23. Dmochowski, L., Proc. Fifth National Cancer Conference, Philadelphia, 1964, 57.
24. McAllister, R. M., Calif. Med., 1965, v102, 344.
25. Girardi, A. J., Slotnick, V. B., Hilleman, M. R., Proc. Soc. Exp. Biol. and Med., 1962, v110, 776.
26. Larson, V. M., Gosnell, P. A., Hilleman, M. R., to be published.
27. Sweet, B. H., Hilleman, M. R., Proc. Soc. Exp. Biol. and Med., 1960, v105, 420.
28. Lwoff, A., Bact. Rev., 1953, v17, 269.
29. Goldberg, I. H., Reich, E., Fed. Proc., 1964, v23, 958.
30. Lockart, R. Z., Jr., Biochem. Biophys. Res. Comm., 1964, v15, 513.
31. Taylor, J., ibid., 1964, v14, 447.
32. Iyer, V. N., Szybalski, W., Proc. Nat. Acad. Sci., 1963, v50, 355.
33. Reich, E., Franklin, R. M., ibid., 1961, v47, 1212.
34. Yarmolinsky, M. B., de la Haba, G. L., ibid., 1959, v45, 1721.
35. Friedman, R. M., Sonnabend, J. A., J. Immunol., 1965, v95, 696.
36. Drake, J. W., J. Cell. Comp. Physiol., 1964, v64, 19.
37. Smart, K. M., Kilbourne, E. D., J. Exp. Med., 1966, v123, 299.
38. Segre, D., Proc. Soc. Exp. Biol. and Med., 1964, v117, 567.
39. Tokunaga, T., Mizuguchi, Y., Murohashi, T., Am. Rev. Resp. Dis., 1964, v90, 431.

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Studies on Induction of Virus from Adenovirus and SV_{40} Hamster Tumors 2. "Helper" Viruses.* (31356)

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Cells of various animal species which have been rendered neoplastic *in vivo* or *in vitro* by DNA viruses and which are free of infectious virus appear to retain viral genetic material based on continued presence in the cells of new transplantation antigens and of "T" antigens which are demonstrable serologically(1-12). Cells rendered neoplastic by the RNA Rous virus may also fail to yield infectious virus but retention of viral genetic material is indicated by the apparent continued presence in the cells of a DNA precursor of Rous sarcoma virus(13) and by release of infectious virus from nonproducer cells by infection with another virus of the avian leukosis group which serves as a helper (14). The demonstration of retained viral genetic material in neoplastic cells is of considerable interest in the pathogenesis of cancer and in the development of an approach to elucidating a viral role in human tumor in which infectious virus of etiologic significance has not been demonstrated.

Extensive investigations carried out in our laboratory have sought to ascertain, in a definitive way, whether infectious virus could be induced or recovered from virus-free SV_{40} or adenovirus tumor cells of hamsters propagated *in vitro*. The first report(15) in the present series dealt with attempts to induce infectious virus by application of chemical or physical agents and by copropagation with indicator cells. The present report relates attempts to release infectious virus from virus-free adenovirus tumor cells in culture by use of potential "helper" viruses including SV_{40}, respiratory syncytial virus and reovirus type 1.

Materials and methods. In the experiments to be described, all cell cultures were incubated in a stationary position at 36°C. Growth and maintenance media contained 100 units of penicillin and 100 μg of streptomycin per ml and were changed at weekly intervals or as frequently as required for maintenance of the cells. Cultures were ex-

