not only with polyoma and F. Sa. no. 3 hamster tumors,[2] but also with concentrated cell suspensions derived from tissue cultures of normal human kidney or of a variety of human tumors (due to the presence of a distinctive antigen in the tissues of about 17% of hamsters that is apparently shared with other animals), special care was taken to use hamster sera which contained only the specific, antitumor CF antibodies. Sera with specific CF antibodies for polyoma tumor antigens were obtainable only from certain adult hamsters that survived for more than a month after transplantation of the Habel strain.

Results.—The results shown in Table 1 indicate that the human embryonic kidney cells as well as the hamster kidney cells that were morphologically transformed by SV40 virus *in vitro*, contained CF antigens that reacted with anti-SV40 tumor hamster serum but not with antipolyoma tumor hamster serum. The specificity of these sera is indicated in the negative reactions with the listed control antigens. It is perhaps of interest to note that the 7 human tumors, which yielded cells that failed to react with the anti-SV40 tumor serum, included 2 neuroblastomas, 2 Wilms' tumors, 1 malignant lymphoma of the thymus, 1 carcinoma of the liver, and 1 carcinoma of the lung.

The data shown in Table 2 indicate (*a*) that the concentration of at least one component of the specific CF tumor antigens (as measured with a 1:16 dilution of the antitumor hamster serum) is the same in the transformed human cells and the hamster tumor cells grown for 16 passages in tissue culture; but (*b*) when extracts

TABLE 1

SPECIFICITY OF SV40 TUMOR CF ANTIGENS IN HUMAN EMBRYONIC KIDNEY AND HAMSTER KIDNEY CELL LINES TRANSFORMED BY SV40 VIRUS

Antigens tested*	Concentration of antigen, %	Result of CF Test with Indicated Sera — Hamster 437 — Before transplantation	Hamster 437 — After SV40 tumor†	Hamster 85-1 after polyoma tumor†
SV40 hamster tumor—trypsinized cells	5	0	+	0
Polyoma hamster tumor				
Trypsinized cells	10	0	0	+
Homogenate	5	n.t.	0	+
SV40-transformed hamster kidney				
15Y	2.5	"	+	0
6F	"	"	+	0
EG	"	"	+	0
SV40-transformed human embryonic kidney				
Passage 63	10	0	+	0
Passage 70	10	0	+	n.t.
Norman human kidney—passage 4	10	n.t.	0	"
7 Different human tumors	10	"	0	"

* Except for one of the polyoma tumor antigens (homogenate) all others consisted of washed trypsinized cells, the % concentration referring to the volume of sedimented cells in the suspending fluid.
† Both sera were used in a dilution of 1:16, which represented 16 units of CF antibody for SV40 tumor antigen (hamster serum 437) and 8 units for polyoma tumor antigen (hamster serum 85-1).
n.t. = not tested.

of about the same number of both kinds of cells were tested against different dilutions of the serum, the titer of CF antibody was only 1:32 with the human cells and 1:128 with the hamster tumor cells. The latter result may be interpreted as indicating that the 5 per cent suspension of the human transformed cells had an insufficient concentration (or none) of another component of the CF tumor antigens which gave rise to the antibody present in highest concentration in the antitumor hamster serum.

TABLE 2

COMPARATIVE CONCENTRATION OF TUMOR CF ANTIGENS*

Antigen used	No. CF units per 0.2 ml of 10% suspension tested with hamster serum 437, diluted 1:16	CF antibody titer of hamster serum 437 in presence of 5% suspension of indicated cells
Transformed human—passage 70	8	32
Hamster tumor—passage 16 in tissue culture	8	128

* In cultures of SV40 virus-transformed human embryonic kidney cells and in cultures of transplanted SV40 hamster tumors, and potency of antitumor hamster serum when tested with similar concentrations of each cell suspension.

Other work has established that the specific, SV40 tumor CF antigens are not a consequence of malignant transformation because serologically identical antigens, that are not a part of the CF antigens of the SV40 viral particles, were shown to be produced early after SV40 virus infection of normal cells that quickly undergo complete destruction.[2, 5-7] Moreover, during the course of tests which demonstrated that all of the CF antibody in anti-SV40 tumor hamster serum can be removed by absorption with the nonviral antigens present in SV40 virus-infected normal cells, it was found that the specific tumor antigens consist of at least two different components that are present in different concentrations in the tumor cells and in the SV40 virus-infected normal cells.[5] In the light of these observations it is possible that the human embryonic kidney cells that were morphologically transformed by

SV40 virus also contain the various components of the specific CF antigens in concentrations that are different from those found in the hamster tumor cells.

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*CONTINUOUS CONDUCTION OF ACTION POTENTIALS BY SINGLE MYELINATED FIBERS OF DESHEATHED NERVE: TYPES OF NORMAL NERVE FIBERS**

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Evidence is presented in this communication that in the fibers of desheathed nerves the action potential is also established in the myelinated internodes.

Technique.—The membrane action current was recorded with the technique described on p. 311 of reference 1*a* using single undissected fibers of desheathed peroneal branches[1c] (*R. catesbiana*). Glucose Ringer's, to which 15 mM sodium chloride had been added, was used to prepare the conducting medium, but the desheathed nerves were not soaked in the glucose solution. During the experiment a certain amount of sodium ions must have passed from the nerve into the medium, but since the amount of fluid in the medium was quite small and since the medium contained 15 mM sodium ions, the loss of sodium by the nerve was too small to be significant.

The longitudinal action current was recorded by placing the nerve in two Ringer pools separated by a 600-μ-wide air gap (Fig. 1*a* in ref. 1*b*), and connecting the amplifier to the two Ringer pools. In all cases the traveling impulses were initiated in the peroneal trunk.

Results.—Membrane action current: Figure 1 presents records obtained with the microelectrode at 27 points of a 13-mm-long segment of nerve, the last point being at 14 mm from the killed end. In that segment of the single conducting fiber 6 nodes of Ranvier were present at 2-mm distances; nevertheless, triphasic deflections (downward, upward, downward) were recorded at all the numerous tested points, indicating that at all points of the fiber the membrane action current was flowing, of course, across the myelin layer in three successive directions, outward, inward, outward. The observations were repeated time and again in this experiment and have also been repeated in numerous other, similar experiments. *Without a single exception,* triphasic records of the membrane action current were obtained at all tested points of the conducting fiber. Therefore, it may be stated with absolute certainty that also in the myelinated fibers of desheathed nerves the emf of the action potential is established at all points of the internodes.