Studies of Oncogenicity of Adenovirus Type 7 Viruses in Hamsters.* (29744)

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Adenovirus type 7 is a principal cause for epidemic acute respiratory illness in military recruit populations(1,2) and is a frequent cause for respiratory tract infection among civilians(3). By adulthood, a majority of persons present serologic evidence of prior infection with this virus(2).

Renewed interest in this particular agent followed the demonstration by this laboratory (4) of tumor induction in newborn hamsters by all of 3 strains of type 7 adenovirus tested. The present report summarizes the findings in extensive investigations to measure the oncogenic quality of type 7 adenoviruses and of the virologic, pathologic and immunologic findings in the adenovirus 7-hamster system.

Materials and methods. Cell cultures. Primary cell cultures of human embryonic kidney (HEK) were purchased from commercial sources. During virus propagation, the cultures were maintained in Basal Medium Eagle (BME) containing 5% calf serum. Primary cell cultures of grivet monkey kidney (GMK) were prepared from trypsinized cell suspensions and were propagated in Melnick's lactalbumin-yeast extract medium containing 5% heat inactivated calf serum. The cells were maintained in medium 199 containing 2% inactivated chicken serum. *Viruses.* The type 7 adenoviruses used in the studies of oncogenicity, *viz.*, Pinckney, Grider, and Champagne, were recovered from frozen and stored respiratory secretions of a child (Pinckney—Children's Hospital of Philadelphia, 1959) or from military recruits (Grider and Champagne, Fort Leonard Wood, 1957) with acute respiratory illness. The cultures were harvested when essentially all cells showed cytopathic change and they were frozen and thawed once to facilitate release of virus. All suspensions were centrifuged at 2,000 rpm for 20 minutes to remove cellular debris. The recovery of Pinckney virus from human specimen was described earlier(4). Pinckney-1 virus was passed 3 times in HEK and 3 times in human diploid cell strains, Wistar(4). Pinckney-2 was passed a total of 3 times in HEK, using, as starting material, the same first HEK passage as Pinckney-1. Details relating to the isolation, identification and properties of the strains are presented in the text. Adenovirus 12 strain Huie (fresh isolate) was recovered from patient's stool as reported previously (4). Type 18 adenovirus strain 14606 was obtained from Dr. R. Chanock and type 7a (S-1058) from Dr. W. Rowe. Adenovirus type 7 strain Goldberg (RI-4-202), type 4 (RI-67), and type 3 (Armstrong) were brought to this laboratory by one of us (MRH). Type 10 strain JJ and type 6 strain "tonsil 99" were from the files of this department. Prototype SV_{40} strain VA 45-54 was recovered here(5). *Serologic procedures.* The *complement-fixation* (CF) test was carried out by methods ordinarily used in this laboratory(6) except that the microtechnic (7) as applied to study of tumor CF antigen by Huebner *et al*(8) was employed. *Hemagglutination* tests with rhesus monkey and rat erythrocytes and hemagglutination-inhibition tests with rhesus red blood cells were performed according to Rosen(9), except that the micro-modification(7) was used for hamster sera. Serum neutralization tests were carried out by ordinary procedures in HeLa or HEK cell cultures, employing 100 TCD_{50} of virus.

Experimental design. Pregnant hamsters obtained from Lakeview Hamster Colony, Newfield, N. J., were housed individually in