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amined for viral cytopathic effect at 1-7 day intervals depending upon the experiment. *Tumor cell cultures.* The tumor cell lines were all derived from virus-induced primary hamster tumors. The Pinckney C1 cell line was originally induced by adenovirus type 7 Pinckney strain, Grider F1 line by adenovirus type 7 Grider strain, adenovirus 18 line A2 by adenovirus type 18 Chanock strain, and F5-1 by SV_{40} virus(9,10). The Pinckney C1 adenovirus 7 tumor cell line was propagated and maintained in basal medium Eagle (BME) with 2% agamma calf serum; Grider adenovirus 7 line F1 and adenovirus 18 line A2 were grown in BME with 10% agamma calf serum and maintained in BME containing 5% agamma calf serum; and SV_{40} tumor line F5-1 was propagated in medium 199 with 5% calf serum and maintained in BME with 2% or 5% agamma calf serum. All the tumor cell lines were free of infectious virus based on extensive tests in sensitive assay cell cultures. All the tumor lines were free of pleuropneumonia-like organisms (PPLO) throughout the study except the Grider adenovirus 7 cell line which was shown to contain PPLO when tested at passages 31 and 115. *Virus assay.* Primary cell cultures of human embryonic kidney (HEK) were used for all adenovirus isolation attempts and titrations. Tube cultures of HEK were purchased from a commercial source and were maintained in BME with 5% agamma calf serum. HEK cultures are highly susceptible to infection with adenoviruses; under the test conditions used, they did not exhibit cytopathic effects when exposed to SV_{40} or respiratory syncytial viruses. SV_{40} virus infects HEK cells but does not produce cytopathic changes except upon prolonged observation and passage(16). In the text, HEK cultures are referred to as assay cultures when used to detect virus in cell culture fluid or in freeze-thaw extracts and as indicator cells when used in copropagation experiments. For detection of infectious adenovirus after exposure of tumor cultures to helper virus, supernatant fluids were incubated for 2 hours with an equal volume of "helper" virus antiserum and 0.2 ml inoculated per HEK assay culture. Mixed cultures for copropagation experiments were prepared in two ways. Thus, trypsinized HEK cells were added to tumor cell cultures in some experiments, while in others the tumor cells were trypsinized after exposure to "helper" virus and added to HEK cell cultures. In either case, the mixed cultures were observed for cytopathology until the cultures began to deteriorate. At this time they were frozen and thawed 3 times and 1 ml aliquots inoculated per HEK assay culture. All HEK assay cultures were observed for cytopathic change for approximately one month, frozen and thawed 3 times, 1 ml quantities inoculated into new HEK cultures and observation continued for a total of 41-50 days. At the end of this period all HEK assay cultures were challenged with 100 $TCID_{50}$ doses of adenovirus type 7 in order to demonstrate continued susceptibility of the cells. In some experiments HEK indicator and assay cultures were maintained in the presence of "helper" virus antiserum. Details relating to the actual conduct of the tests are given in the text.

Adenovirus was titrated in HEK cultures by serial 10-fold dilutions with 0.1 ml inoculated into each of 4 tubes per dilution. Cultures were observed for 35 days and the 50% titer endpoint was calculated by the Reed and Muench method.

Hamster renal cell cultures. Primary cell cultures of suckling hamster kidney were grown in lactalbumin hydrolysate-yeast extract medium containing 10% agamma calf serum and were maintained in BME containing 5% agamma calf serum. *"Helper" viruses.* Strain 45-54 of SV_{40} virus, the Long strain of respiratory syncytial virus, the Wenner 106 strain of reovirus type 1 and the Pinckney strain of adenovirus 7 were from the virus stocks of this laboratory. The infectivity titers of the 4 viruses were $10^{-5.9}$, $10^{-4.0}$, $10^{-5.9}$ and $10^{-7.5}$ $TCID_{50}$/0.1 ml, respectively. The heated preparation of SV_{40} virus which had been exposed to 65°C for 5 hours and at 75°C for an additional 5 hours contained a residual trace of live virus. The inactivated control RS virus was prepared by heating at 56°C for 2 hours and contained no detectable live virus. *Viral antisera.* The calf antiserum against SV_{40} was obtained

TABLE I. Demonstration of Complement-Fixing "T" Antigen in Hamster Tumor Cell Lines.

Adenovirus tumor	No. of times passed in cell culture: Passage levels used for helper virus release experiments	Passage tested for "T" antigen: Level	Passage tested for "T" antigen: CF titer
Type 7			
Pinckney C1	54, 96 & 113	113	1:8
Grider F1	71 & 97	117	1:4
Type 18			
Chanock A2	63 & 93	112	1:16
SV_{40} (Control) F5-1	43 & 54	67	1:16

from Flow Laboratories, Rockville, Md., and had a neutralizing antibody titer of 1:1280. The RS antiserum was prepared in horses in these laboratories and had a neutralizing antibody titer of 1:320. The reovirus 1 antiserum was obtained from Microbiological Associates, Bethesda, Md., and had a neutralizing antibody titer of 1:1280.

