suggestion of a strong chemical interaction is the observation that the deposition of the colorless compound (I) on a vanadium film held at 4.2°K produces a deep purple color.

A preliminary experiment with 5-nitro-1,10-phenanthroline (II) gave qualitatively similar increases. Condensation of oxygen produced by the decomposition of silver oxide produced an increase of transition temperature of 0.07°K and a twofold increase in critical current. On the other hand, metal-free phthalocyanine (III) *decreased* the transition temperature by about 0.05°K and depressed the critical current by a factor of four. The *normal* resistance of the film at 4.2°K and below did not change upon the addition of (III). The superconducting transition temperatures of the vanadium films were about 2°K; the transition temperature appears to increase with increasing film thickness for these films estimated to be <200 A.

Preliminary experiments with indium films indicated that (III), copper phthalocyanine, tetracyanoquinodimethan, chloranil, and nitrogen dioxide all depress the critical current and transition temperature. Further experiments are in progress.

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IN VITRO TRANSFORMATION OF HAMSTER AND HUMAN CELLS WITH THE ADENO 7-SV40 HYBRID VIRUS

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Huebner *et al.*[1] have reported the occurrence of tumors in hamsters inoculated with adenovirus type 7 (Ad. 7) strain L.L. Such tumors have the virus-specific antigen(s) characteristic of that produced by SV40 virus (hereafter referred to as the SV40 neoantigen) as determined by complement fixation (CF)[2] and fluorescent

antibody studies (FA).[3] Rowe and Baum[4] and Rapp *et al.*[5] have demonstrated that a passage line (E46) of L.L. Ad. 7 induces the SV40 neoantigen in acutely infected monkey and human tissue culture cells. Since the factor which induces the SV40 neoantigen is integrally associated with the Ad. 7 infectious particle, it has been postulated that a portion of the SV40 genome is incorporated within the adeno virion.[4-6] This article reports the *in vitro* transformation of primary hamster kidney cultures and diploid human skin fibroblasts with the hybrid virus.

Materials.—Virus: The history and characterization of the E46 preparation of the L.L. strain of Ad. 7 have been described.[1] Subsequent passages of LLE46 in primary African green monkey kidney (AGMK) cells were carried out,[4] and the pool from the 8th (LLE46 AG8) AGMK passage was used; this pool titered $10^{7.2}$ tissue culture infectious doses 50 ($TCID_{50}$) per ml in human embryonic kidney (HEK) cultures. Another LLE46 subline, freed of the SV40 antigen-inducing component by limiting dilution passage in HEK will be referred to as $LLE46^-$;[4] a pool of this subline grown in HEK (pool 970) had a titer of $10^{8.2}$ $TCID_{50}$/ml. This pool has not produced tumors in hamsters over a 6-month observation period. SV40 virus strain 777,[7] purified in a RbCl preparative density gradient,[8] was used to infect BSC-1 cultures. The virus obtained was employed after freezing and thawing twice and titered $10^{9.2}$ $TCID_{50}$/ml.

Infection of hamster cells: Roller tube cultures of weanling hamster kidney (WHK) obtained from Microbiological Associates, Inc., and Flow Laboratories were washed twice with phosphate buffered saline pH 7.2 (PBS), and 0.1 ml of virus or Eagle's basal medium (BME) was added to each of 4 replicate cultures. After a 1-hr adsorption period, during which time the tubes were frequently rotated, 1.5 ml of N.C.T.C. 109 containing 10% fetal calf serum (heated 56° 30 min) (Microbiological Associates, Inc.), 2 mM glutamine, penicillin, and streptomycin, 100 units and 100 mcg, respectively, were added to each tube. In neutralization tests with SV40 and Ad. 7 hyperimmune rabbit sera (*vide infra*) equal volumes of virus and serum dilutions were mixed and kept at room temperature for 45 min; 0.2 ml was then adsorbed on a washed WHK monolayer, as described. Maintenance media for these latter tests, in addition to the ingredients described, contained 0.5% of SV40 or Ad. 7 antiserum having neutralization titers of 1024 and 512, respectively. All cultures were incubated at 36°C in a stationary position; culture medium was changed and tubes were read twice a week.