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isolation filter cages.‡ Newborn hamsters less than 24 hours of age were inoculated with 0.2 ml of undiluted virus subcutaneously into the nuchal region and with 0.05 ml into the thoracic cavity with intent to expel the virus into the lungs. Animals for control purpose were given extracts from noninfected cell cultures passed serially in the same lots of cells used to prepare the virus pools. The baby hamsters were weaned at 18 days of age, housed in filter cages during the first 2 months following inoculation, and then transferred to ordinary cages. The animals were observed at least once each week for development of palpable masses. The cages were examined twice daily for dead animals and autopsies were performed on those which had died. The animals were sacrificed and the tumors removed as appropriate and as described in the text. Hamsters which received tumor transplant material were housed in conventional cages and observed in the manner described above. Sera were collected and the tumor tissue was submitted for histopathologic diagnosis, transplanted into new hamsters, or suspensions were prepared for virus isolation or complement-fixation tests. Tissues for *histopathology* were fixed in 10% formalin and the paraffin sections were stained with hematoxylin-eosin. Tumors for *transplant* into newborn or suckling hamsters were finely minced in Hanks' balanced salt solution (BSS) and inoculated *via* hypodermic needle into the dorsal subcutaneous space. Tumor was implanted into 1- to 3-month-old hamsters *via* a trocar. *Tumor homogenate* was prepared for serologic and virus recovery purpose. The tumors were usually homogenized for 10 minutes at 16,000 rpm in an Omnimix cup held in an icebath, employing sufficient Hanks' BSS to give a 15% suspension. On occasion, small tumors were triturated in Ten Broek grinders or with mortar and pestle. The homogenates were centrifuged at 2,000 rpm for 20 minutes and the supernates examined for presence of CF antigen and infectious virus. Normal hamster muscle antigen for control purpose was prepared in the same manner. All tumor antigens and antisera were prepared here except for polyoma HE-11 reagents kindly furnished by Dr. K. Habel. Attempts to *recover virus* from tumor were carried out using cultures of HEK and GMK. The HEK cultures were observed for about 60 days before discard. The GMK cultures were observed for approximately 30 days at which time transfer was made to fresh cultures and observation was continued for approximately 30 additional days. *Cell cultures* of Pinckney-2, adenovirus 18 and SV_{40} hamster tumor were prepared by the general procedure described earlier(10). To prepare tumor cell culture antigen for CF tests, the cultures were washed with pH 7.2 phosphate buffered saline (PBS), trypsinized, resuspended in PBS and the cells collected by centrifugation. Ten to 20% suspensions of cells were prepared (V/V) in PBS, and frozen and thawed 3 times. The clarified supernate was the antigen.

Results. Tumor induction by type 7 adenoviruses. Table I presents the findings to date in tests to measure the oncogenic potential of adenovirus type 7 strains. All 3 viruses, including 2 separate virus pools of strain Pinckney, were tumorigenic. The tumor incidence was consistently low for all 3 agents, (7 to 21%) and the latent period was long (155 to 405 days). Within the limits tested, the amount of virus given did not appear to influence tumor incidence or time of occurrence. None of the animals which received placebo control material developed tumor during 13 months of observation. *Transplantability of adenovirus 7 tumor* (Table II). Pinckney-2 tumor was carried through 6 transplant passages in newborn, suckling or adult hamsters. Positive takes were obtained in essentially all recipients and the tumors usually appeared within 3 weeks following transplantation. Tumor brei stored at —70°C as a 10% suspension in BME containing 10% glycerol and 10% calf serum retained its capability of initiating tumors in hamsters. The findings in studies to compare the time of appearance of primary or first transplant tumor are summarized in Table III. There was no apparent difference between strains. The primary tumors required 155 to

‡ Modification of the basic design by Dr. L. Kraft, Yale University, for work with gastroenteritis and newborn mice.

322 days to appear with an average of about 230 days. First transplant tumors, by contrast, developed rapidly in 12 to 24 days in suckling and in 13 to 60 days in adult hamsters. It appeared that suckling hamsters, on the average, developed palpable tumor more rapidly than did adults. Additionally, the tumor transplant sometimes failed to take in adult animals.

Tumor pathology. The primary tumors which developed were usually located in the dorsal subcutaneous space in close proximity

TABLE I. Induction of Tumor in Hamsters Inoculated Subcutaneously when Newborn with Type 7 Adenoviruses.

Virus used			Tumor occurrence in hamsters				
Strain	Passage No.*	Infectivity titer in HEK (0.2 ml)	No. inoc	No. weaned	No. non-cancer deaths	No. with tumor/Total survivors (%)	Tumor appearance range (days)
Pinckney–1	HEK 3/HDCS 3	$10^{-5.5}$	30	30	11	3/19 (16)†	231–405‡
Control	" "	—	31	31	6	0/25 (0)	—
Pinckney–2	HEK 3	$10^{-7.0}$	63	60	22	8/38 (21)	155–299
Control	"	—	67	50	19	0/31 (0)	—
Grider	HEK 2	$10^{-7.5}$	55	45	19	5/26 (19)	201–321
Champagne	"	$10^{-8.0}$	74	61	20	3/41 (7)	228–292
Control	"	—	78	65	16	0/49 (0)	—

Pinckney–1 animals were inoculated subcutaneously. All others were inoculated subcutaneously and into the thorax.