Results. Evidence for presence of viral genome in the tumor cell cultures. The tumor cell cultures employed in the experiments represented diverse passage levels as shown in Table I. It was necessary, therefore, to demonstrate that the viral genetic material had been retained on serial cell culture passage and was available for "rescue." Retention of viral genome was indicated by continued presence of "T" antigen in the cultures at passage levels greater than those employed in the experiments. Tests for "T" antigen were carried out by the micro complement-fixation method using appropriate anti-

TABLE II. Summaries of Procedures Used in Attempted Adenovirus Rescue Experiments.

Exp No.	Procedure
1 (Table III)	(A) "Helper" SV_{40} virus was added to groups of tumor cell tube cultures. (B) Following 24 hr incubation, the tumor cell cultures were washed and copropagated by addition of HEK cells. Half the mixed cultures were maintained during copropagation with and half without added SV_{40} antiserum. (C) After 9-18 days incubation, the mixed cultures were freeze-thawed to disrupt the cells, passed to HEK assay cell cultures with and without added SV_{40} antiserum, and observed for presence of infectious adenovirus. In the experiments, uninfected tissue culture fluid was substituted for helper virus for control purpose. The SV_{40} hamster tumor F5-1 system was employed as a cell control.
2 (Table IV)	(A) "Helper" SV_{40} virus was added to tumor cell cultures in 2 oz prescription bottles. After 3-5 days incubation, the cultures were harvested and the supernatant fluid (B) and cells (C) treated separately. (B) The cell culture fluid from (A) cultures was neutralized with SV_{40} virus antiserum and tested in HEK assay cell cultures for presence of adenovirus. (C) The cells from the (A) cultures were trypsinized and added to HEK indicator cell cultures. These were observed for development of viral CPE for 10-18 days. (D) Following observation, the (C) cultures above were freeze-thawed to disrupt the cells, passed to HEK assay cell cultures, and observed for presence of infectious adenovirus.
3 (Table V)	Experiment performed in the same manner as Exp 1 above except that respiratory syncytial (RS) virus was substituted for SV_{40} virus as the "helper" and the homologous antiserum was used.
4 (Table VI)	Experiment performed in the same manner as Exp 2 above except that respiratory syncytial (RS) virus was substituted for SV_{40} virus as the "helper" and homologous antiserum was used.
5 (Table VII)	(A) "Helper" reovirus 1 was added to type 7 Pinckney C1 tumor cells and the cultures incubated until cytopathology was complete. (B) At 6 days, the (A) cultures were frozen and thawed, the reovirus neutralized with homologous antiserum, and passage made to assay HEK cell cultures which were observed for presence of infectious adenovirus.
6 (Table VIII)	(A) Adenovirus 7, adenovirus 7 plus SV_{40} virus, or SV_{40} virus alone was inoculated into cell cultures of normal hamster kidney and were observed for adenovirus CPE. (B) After 13 days incubation, the (A) cultures were freeze-thawed and the content of adenovirus was titrated in HEK assay cell cultures.

TABLE III. Attempt to Induce Infectious Virus from Adenovirus Hamster Tumor Cell Cultures by SV_{40} "Helper" Virus with Test for Induced Virus by Copropagation with Indicator Cells. Exp 1.

	"Helper" virus added (A)			Copropagation with indicator HEK (B)* cells		Subpassage into HEK assay cells (C)§	
Tumor cell line	Kind	Amt ($TCID_{50}$)	Period (days)	Observation period (days)	Cytopathology	Observation period (days)	Result
Adenovirus							
Type 7	SV_{40}	$10^{5.9}$ & $10^{2.9}$	1	18	Negative	47	Negative
(Pinckney) C1	heated SV_{40}†	Trace	"	"	"	"	"
	T.C. fluid (control)‡	—	"	"	"	"	"
Type 7	SV_{40}	$10^{5.9}$ & $10^{2.9}$	"	10	"	49	"
(Grider) F1	heated SV_{40}	Trace	"	"	"	"	"
	T.C. fluid (control)	—	"	"	"	"	"
Type 18	SV_{40}	$10^{5.9}$ & $10^{2.9}$	"	9	"	41	"
(Chanock) A2	heated SV_{40}	Trace	"	"	"	"	"
	T.C. fluid (control)	—	"	"	"	"	"
SV_{40} (control)							
F5-1	SV_{40}	$10^{5.9}$ & $10^{2.9}$	"	10	"	49	"
	heated SV_{40}	Trace	"	"	"	"	"
	T.C. fluid (control)	—	"	"	"	"	"

* Half the cultures were copropagated with primary human embryonic kidney (HEK) cells in presence of SV_{40} antiserum and half in absence of SV_{40} antiserum.
† $TCID_{50}$ before heating same as above.
‡ Uninfected tissue culture fluid from same lot of cells as used to prepare the SV_{40} virus.
§ Half the subcultures were made in presence and half in absence of added SV_{40} antiserum.

sera from tumor-bearing hamsters.