Infection of adult human skin fibroblasts: The growth properties and methods of cultivation of the human skin fibroblasts (strain A) have been described.[9] Originally obtained from a skin biopsy from an adult female, the cells maintain a constant 25-hr doubling time for the first 50 cell generations, then enter a phase of progressively lengthening doubling time (phase 3[10]), and finally cease dividing after 71–74 cell generations. Toward the end of their *in vitro* lifetime such cells have been demonstrated to be particularly sensitive to the transforming effect of SV40.[9, 11, 12]

The cells were grown in 50-mm plastic Petri dishes in Dulbecco and Vogt's modification of Eagle's medium supplemented with 10% unheated calf serum (Colorado Serum Co.). Petri dishes containing approximately 2.5×10^5 cells at the 68th–70th cell generation (33rd–40th transfer) were washed twice with PBS and then exposed for 3 hr to 0.5 ml of virus or medium. During the adsorption period the plates were tilted back and forth several times every 15 min. The cultures were then washed and fresh medium was added; culture medium was changed three times a week. The neutralization tests were performed as described above.

Tests for virus in E46-transformed lines: Supernatant fluids were examined for virus by inoculating 0.2 ml into each of 4 AGMK and HEK cultures. After 4 weeks the cultures were thrice frozen and thawed, the fluids pooled, and 0.2 ml passed to fresh cultures which were observed for an additional 4 weeks. In the absence of detectable virus by the above method the cell overlay technique was used.[13] On each of three monolayer cultures 3×10^5, 3×10^4, and 3×10^3 transformed cells were overlaid per dilution; both AGMK and HEK cells were used as the indicator system. After 15 days the dual cultures were thrice frozen and thawed, the replicate cultures of each dilution pooled, extracted with chloroform, and all the extracts passed to fresh AGMK and HEK cultures. These latter cultures were observed for 6 weeks.

Fluorescent antibody studies: Fluorescent antibody tests were done with cells grown on 11 × 22-mm coverslips as previously described.[3] For detection of SV40 and Ad. 7 neoantigens the indirect FA test was used, employing sera from hamsters bearing SV40 and adeno type 7 virus-

induced tumors and fluorescein conjugated goat antihamster serum. The hamster sera were diluted 1–10 in a 20% suspension of normal hamster brain. The goat antihamster conjugate was adsorbed with monkey liver tissue, and a 1:5 dilution of the conjugate was mixed with an equal volume of rhodamine (final concentration 1–100) prior to use. For detection of SV40 and Ad. 7 viral antigens the direct FA test was used, employing a fluorescein conjugated African green monkey hyperimmune anti-SV40 serum and a rabbit hyperimmune anti-Ad. 7 serum, respectively.

Complement fixation: The microtechnique described previously for tumor antigen studies was used,[14] employing 1.6–1.8 exact units of complement. SV40 and Ad. 7 neoantigens were tested for with 4–8 units of antibody from hamsters with SV40 and Ad. 7 tumors, respectively. Four to eight units of anti-SV40 hyperimmune African green monkey serum were used in tests for SV40 virion antigen. In tests for Ad. 7 virus antigen, 4–8 units of both individual and pooled convalescent human adenovirus sera were employed.

Results.—Transformation of WHK monolayer cultures by LLE46: Table 1 gives the results of three experiments using different batches of WHK cultures. A variable and nonprogressive cytopathogenic effect (CPE) occurred in cultures inoculated with both LLE46 and LLE46$^-$ pools of virus. This was characterized by a typical adenovirus-type rounding of the cells with some destruction of the tissue varying from 10 to 30 per cent and occurring mainly at the periphery of the monolayer. However, after 3–4 days no further CPE was noted. During this time or soon after, multinucleated, syncytial giant cells appeared. In addition, large single nucleated as well as bizarre-shaped, and multinucleated giant cells were present.