* HEK = Primary human embryonic kidney; HDCS = human diploid cell strains, Wistar.

† Survivors including all animals which developed tumor at a time when tumorigenesis was complete.

‡ One of 21 additional animals inoculated intrathoracically developed tumor at day 231.

TABLE II. Serial Passage of Adenovirus Type 7 (Pinckney–2) Tumor in Hamsters.

Passage No. of tumor used as inoculum	Age of hamster recipients	No. developed tumor/No. inoculated	Day of first tumor detection
Primary tumor	<24 hr	13/13	12 days
1st passage	1–3 mo	6/6	8–22
" " *	"	3/5	41–99
" " *	<24 hr	26/26	18–24
2nd "	1.5 mo	5/5	21
3rd "	11 days	4/5	9–22
4th "	1 mo	5/5	12
5th "	1–3 mo	5/5	6
6th "	1–3 mo	5/5	12

* Tumor brei stored frozen –70°C was used.

TABLE III. Time of Appearance of Palpable Primary or Transplant Adenovirus Type 7 Tumor in Hamsters Following Inoculation with Virus or Transplant Tumor Tissue.

Type 7 adenovirus strain	Passage	Age of hamster recipients	Time following virus or transplant		
			No. tumors	Range (days)	Avg (days)
Pinckney–2	Primary (virus)	Suckling	8	155–299	225
	First transplant	Suckling	24	12– 21	14
		Adult	8	13– 56	23
Grider	Primary (virus)	Suckling	5	201–322	224
	First transplant	Suckling	13	24	24
Champagne	Primary (virus)	Suckling	3	228–292	252
	First transplant	Suckling	14	18– 20	20
		Adult	8	18– 60	37