Attempted rescue of tumor virus by use of "helper" viruses. General and specific details concerning the conduct of the experiments are presented in the section on *Materials and methods* and in the text. An abbreviated outline relating specifically to each experiment is given in Table II and should be followed for purpose of explanation. The capital letters in Tables III to VIII refer to the appropriate sections of the experiments designated in Table II.

Experiment 1. (Table III). This experiment was designed to ascertain whether live- or heat-treated SV_{40} virus was capable of rescuing residual adenovirus genetic material from adenovirus tumor cells with release of infectious adenovirus. It is seen that the HEK indicator cells which were copropagated with the SV_{40} virus-treated adenovirus tumor cells failed to show presence of infectious adenovirus during the 9-18 day period of observation (B). This was true of cultures copropagated in the presence of added SV_{40} antiserum as well as of those in which antiserum was not added to neutralize residual SV_{40} which might have infected the indicator cells and rendered them less sensitive to infection with adenoviruses. Subpassage of the freeze-thawed extracts of the copropagated cell cultures to HEK assay cell cultures failed to reveal infectious adenovirus either in the presence or absence of added SV_{40} virus antiserum (C). Cultures in the uninfected tissue culture fluid control series and in the SV_{40} tumor cell control system remained normal throughout the observation periods shown. At the end of the observation period all subcultures showed typical adenovirus cytopathic effect upon challenge with adenovirus indicating continued susceptibility of the HEK cells to adenovirus. It was concluded that infectious adenovirus was not released from any of the adenovirus tumor cell lines which were studied.

Experiment 2. (Table IV). The approach in this experiment was essentially the same as for Exp. 1 except that, in addition to copropagation and subpassage, tests for infectious adenovirus in supernatant fluids were made directly into the HEK assay cells. It is seen that the adenovirus tumor cell lines

TABLE IV. Attempt to Induce Infectious Virus from Adenovirus Hamster Tumor Cell Cultures by SV_{40} "Helper" Virus with Test for Induced Virus by Copropagation with Indicator Cells and by Subpassage into HEK Assay Cell Cultures. Exp 2.

	"Helper" virus added (A)			Test for infectious adenovirus in fluid from (A) tumor culture in assay HEK (B)		Copropagation by addition of (A) cells to indicator HEK cell cultures (C)			
						Cytopathology in indicator cells		Subpassage to assay HEK (D)	
Tumor cell line	Kind	Amt ($TCID_{50}$)	Period (days)	Observation period (days)	Result	Observation period (days)	Result	Observation period (days)	Result
Adenovirus									
Type 7 (Pinckney) C1	SV_{40}	$10^{6.5}$ & $10^{3.5}$	5	54–56	Neg	ND‡	—	ND	—
	heated SV_{40}*	Trace	"	"	"	"	—	"	—
	T.C. fluid (control)†	—	"	"	"	"	—	"	—
" " (Grider) F1	SV_{40}	$10^{6.5}$ & $10^{3.5}$	3	49	"	18	Neg	49	Neg
	heated SV_{40}	Trace	"	"	"	"	"	"	"
	T.C. fluid (control)	—	"	"	"	"	"	"	"
" 18 (Chanock) A2	SV_{40}	$10^{6.5}$ & $10^{3.5}$	"	"	"	14	"	54	"
	heated SV_{40}	Trace	"	"	"	"	"	"	"
	T.C. fluid (control)	—	"	"	"	"	"	"	"
SV_{40} (Control)									
F5-1	SV_{40}	$10^{6.5}$ & $10^{3.5}$	"	"	"	10	"	49	"
	heated SV_{40}	Trace	"	"	"	"	"	"	"
	T.C. fluid (control)	—	"	"	"	"	"	"	"

* $TCID_{50}$ before heating same as above.
† Uninfected tissue culture fluid from same lot of cells as used to prepare the SV_{40} virus.
‡ Not done.

TABLE V. Attempt to Induce Infectious Virus from Adenovirus Hamster Tumor Cell Cultures by RS "Helper" Virus with Test for Induced Virus by Copropagation with Indicator Cells. Exp 3.