Beginning approximately 8 days after inoculation with LLE46 virus, foci of proliferation of triangular to polygonal-shaped "epithelioid" cells appeared; foci of cells with a fibroblastic morphology also were noted. Syncytial giant cells containing 20–30 nuclei were associated mainly with the epithelioid cells. The new tissue piled up into several layers, overgrew what remained of the original monolayer, and caused increased acidification of the medium. Over the next 60 days, the growth continued with retractions of part of the cell sheet and subsequent regrowth; cultures have been observed for over 135 days with this recurring pattern. In some areas of the culture, foci of tightly packed, piled up cells were observed.

TABLE 1

SUMMARY OF TRANSFORMATION EXPERIMENTS WITH WEANLING HAMSTER KIDNEY

Tissue	Expt. no.	Inoculum	$TCID_{50}$/-0.1 ml	First day of appearance of giant cells	Frequency of transformation, no. tubes pos./no. tubes inoc.	Day transformation first observed in positive tubes	Length of observation of neg. tubes
WHK	11–25	LLE46 AG 8	$10^{6.2}$	2–5	4/4	8, 8, 15, 15	—
	11–25	LLE46$^-$ pool 970	$10^{6.2}$	—	0/4	—	55
	11–25	SV40	$10^{6.2}$	5–19	4/4	19, 26, 26, 40	—
WHK*	12–17	LLE46 AG 8	$10^{6.2}$	7	4/4	9, 9, 9, 12	—
		LLE46$^-$ pool 970	$10^{7.2}$	—	0/4	—	>63
WHK*	12–17	LLE46 AG 8	$10^{6.2}$	7–12	4/4	12, 18, 20, 23	—
		LLE46$^-$ pool 970	$10^{7.2}$	—	0/4	—	>63
		LLE46 AG 8 + SV40 antiserum†	$10^{6.2}$	9–12	3/4	18, 18, 20, —	>63
		LLE46 AG 8 + adeno 7 antiserum†	$10^{6.2}$	—	0/4	—	>63

* These represent two different lots of WHK obtained from different sources.
† Virus was exposed to approximately 50 units of SV40 or Ad. 7 antiserum at room temperature for 45 min.; maintenance media contained antiserum (see *Materials and Methods*).

These thickened, homogeneous areas were clearly distinct from the other foci and ultimately coalesced with the latter. It is not clear whether these represent an additional cell type transformed or a variation in the type of growth pattern of the transformed cells. In general, the pattern was similar to that observed with SV40 transformation of WHK;[13] however, transformation occurred earlier (Table 1) and the spectrum of giant cells was much greater. Ad. 7 antiserum treatment of LLE46 prevented transformation, while treatment of LLE46 with SV40 antiserum did not prevent transformation from occurring in 3 of 4 cultures (Table 1).

Uninoculated control cultures underwent nonspecific, progressive deterioration from approximately the 20th to the 40th day. No proliferation was noted in any of the cultures inoculated with LLE46$^-$; after the minimal CPE described above, they underwent deterioration similar to the controls.

Characteristics of transformed WHK cells: Two cell lines were initiated at the 18th and 88th day after inoculation with LLE46 (expt. 11–25, Table 1). These lines will be designated E46HK-1 and E46HK-2; at present they are in their 12th and 6th passages, respectively. A cell line, presently in its 6th serial passage (THK-3), was established from a WHK culture inoculated with SV40 88 days earlier (expt. 11–25, Table 1). Cells from uninfected cultures, when trypsinized and transferred, could not be maintained in serial culture for more than 2–3 passages.

Microscopic examination of H & E stained coverslips of the E46-transformed cell lines revealed many similarities with THK-3 and a previously described SV40-transformed hamster line.[13] The predominant cell type was "epithelioid"; however, there was considerable pleomorphism, the cells ranging from a polygonal to a triangular or fibroblastic morphology. The cells of both the E46- and SV40-transformed lines had large, pleomorphic nuclei, and an increased nuclear-cytoplasmic ratio. They tended to be smaller in the E46-transformed lines, and many more single and multinucleated giant cells were present.