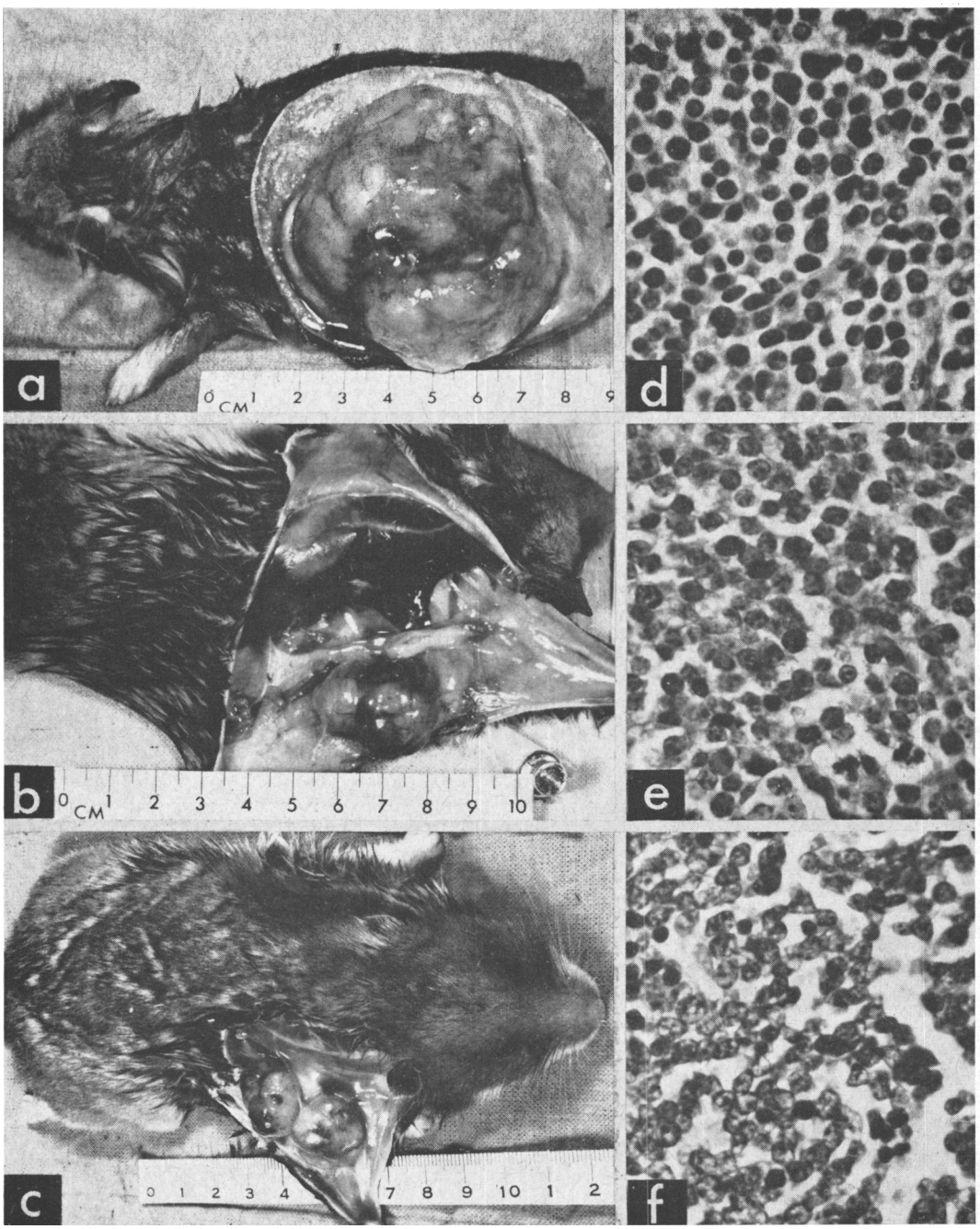

FIG. 1. Gross and micropathology of tumors induced in hamsters by type 7 adenoviruses. a. Pinckney-2 tumor *in situ* 292 days following virus. b. Grider tumor *in situ* 287 days following virus. c. Champagne tumor *in situ* 251 days following virus. d. Malignant lymphoma, 19 days following transplant with 7th cell culture passage of Pinckney-2 tumor. H & E. 500 × magnification. e. Malignant lymphoma, 34 days following implantation of Grider primary tumor. H. & E. 500 × magnification. f. Malignant lymphoma, 251 days following inoculation of Champagne virus. H & E. 500 × magnification.

TABLE IV. Pathologic Diagnoses Among Type 7 Adenovirus Tumors of Hamsters.

Pathologic type of tumor	Primary tumors induced by virus				Transplant tumors			
	Pinck-ney–1	Pinck-ney–2	Grider	Cham-pagne	Pinck-ney–1	Pinck-ney–2	Grider	Cham-pagne
Undifferentiated sarcoma	4*	0	0	0	9	0	0	0
Idem resembling malignant lymphoma	0	2	0	0	0	1	1	0
Malignant lymphoma	0	6	4	2	0	17†	6	2
Lymphosarcoma	0	0	1	1	0	0	1	0

* Includes one tumor which occurred in subcutaneous site following intrathoracic inoculation.
† Five of these tumors were derived from transplant of tissue culture passage cells.

to the site of deposition of virus inoculum. One animal which received Pinckney-1 virus by the thoracic route only(4) developed a subcutaneous tumor at the corresponding site. One animal which was given Champagne virus developed a lung tumor at the approximate inoculation site. Malignancies developed both in male and female animals and, except for one instance, were single. Once palpable, the tumors grew at various rates necessitating early harvest in some animals or permitting extension of observation for as long as 3 months before sacrifice in others.