		"Helper" virus added (A)		Copropagation with indicator HEK (B)* cells		Subpassage into HEK assay cells (C)§	
Tumor cell line	Kind	Amt ($TCID_{50}$)	Period (days)	Observation period (days)	Cytopathology	Observation period (days)	Result
Adenovirus							
Type 7	RS	10^4	1	8	Negative	50	Negative
(Pinckney) C1	heated RS†	—	"	"	"	"	"
	T.C. fluid (control)‡	—	"	"	"	"	"
Type 7	RS	10^4	"	10	"	47	"
(Grider) F1	heated RS	—	"	"	"	"	"
	T.C. fluid (control)	—	"	"	"	"	"
Type 18	RS	10^4	"	"	"	48	"
(Chanock) A2	heated RS	—	"	"	"	"	"
	T.C. fluid (control)	—	"	"	"	"	"
SV_{40} (control)							
F5-1	RS	10^4	"	"	"	"	"
	heated RS	—	"	"	"	"	"
	T.C. fluid (control)	—	"	"	"	"	"

* Half the cultures were copropagated with primary human embryonic kidney (HEK) cells in presence of RS antiserum and half in absence of RS antiserum.

† $TCID_{50}$ before heating same as above.

‡ Uninfected tissue culture fluid from same lot of cells as used to prepare the RS virus.

§ Half the subcultures were made in presence and half in absence of added RS antiserum.

which had been incubated (A) with live- or heat-treated SV_{40} "helper" virus failed to show presence of infectious adenovirus when the tissue culture fluid was removed from the cultures, treated with SV_{40} antiserum and tested in HEK assay cells (B). Likewise, the trypsin-dispersed cells from the SV_{40} virus-treated tumor cultures (A) failed to show presence of infectious adenovirus when added to the assay HEK cells (C) or upon subpassage (D) of this material to assay HEK following freeze-thaw. Cultures in the uninfected tissue culture fluid control series and in the SV_{40} tumor cell control system remained normal throughout the observation periods shown. At the end of the observation period, assay cultures (B and D) were challenged with adenovirus 7 to prove the susceptibility of the HEK cells to adenovirus and all showed typical adenovirus cytopathic effect. It was concluded that infectious adenovirus was not released from any of the adenovirus tumor cell lines which were studied.

Experiments 3 and 4. (Table V and VI). These experiments were designed to ascertain whether live- or heat-treated RS "helper" virus was capable of rescuing residual adenovirus genetic material from adenovirus tumor with release of infectious virus. The conduct of the experiments was the same as for Exp. 1 and 2 employing SV_{40} virus except that the RS virus was substituted for SV_{40} and antiserum against RS virus was employed for purpose of neutralizing the "helper". The data in Tables V and VI support the conclusion that exposure to RS virus did not bring about release of infectious adenovirus from the adenovirus tumor cell lines which were studied nor did it affect the susceptibility of HEK assay cultures to adenovirus infection upon challenge at the end of the experiment. In preliminary studies, RS virus had been found to replicate in HEK cells with only slight cytopathogenic effect (CPE). Primary HEK cultures which were inoculated with RS virus and observed for 15 days showed minimal CPE but, upon subpassage to Hep-2 cells, typical syncytial formation occurred. The latter was attributed to virus replication since RS virus infectivity is quite labile(17) and since the virus which was present in the

TABLE VI. Attempt to Induce Infectious Virus from Adenovirus Hamster Tumor Cell Cultures by RS "Helper" Virus with Test for Induced Virus by Copropagation with Indicator Cells and by Subpassage into HEK Assay Cell Cultures. Exp 4.

Tumor cell line	"Helper" virus added (A)			Test for infectious adenovirus in fluid from (A) tumor culture in assay HEK (B)		Copropagation by addition of (A) cells to indicator HEK cell cultures (C)			
						Cytopathology in indicator cells		Subpassage to assay HEK (D)	
	Kind	Amt ($TCID_{50}$)	Period (days)	Observation period (days)	Result	Observation period (days)	Result	Observation period (days)	Result
Adenovirus									
Type 7 (Pinckney) C1	RS	$10^{4.6}$	3	49	Neg	11	Neg	49	Neg
	heated RS*	—	"	"	"	"	"	"	"
	T.C. fluid (control)†	—	"	"	"	"	"	"	"
" " (Grider) F1	RS	$10^{4.6}$	"	"	"	"	"	50	"
	heated RS	—	"	"	"	"	"	"	"
	T.C. fluid (control)	—	"	"	"	"	"	"	"
" 18 (Chanock) A2	RS	$10^{4.6}$	4	"	"	10	"	49	"
	heated RS	—	"	"	"	"	"	"	"
	T.C. fluid (control)	—	"	"	"	"	"	"	"
SV_{40} (control)									
F5-1	RS	$10^{4.6}$	"	"	"	"	"	"	"
	heated RS	—	"	"	"	"	"	"	"
	T.C. fluid (control)	—	"	"	"	"	"	"	"