Saturation densities (maximum cell number per plate) of the cell lines THK-3 passage #2 (p #2) and p #5 and E46HK-2 p #2 and p #5 were found to be approximately the same, varying from 3.5 to 5.0×10^6 per plate, while normal WHK had a saturation density of 8×10^5 to 1×10^6 per plate. Preliminary cytogenetic studies on the cell lines THK-3 and E46HK-2 revealed that both were aneuploid by the 3rd passage with occasional massive polyploidy.

Transformation of adult human fibroblasts by LLE46: In the first two weeks following exposure of strain A human fibroblasts to the virus, no differences between infected and control cultures could be seen. In the third week a self-limiting, focal CPE was seen on plates inoculated with either LLE46 or LLE46$^-$. Foci of rounded, granular cells were seen; these cells ultimately detached from the monolayer and left plaquelike regions. Approximately 10–20 per cent of the cells from LLE46$^-$ infected cultures and well under 5 per cent of the cells from LLE46 infected cultures were involved. During the third and fourth weeks clearly delineated foci of increased mitotic activity were seen in the LLE46 infected cultures; this constituted the first evidence of transformation (Table 2). These foci of high proliferative activity bore no relation to the foci of degenerating cells also present in the cultures. In the next two weeks the cells in the proliferating regions formed multiple cell layers consisting of small, tightly packed epithelioid cells; single and multinucleated giant cells were frequently observed. The newly transformed cells, however, showed little tendency

TABLE 2

SUMMARY OF TRANSFORMATION EXPERIMENTS WITH HUMAN SKIN FIBROBLASTS

Expt. no.	Inoculum	TCID50 (0.1 ml)	Cell generations at infection*	Day transf. first observed
1	LLE46 AG 8	$10^{6.2}$	68	28, 28†
	LLE46⁻ pool 970	$10^{6.2}$	68	—‡
2	LLE46 AG 8	$10^{6.2}$	68	18, 25
	LLE46⁻ pool 970	$10^{7.2}$	68	—
	SV40 strain 777	$10^{6.2}$	68	28, 30
3	LLE46 AG 8	$10^{6.2}$	70	16, 20
	LLE46 AG 8 + SV40 antiserum	$10^{6.2}$	70	15, 18
	LLE46 AG 8 + adeno 7 antiserum	$10^{6.2}$	70	—
	LLE46⁻ pool 970	$10^{7.2}$	70	—
	SV40 strain 777	$10^{6.2}$	70	26, 35
	SV40 strain 777	$10^{8.2}$	70	4, 6

* Uninfected cultures die at 71–74 cell generations.
† Two plates were inoculated per test.
‡ Negative plates were observed for 60 days.

to grow over the adjacent untransformed cells and there was little change in the condition of the cultures over the next 60 days. However, in cultures that were transferred after the third week and then once or twice a week thereafter, a marked increase in cell number in the LLE46 infected cultures was evident by the fifth week. Uninfected and LLE46⁻ infected cultures could not be carried through more than 2–3 additional cell generations.

Treatment of the LLE46 preparations with Ad. 7 antiserum prevented the appearance of transformed cells over the 60-day observation period, while SV40 antiserum had no effect on the transformation process (Table 2). In experiments 2 and 3, duplicate cultures were also exposed to SV40 strain 777. At the same multiplicity of infection as used for LLE46, transformation was first observed between the 26th and 35th day, while with a 100-fold greater multiplicity of infection an increase in mitotic activity could clearly be seen within one week, as has previously been reported with SV40 strain 776.[9, 12] In addition, at both multiplicities the number of cells dying and detaching from the monolayer was greater with SV40 than with LLE46 infection.

Characteristics of transformed human fibroblast cells: E46-STRA-1, a transformed cell line derived from the infection of strain A cells at the 68th generation was carried through 16 passages and 36 additional cell generations; it was a uniform population of rapidly dividing epithelioid cells with a doubling time of close to 30 hr which then went through a phase of progressively declining growth characteristic of SV40-transformed human cells.[15, 16] E46-STRA-2, derived from experiment 2, is in its 10th passage and is still rapidly dividing with a 36-hr doubling time.