Fig. 1 shows examples of primary tumor in hamsters which received the Pinckney-2, Grider or Champagne adenovirus strain and the histopathology of primary or transplant tumor caused by these agents. *Gross pathology.* The tumors at autopsy appeared grossly as soft, whitish, amorphous masses which usually were nonadherent. Cut surfaces presented a homogeneous, dull, white appearance and it was not unusual to observe central necrosis and hemorrhage. Metastases were not found in animals bearing primary tumors. *Histopathology.* The adenovirus 7 primary and transplant tumors were composed of pleomorphic cells with scanty cytoplasm and poorly defined cell walls. Microscopically, the tumors were highly cellular. The nuclei were vesicular, usually round or oval, and were found occasionally in mitosis. Large, multinucleated giant cells were noted infrequently. The pathology was not significantly different among tumors caused by any of the 3 virus strains. Previously described tumors(4) caused by Pinckney-1 adenovirus were designated undifferentiated sarcoma, some of which presented areas suggestive of lymphoma. Sections from more recent tumors showed sufficient differentiation to permit a diagnosis of malignant lymphoma or, when invasion of contiguous tissue was evident, lymphosarcoma. The pathologic diagnoses among 20 primary and 37 transplant adenovirus 7 tumors are summarized in Table IV. There did not appear to be any appreciable difference between the distribution of the kinds of tumors caused by the 3 different viruses, either on primary induction or on transplantation. *Serologic studies of adenovirus 7 hamster tumors.* As noted previously (4), tumors induced by type 7 adenoviruses commonly contained CF antigen and sera from hamsters bearing such tumors commonly presented antibody against such antigen. The tumor antigen preparations were often highly anticomplementary, making them unsuitable for test purpose. In certain instances this appeared to be due to union of antibody with antigen in the tumor in the animal and it was necessary to screen tumor preparations to find those which were suitable for study purpose. Table V presents the representative findings in a large series of tests to compare the CF antigens and antibodies formed in response to adenovirus type 7, 12, 18 or SV_{40}, or polyoma tumor. The antigens and antibodies formed in response to type 7, Pinckney-2, Grider or Champagne material were serologically homogeneous and quite distinct from adenovirus 12 or 18 or papovavirus (SV_{40} or polyoma) antigens. Type 7 tumor or tumor cell culture antigens showed no relationship to type 12 or 18 antigen, even though all these viruses were tumorigenic.

Figs. 2, 3 and 4 summarize some of the relationships between time, tumor weight, tumor antigen content and serum antibody titer observed in studies of the type 7 adenovirus

hamster system. Figure 2 shows that less than half of the hamsters developed detectable CF antibody to transplant tumor by the time of autopsy. Antibody appeared as early as 18 days following transplant. Hamsters which received transplant as adults appeared somewhat more efficient in producing antibody than did those which were given transplant when of suckling age. There was no apparent difference between virus strains. Fig. 3 shows that there was generally more tumor antigen and greater antibody titer in animals with large primary tumors than in those with small primary tumors. Such dif-

TABLE V. Virus Strain-specific Complement Fixation Reactions Obtained in Tests with Antigens of Several Primary and Transplant Tumors of Hamsters and Their Corresponding Antisera.

Tumor antigen: Virus		Tumor antigen: Transplant or tumor cell culture passage		CF antigen titer obtained in tests with tumor-bearing hamster serum							
				Adeno type 7			Adeno type 12	Adeno type 18			
Type	Strain	Kind	Passage No.	Pinkney–2	Grider	Champagne	Huie	14606	SV_{40}	VA 45–54	Polyoma HE–11
7	Pinckney–2	Hamster X'plant	2nd	2	2	8	0	0	0	0	0
"	"	Cell culture	19th	16	16	16	0	0	0	0	0
"	Grider	Hamster X'plant	2nd	2	2	4	0	0	0	0	0
"	"	Cell culture	12th	4	4	4	0	0	0	0	0
"	Champagne	Hamster X'plant	2nd	4	4	4	0	0	0	0	0
12	Huie	" "	1st	0*	0	0	16	8	0	0	0
18	14606	" "	"	0	0	0	16	64	0	0	0
"	"	Cell culture	7th	0	0	0	16	32	0	0	0
SV_{40}	VA 45–54	" "	19th	0	0	0	0	0	16	0	0
Polyoma	HE–11	Hamster	Unknown	0	0	0	0	0	0	0	4 or >
Control	—	Normal hamster muscle tissue	—	0	0	0	0	0	0	0	0

* Zero titer = <1:4 except for tests with type 18 adenovirus tumor antigen in which antigen was anticomplementary at 1:8 and 0 = <1:16.

ference was less marked in animals which developed tumor following transplant. Fig. 4 reveals a general correspondence between the amount of antigen in primary tumor and the level of antibody in the host. Such correspondence was not apparent in animals with transplant tumor. There was no evident difference between viral strains or between age at transplant.