* $TCID_{50}$ before heating same as above.
† Uninfected tissue culture fluid from the same lot of cells as used to prepare the RS virus.

TABLE VII. Attempt to Induce Infectious Virus from Adenovirus 7 Hamster Tumor Cell Culture by Reovirus 1 "Helper" Virus. Exp 5.

Helper virus added			Subpassage to HEK assay cells (B)*	
	Observation (A)			
Kind & (amount)	Period (days)	Cytopathology	Observation period (days)	Result
Reovirus type 1 ($10^{5.9}$ $TCID_{50}$)	6	Complete (Reovirus type)	46	Negative
None	6	None	"	"

* HEK cultures maintained in the presence of reovirus 1 antiserum.

TABLE VIII. Effect of Simultaneous SV_{40} Virus Infection on Replication of Adenovirus 7 (Pinckney) in Hamster Kidney Cells. Exp 6.

Normal hamster kidney cell culture				
	Inoculum	Observation (A)		Adenovirus titer measured on subpassage in assay HEK (B)
Virus	$TCID_{50}$	Period (days)	Cytopathology	$TCID_{50}$/0.1 ml at 35 days
Adenovirus 7	$10^{7.5}$	13	±*	$10^{1.3}$
" " plus SV_{40}	$10^{7.5}$ + $10^{5.9}$	"	"	$10^{1.3}$
SV_{40}	$10^{5.9}$	"	0	None
None	0	"	"	"

* Cytopathology was of the adenovirus type.

original inoculum would have been inactivated during the incubation period at 36°C. Slow growth of RS virus strains in primary or secondary HEK cells with minimal CPE was reported by Bennett *et al*(17) and by Beem *et al*(18).

Experiment 5. (Table VII). The ability of reovirus 1 to "rescue" adenovirus from Pinckney adenovirus 7 hamster tumor cell line C1 was measured. The reovirus which was added as a "helper" virus caused total destruction of the C1 tumor cells within 6 days(A). Subpassage of the freeze-thawed (A) cultures to HEK assay cell cultures (B) in the presence of reovirus 1 antiserum failed to reveal the presence of adenovirus. Again, the assay cells retained their susceptibility to adenovirus upon challenge. It was concluded that superinfection of adenovirus tumor cells with cytopathogenic reovirus 1 did not bring about release of infectious adenovirus from the C1 tumor line.

Effect of SV_{40} virus on replication of adenovirus in normal hamster cells. Experiment 6. (Table VIII). An experiment was carried out to ascertain whether SV_{40} virus would facilitate propagation of adenovirus in hamster cell cultures as had been shown for monkey renal cell cultures(19-22). Normal hamster kidney cultures were simultaneously inoculated with adenovirus type 7 strain Pinckney and SV_{40} virus or each virus alone. The cultures were observed daily for cytopathic effect throughout 13 days of incubation at the end of which time the cultures were freeze-thawed and titrated in HEK assay cultures. Adenovirus type 7 virus inoculated either alone or in the presence of added SV_{40} virus produced a minimal cytopathic effect after 3 days and this did not progress. The small amount of adenovirus detected by subpassage on the thirteenth day after inoculation may have been residual virus from the initial inoculum or might have been derived from minimal adenovirus replication in the hamster kidney cells. It was concluded that the SV_{40} virus did not enhance the replication of adenovirus type 7 in normal hamster kidney cells.

Discussion. The present experiments were undertaken to attempt release of infectious virus from adenovirus tumor cells of ham-

sters which were carried in cell culture. Infectious virus had never been detected in any of the tumor cell lines during a long and exhaustive test period. The cultures did, however, carry "T" antigen marker which is considered indicative of the continued presence of viral genetic material. The choice of tumors in the present experiments was purposeful in attempting to simulate the hypothetical situation as it might apply to adenovirus-caused tumor in man.