The morphology of the E46 cells is not distinguishable from that of the SV40-transformed cells;[17, 18] the predominant cell type is epithelioid with syncytial cells and giant cells with bizarre nuclei also prevalent. The parallel orientation of fusiform cells with gentle whorls, characteristic of human fibroblast cultures, was replaced by randomly oriented cells, epithelioid and polygonal in shape, that had an increased nuclear-cytoplasmic ratio and were less firmly adherent to the plastic. While strain A fibroblasts at the end of their *in vitro* lifetime reach a maximum cell density of 6–8 × 10^5 cells per plate, the transformed cells reached saturation densities of 3–5 × 10^6 cells per plate. Chromosome preparations on E46-STRA-1 cells at the 4th and 12th passage show a modal number in the hypotetraploid region and

extensive chromosomal rearrangements similar to those previously described for SV40-transformed strain A cells.[12]

Fluorescent antibody studies: Coverslip preparations of cells from E46HK-1 p #2, p #7, and E46HK-2 p #3 were stained with SV40 and Ad. 7 "tumor" and viral antisera in the direct or indirect FA test. Approximately 90–100 per cent of nuclei from the 3 preparations fluoresced with the SV40 tumor antiserum. Most nuclei contained within the giant cells also showed specific fluorescence; however, some nuclei, adjacent to fluorescent ones, were negative. No fluorescence occurred with the Ad. 7 tumor sera or with the SV40 and Ad. 7 viral antisera. There was no staining with any of the 4 sera of 2nd passage cells from either control WHK or WHK inoculated with $LLE46^-$ pool 970. One hundred per cent of the cells from THK-3 p #2 showed specific nuclear fluorescence only with the SV40 tumor antiserum, confirming previous studies.[3, 19] Nearly all the nuclei from the two lines of E46-transformed human fibroblasts were also positive for SV40 neoantigen and negative with the other 3 sera. Approximately one per cent of third passage human fibroblast cells infected with $LLE46^-$ contained both Ad. 7 viral and neoantigens.

Complement fixation: Ten per cent suspensions containing 1×10^7 cells per ml from E46HK-2 p #2 and THK-3 p #2 gave positive CF reactions with a specific SV40 tumor serum; greater than 16 units of antigen were present in such preparations. Each of two similar cell suspensions from the E46-transformed human lines contained 4 units of SV40 neoantigen. Neither the E46-transformed hamster or human lines gave reactions with any of the following: SV40 monkey antiserum, Ad. 7 hamster tumor serum, and pooled or individual human adenovirus convalescent serum.

Tests for virus in the transformed lines: No infectious SV40 or Ad. 7 virus was found in the supernatant fluid of a culture of E46HK-1, p #2, or in 2nd passage cells of this line tested by the overlay technique (see *Methods*). Infectious, hybrid virus was found, however, in the supernatant fluids of both lines of E46-transformed human cells at p #4 and p #8. The virus titers, determined in HEK cultures, varied from 10^3 to 10^4 $TCID_{50}$/ml. At the 15th passage of E46-STRA-1 hybrid virus could only be detected by the overlay technique using HEK cells.

Discussion.—From the data presented, it is concluded that the Ad. 7-SV40 hybrid virus is capable of inducing *in vitro* transformation of both hamster and human cells. These findings provide *in vitro* confirmation of the oncogenic potential of this virus in hamsters as described by Huebner *et al.*[1] At present, all available evidence indicates that the portion of the SV40 genome in LLE46 responsible for the SV40 neoantigenic and neoplastic activity is enclosed in the Ad. 7 protein coat.[4–6] The findings that Ad. 7 antiserum prevented both the hamster and the human cell transformation, while SV40 antiserum did not, are in agreement with this hypothesis.