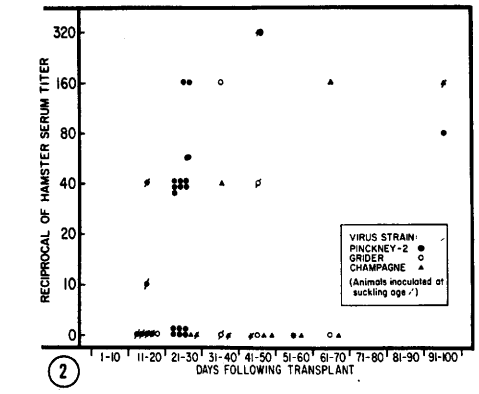

FIG. 2. Relationship between time following transplant and CF titer in hamsters.

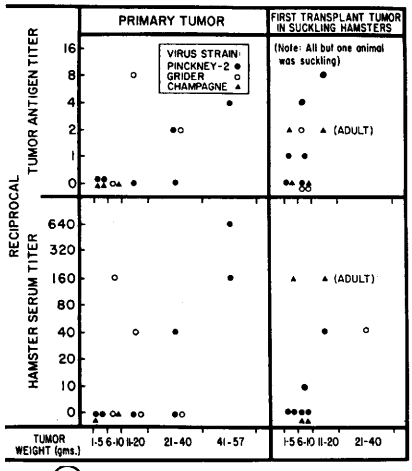

FIG. 3. Relationship between weight of tumor and titer of tumor antigen or host antibody as measured in the hamster-adenovirus 7 system.

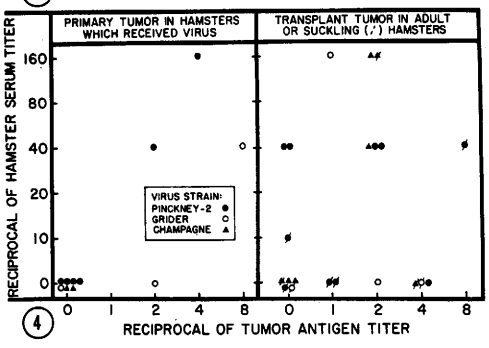

FIG. 4. Relationship between CF titers of antigen and antibody in individual hamsters bearing primary or transplant adenovirus 7 tumors.

In addition to CF tests, the sera of hamsters bearing primary or transplant adenovirus 7 tumors derived for all 3 strains were tested for neutralizing antibody against adenovirus type 7 (33 sera) and for hemagglutination-inhibitory antibody against adenovirus types 3 and 7 virus (24 sera) by ordinary procedures. Neither kind of antibody was detected at 1:5 or 1:10 dilution of serum and this included animals with tumors weighing as little as 1.5 g or as much as 56.8 g.

Attempts to recover virus from type 7 adenovirus tumors. Attempts were made to recover virus from 3 Pinckney-2, one Grider and 2 Champagne primary tumors and from one first transplant Pinckney-2 tumor using primary HEK and GMK cell cultures for propagation. The total time on test ranged from 56-61 days and no virus was recovered. Additionally, sixth passage cell culture of Pinckney-2 tumor was cultivated simultaneously with HEK for 2 passages by the feeder technic of Gerber(11). No virus was found during the 89-day test period.

Identification and characterization of the type 7 adenovirus strains. Table VI summarizes the findings in serum neutralization tests to prove the identity of the type 7 virus strains employed. Standard reference antisera prepared in rabbits in these laboratories or obtained from Dr. Rowe (type 7a) and the paired sera from patient Pinckney were used. The type 7 isolate revealed the appropriate identity even when 10,000 TCD_{50} of virus were used and the tests with homologous serum were held for 14 to 26 days, a time period well beyond that when cytopathic effect was observed in cultures inoculated with virus but without antiserum. No other virus was detected in the cultures and the type 7 antisera used did not neutralize either type 12 or 18 adenovirus. Types 12 and 18 adenovirus showed "one-way" cross. Tests for presence of SV_{40} virus were performed in GMK cell cultures and were not considered

TABLE VI. Cross-neutralization Tests with Several Adenovirus Strains.