The methods employed for testing for infectious virus in the experiments were aimed at maximizing the chance for demonstrating infectious virus, especially through employment of long incubation periods. The viruses which were chosen to act as "helper" agents for rescue purpose were selected for various reasons. SV_{40} virus was employed because of its known activity in making possible or in enhancing replication of adenoviruses in monkey renal cell cultures(19-22), in potentiating the oncogenicity of adenovirus type 12(23) and because of its demonstrated ability to hybridize with adenoviruses(24-27). Respiratory syncytial virus was chosen because of the frequent association of adenovirus and RS viruses in patients with acute respiratory illnesses(28). This suggested possible evoking of latent adenoviruses by the infection with RS virus. Reovirus 1 was chosen because it propagates readily in hamster cells in culture and because it has an unusual association with the mitotic apparatus of the host cell(29,30). During replication, the reovirus antigen apparently has an affinity for the spindle and centriole material and can be carried along through mitosis. Reovirus type 3 shares a number of properties with the wound tumor virus of plants, one of which is double-helical RNA(31), and has been recovered from cell cultures of Burkitt's lymphoma(32) though its relationship to human tumor is unknown.

It has been reported that certain adenoviruses may undergo an abortive growth cycle in monkey renal cells with formation of "T" antigen but not of infectious virus(22). Adenovirus 7 probabbly undergoes such abortive replication in primary hamster renal cells in culture since slight cytopathic effect may develop on primary virus passage and since "T" antigen has been detected by fluorescent antibody staining in hamster embryo cells infected with the virus(12). In contrast to the monkey kidney cell system, added SV_{40} virus failed to enhance propagation in hamster cells.

One factor of importance for any "helper" virus to enhance replication or to "rescue" residual genome of another virus in a particular cell might be related to the ability of the "helper" to replicate freely in that cell. Thus, the RAV "helper" of Rous sarcoma genome (14) in chick embryo and SV_{40} "helper" of adenovirus in monkey kidney culture both propagate freely in the cell in which the assistance is observed. In the adenovirus tumor cells employed in the present study, SV_{40} and RS viruses replicated poorly, if at all, while reovirus 1 was replicated freely. SV_{40} virus also fails to cause lysis or to produce cytopathology in normal hamster cells(6,33-35).

The ability of a "helper" virus to replicate in the host cell may not be the only requisite for successful "rescue" or enhanced propagation since it has been shown that measles and herpes simplex viruses, which reproduce freely in monkey kidney cells, do not enhance the growth of adenovirus 7 in these cells(22). Further, virus growth enhancement may be quite virus-specific since it has been shown that herpes simplex, rabbit papilloma, vaccinia, human wart and measles viruses all fail to enhance replication of adenovirus in monkey renal cells(22,27).

The findings in the present study provide convincing evidence for the failure of the various candidate "helper" viruses to "rescue" residual adenovirus genetic material from adenovirus-induced tumor cells in the form of infectious adenovirus. It may be speculated that the viral genetic material retained in the tumor cell which is sufficient to produce "T" antigen may not be sufficient to replicate the infectious virus or is too highly "integrated" in the cell to be capable of rescue. The possibility remains, however, that agents other than those tested might have been capable of adenovirus rescue. Whatever the factors involved, it appeared quite clear in the present studies that

the candidate "helper" viruses employed failed to afford sufficient or proper complementation or facilitation to effect release of infectious adenovirus from hamster tumor cells induced by types 7 or 18 adenovirus.

Summary. Cell culture lines of virus-free hamster tumors which were initiated by adenovirus 7 (Pinckney and Grider) or by adenovirus 18 and which synthesized "T" antigen were tested exhaustively for release of infectious adenovirus following addition of candidate "helper" viruses including SV_{40}, respiratory syncytial virus and reovirus type 1. Attempts to detect adenovirus were by copropagation with susceptible indicator cells and by subculture of supernatant fluid or of disrupted cells in a sensitive assay cell system. In no case was infectious adenovirus recovered. The data permit speculation that the adenovirus genetic material retained in the tumor cells which was sufficient to code for "T" antigen might have been too highly integrated in the cell or might have been insufficient in amount to produce infectious virus. Alternatively, the "helper" viruses chosen might not have been appropriate to demonstrate the effect. Whatever the factors involved, it appeared quite clear that SV_{40}, RS and reovirus 1 agents failed to afford sufficient or proper complementation or facilitation to effect release of infectious adenovirus from adenovirus 7 and 18 tumor cells of hamsters.