The transformations of hamster and human cells by LLE46 are in most ways similar to those described with SV40.[7, 9] The hybrid virus produces a morphological transformation characterized by the emergence of epithelioid cells with altered growth characteristics. Cells transformed by both viruses grow to similar saturation densities, have similar chromosomal aberrations, and have the SV40 neoantigen in virtually every cell. Transplantation studies of the E46-HK lines are in progress

TABLE 3

COMPARISON OF TRANSFORMATION WITH SV40 AND LLE46 VIRUSES

		Time transformation observed (days)*	Characteristics of cell lines	Presence of SV40 Neoantigen C. F.	F. A.	Presence of infectious virus
Hamster kidney cells	SV40	19–40	Cells triangular to epithelioid; much piling up; giant cells	+	+	+†
	LLE46	8–23	Cells similar in shape but smaller; less piling up; many more giant cells	+	+	—
Human skin fibroblasts	SV40	25–40	Cells epithelioid, closely packed; loss of parallel orientation, many dying and detaching cells	+	+	+
	LLE46	16–28	Cells similar in morphology and growth pattern; few detaching cells	+	+	+‡

* Following exposure to $10^{6.2}$ $TCID_{50}$ of virus.
† Based on studies carried out with a previous SV40 transformation (THK-1).[13]
‡ Ad. 7-SV40 hybrid virus.

to determine whether the transplantation rejection antigen is also present.[20-22] Some differences between the SV40 and E46 transformation were observed; at comparable multiplicites of infection transformation was observed earlier with LLE46 in both systems; more and varied giant cells and greater morphological variability were present with LLE46 transformation of the hamster cells as compared with the SV40 transformation. A further difference noted with LLE46-transformed hamster cells is the inability to recover infectious SV40; SV40 has repeatedly been found in a line of WHK transformed by SV40 and in 12 clones derived therefrom.[13, 23] No SV40 viral antigen has ever been found by FA or CF testing in either cell system at various time intervals after acute infection with E46.[4, 5] It is probable that the SV40 DNA present in the hybrid represents only a portion of the genome which is insufficient to code for virion antigen.

The failure to demonstrate Ad. 7 viral or neoantigens in the transformed human cell lines which yield some infectious hybrid virus by FA or CF testing probably reflects the difficulty in detecting the rare virus producing cells by these methods. Since only a small proportion (1%) of LLE46-infected cells have Ad. 7 viral and neoantigens by fluorescence, and the titers of infectious virus in these cultures are 100 times those found in LLE46-transformed cultures, detection of any such cells in the transformed lines by FA or CF tests would be exceedingly difficult.

To date the only viruses that have been shown capable of transforming human cells in culture are SV40[17, 18] and Rous sarcoma virus.[24] The present report demonstrates that a human virus that has apparently acquired a portion of the SV40 genome is also able to transform adult human cells in culture.

Summary.—The adeno 7-SV40 hybrid virus produced *in vitro* transformation of primary hamster kidney cells and diploid human skin fibroblasts. Both of these transformations resembled those caused by SV40 in almost all respects. Differences between SV40 and the hybrid transformation included a shorter latent period for transformation with the hybrid virus at comparable multiplicities of infection as well as absence of infectious virus from hybrid-transformed hamster cells. The transformation was inhibited by adeno 7 antiserum but not by SV40 antiserum. With both cell systems the virus-specific SV40 neoantigen could be

demonstrated in practically every transformed cell. The *in vitro* transformations confirm the oncogenic potential of the hybrid virus.

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*TUMORS INDUCED IN HAMSTERS BY A STRAIN OF ADENOVIRUS TYPE 3: SHARING OF TUMOR ANTIGENS AND "NEOANTIGENS" WITH THOSE PRODUCED BY ADENOVIRUS TYPE 7 TUMORS**

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Recently, we observed tumors induced in hamsters by a strain of type 3 adenovirus which had been isolated from a child with acute respiratory illness. The type 3 inoculum designated as strain 15520 had been carried for seven passages in human diploid cells prior to injection of newborn hamsters.[1] A subcutaneous tumor appeared 275 days later at the site of injection in one of eight hamsters. When the