Adenovirus tested		Findings in tests with adenovirus antisera						
		Type 7				Type 7a	Type 12	Type 18
Type	Strain	Goldberg (rabbit—20 units*)	Stanfield (rabbit—20 units)	Pinckney (human) Acute 1:5	Pinckney (human) Convalescent 1:10	(Rabbit‡—20 units)	Huie (rabbit—64 units)	D.C. (rabbit—64 units)
7	Pinckney–2	+†	+	0	+	+	0	0
"	Grider	+	+	0	+	+	0	0
"	Champagne	+	+	0	+	+	0	0
"	Goldberg	+	+	0	+	+	0	0
7a	S–1058	+	+	0	+	+	0	0
12	Huie	0 †	0	0	0	0	+	0
18	14606	0	0	0	0	0	+	+

* A unit was the least amount of antibody required to neutralize 100 TCD_{50} of homologous virus.
† + indicates neutralization, 0 indicates failure to neutralize.
‡ Serum obtained from Dr. W. Rowe.

to be negative until 56 days after inoculation of undiluted adenovirus-serum mixtures.

Hemagglutination tests were performed with rhesus monkey and rat erythrocytes to fix the hemagglutination group of the tumorigenic adenovirus 7 strains (Table VII). The viruses employed in the tests were propagated in HeLa cell cultures. The oncogenic type 7 strains displayed group 1 hemagglutination typical for that of type 7 viruses and differed from that of the other groups including oncogenic type 12 virus which failed to cause agglutination of either rhesus or rat erythrocytes.

Discussion. Present information relative to virology, oncology, pathology, and immunology of type 7 adenovirus tumors is summarized in Table VIII. Tumorigenicity for hamsters appears to be a common, if not uniform quality for type 7 adenoviruses. Failure by others(12,13) to demonstrate tumor induction by type 7 virus might be due to failure to hold and observe the inoculated animals for the necessary time period for tumor to appear and also to the apparent small numbers of animals employed in the tests.

The type 7 adenoviruses exhibit ordinary properties of ordinary type 7 virus including type-specificity in serum neutralization tests and hemagglutination of rhesus monkey but not rat erythrocytes(14). Green and Pina (15) have reported that adenovirus types 12 and 18 share with the polyoma and Shope papilloma papovaviruses the quality of low guanine-cytosine (48-49%) amount compared with adenovirus types 2 and 4 (56-57%). More recently, Green stated(16) that the guanine-cytosine content of oncogenic type 7 adenovirus is intermediate between that of the 2 values noted above. Such finding, if substantiated, might provide evidence for a chemical difference between oncogenic

TABLE VII. Hemagglutination Characteristics of Adenovirus Strains.

Adenovirus tested			Hemagglutination titer		Summary	
Hemagglutination group	Virus Type	Virus Strain	Rhesus monkey	Rat	Rhesus monkey	Rat
1	3	Armstrong	320	0*	+	0
	7	Pinckney–2	80	0	+	0
	"	Grider	320	0	+	0
	"	Champagne	320	0	+	0
	"	Goldberg	160	0	+	0
2	10	JJ	0	320	0	+
3	4	RI–67	0	160 (partial†)	0	±
	6	Tonsil 99	0	320 (")	0	±
Nonhemagg	12	Huie	0	0	0	0

* 0 = <1:20. † Partial agglutination, all dilutions.

type 7 and other adenoviruses. Type 7 virus tumor to date, like that for adenovirus 12 and 18(13,17), has failed to reveal presence of infectious virus. This is unlike most other virus-induced tumors which commonly, or at least on rare occasions(18,19), present detectable virus. Adenovirus 7 tumor incidence was very low and the latent period rather long, as for SV_{40}(19,20).

The tumors induced by type 7 adenovirus, like those for adenovirus 12 and 18(12,13), were highly malignant and readily propagable on transplant in hamsters and on passage in cell cultures. Adenovirus 12 tumors induced in hamsters by Trentin *et al*(12) were regarded as undifferentiated sarcomas and the types 12 and 18 adenovirus tumors recorded by Huebner *et al*(13) were considered to be "primitive undifferentiated mesenchymal neoplasms with some definite epithelioid characteristics." Adenovirus type 12 tumor induced in hamsters was regarded by Pereira *et al*(21) as a lymphosarcoma suggestive of reticulosarcoma. Type 7 tumors of hamsters seen in the present study were generally sufficiently differentiated to permit the designation of malignant lymphoma, or lymphosarcoma when evidence for invasiveness was presented.

As for many virus-induced tumors, type 7 adenovirus tumor commonly presented a specific antigen of low titer which reacted in CF tests with sera from hamsters bearing homologous tumor from any of the 3 strains. There was no cross-reaction with other tumor systems such as adenovirus 12 or 18, SV_{40} or polyoma. Tumors generally gained in antigen with increase in time and size and

TABLE VIII. Summary, Properties of Oncogenic Type 7 Adenoviruses.