1. Habel, K., Yale J. Biol. & Med., 1965, v37, 473.
2. Sjögren, H. O., Hellström, I., Klein, G., Cancer Res., 1961, v21, 329.
3. Habel, K., Proc. Soc. Exp. Biol. and Med., 1961, v106, 722.
4. Huebner, R. J., Rowe, W. P., Turner, H. C., Lane, W. T., Proc. Nat. Acad. Sci., 1963, v50, 379.
5. Habel, K., Virology, 1965, v25, 55.
6. Black, P. H., Rowe, W. P., ibid., 1965, v27, 436.
7. Tevethia, S. S., Katz, M., Rapp, F., Proc. Soc. Exp. Biol. and Med., 1965, v119, 896.
8. Gilden, R. V., Carp, R. I., Taguchi, F., Defendi, V., Proc. Nat. Acad. Sci., 1965, v53, 684.
9. Larson, V. M., Girardi, A. J., Hilleman, M. R., Zwickey, R. E., Proc. Soc. Exp. Biol. and Med., 1965, v118, 15.
10. Goldner, H., Girardi, A. J., Larson, V. M., Hilleman, M. R., ibid., 1964, v117, 851.
11. Black, P. H., Rowe, W. P., Turner, H. C., Huebner, R. J., Proc. Nat. Acad. Sci., 1963, v50, 1148.
12. Pope, J. H., Rowe, W. P., J. Exp. Med., 1964, v120, 577.
13. Temin, H. M., Proc. Nat. Acad. Sci., 1964, v52, 323.
14. Rubin, H., J. Cell. Comp. Physiol., 1964, v64, 173.
15. Landau, B. J., Larson, V. M., Devers, G. A., Hilleman, M. R., to be published.
16. Shein, H. M., Enders, J. F., Proc. Soc. Exp. Biol. and Med., 1962, v109, 495.
17. Bennett, C. R., Jr., Hamre, D., J. Infect. Dis., 1962, v110, 8.
18. Beem, M., Wright, F. H., Hamre, D., Egerer, R., Oehme, M., New Eng. J. Med., 1960, v263, 523.
19. Rabson, A. S., O'Conor, G. T., Berezesky, I. K., Paul, F. J., Proc. Soc. Exp. Biol. and Med., 1964, v116, 187.
20. Beardmore, W. B., Havlick, M. J., Serafini, A., McLean, I. W., Jr., J. Immunol., 1965, v95, 422.
21. Feldman, L. A., Melnick, J. L., Rapp, F., J. Bact., 1965, v90, 778.
22. Feldman, L. A., Butel, J. S., Rapp, F., ibid., 1966, v91, 813.
23. Schell, K., Lane, W. T., Casey, M. J., Huebner, R. J., Proc. Nat. Acad. Sci., 1966, v55, 81.
24. Rowe, W. P., Baum, S. G., ibid., 1964, v52, 1340.
25. Huebner, R. J., Chanock, R. M., Rubin, B. A., Casey, M. J., ibid., 1964, v52, 1333.
26. Boeye, A., Melnick, J. L., Rapp, F., Virology, 1966, v28, 56.
27. O'Conor, G. T., Rabsin, A. S., Malmgren, R. A., Berezesky, I. K., Paul, F. J., J. Nat. Cancer Inst., 1965, v34, 679.
28. Hilleman, M. R., Hamparian, V. V., Ketler, A., Reilly, C. M., McClelland, L., Cornfeld, D., Stokes, J., Jr., J.A.M.A., 1962, v180, 445.
29. Lenahan, M. F., Wenner, H. A., J. Infect. Dis., 1960, v107, 203.
30. Spendlove, R. S., Lennette, E. H., John, A. C., J. Immunol., 1963, v90, 554.
31. Gomatos, P. J., Tamm, I., Proc. Nat. Acad. Sci., 1963, v50, 878.
32. Bell, T. M., Massie, A., Ross, M. G. R., Williams, M. C., Brit. Med. J., 1964, v1, 1212.
33. Ashkenazi, A., Melnick, J. L., J. Nat. Cancer Inst., 1963, v30, 1227.
34. Rabson, A. S., Kirschstein, R. L., Proc. Soc. Exp. Biol. and Med., 1962, v111, 323.
35. Black, P. H., Rowe, W. P., Proc. Nat. Acad. Sci., 1963, v50, 606.

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