1. *Identification of oncogenic type 7 viruses*
   a) Exhibit proper type-specificity in tests with homotypic and heterotypic antisera.
   b) Fail to show cross-reaction in neutralization tests with oncogenic adenovirus type 12 or 18.
   c) Belong to hemagglutination group 1 (unlike types 12 and 18 which fail to cause agglutination).
2. *Oncogenicity of type 7 adenoviruses for hamsters*
   a) All of 3 strains tested proved oncogenic.
   b) Virus not recoverable from tumors induced by type 7 adenovirus strains.
   c) Latent period for tumor appearance, 155-405 days.
   d) Only a portion of virus-inoculated animals developed tumor, 7-21%.
   e) As little as 300,000 TCD_{50} of virus induced tumor.
   f) Primary tumors were highly malignant on transplant in suckling or adult hamsters, producing palpable tumor in as few as 6 days.
   g) Primary or transplant tumor was readily propagable in cell culture with retention of malignant quality and production of CF antigen.
3. *Pathology*
   a) Nearly all tumors were subcutaneous at virus injection site. Metastases not observed grossly.
   b) Tumors grossly were soft, whitish, amorphous masses with dull white appearance of the cut surface. Tumors usually nonadherent.
   c) Individual tumor cells were pleomorphic with scanty cytoplasm and poorly defined cell walls. Nuclei were vesicular, round or oval and frequently mitotic, and multinucleated giant cells were sometimes seen.
   d) Most tumors were designated malignant lymphoma or lymphosarcoma if invasion of contiguous tissue was evident. Some tumors were mostly undifferentiated and were classed as undifferentiated sarcoma resembling malignant lymphoma. Pathologic type was retained on transplantation and in cell culture.
4. *Serology*
   a) Tumors present CF antigen reacting only with antisera from animals with homotypic adenovirus tumor caused by any of the 3 type 7 strains.
   b) In animals with primary tumor, there was generally a greater amount of antigen and antibody with increase in tumor size. Such tendency was less in transplant tumor.
   c) CF antibody against tumor antigen appeared as early as 18 days following transplant.
   d) There was a general but not absolute correspondence between amount of tumor antigen and of antibody in hamsters bearing primary tumor. Such relationship was not apparent with transplant tumor.
   e) Animals with primary or transplant tumor failed to develop viral neutralizing or hemagglutination-inhibiting antibody.

the host gained in amount of CF antibody but these relationships were not absolute. Many tumor antigen preparations were anti-complementary, possibly due to union of antibody with antigen in tumor rendering them adsorptive of complement.

The question of oncogenicity as a general property of adenoviruses may reasonably be raised in view of the proved tumorigenicity of at least 3 serologic types, *viz.*, 7, 12 and 18, and of the suggestive evidence for tumor induction by type M-1 virus(13). Large virus dose, long periods of observation, and tests in a variety of animal species will be required to clarify this point. Even more important, is the possible role of adenoviruses in tumor induction in man. While no convincing data have been produced to date, it is worthy of note that Sohier *et al*(22) and McAllister *et al*(23) have recovered type 1 adenovirus from a total of 4 solid tumors of man. The frequent finding of adenoviruses in latent form in tissues of human subjects and the chance for contamination of specimens or cultures with such virus during manipulations in the laboratory precludes definitive judgment of the significance of such data.

Summary. All of 3 strains of type 7 adenovirus isolated in primary human embryonic kidney cell cultures from respiratory secretions of human subjects with acute respiratory illness proved oncogenic for newborn hamsters. The type 7 viruses were isolated and propagated in human embryonic cell cultures and were free of extraneous agents. Detailed information relative to the virology, oncology, pathology and immunology of the type 7 adenovirus-hamster tumor system is presented. The tumors appeared after a long latent period, were highly malignant, were readily transplantable and were free of detectable infectious virus. The cells in most of the tumors were sufficiently differentiated to permit a pathologic diagnosis of malignant lymphoma, or lymphosarcoma when evidence of invasiveness was present. A majority of tumors induced by the virus presented a specific complement-fixing antigen which reacted with sera from hamsters bearing homologous tumor.

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