Tests for Oncogenicity of Viruses Under Conditions of Altered Host and Virus[1] (35481)

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It is well established that variables relating both to host and to virus may affect profoundly the development of tumor in animals inoculated with viruses (1–7). The present report describes the findings in studies to measure the enhancement of tumorigenicity for hamsters of known oncogenic viruses by thymectomy of the host and to employ the thymectomized hamster as a tool for testing for oncogenic potential of "non-oncogenic" agents. Additionally, the results of tests to measure the effect on oncogenicity of host age and of virus passage history are presented.

Materials and Methods. Hamsters. The pregnant random-bred golden Syrian hamsters were purchased from the closed breeding unit of Lakeview Hamster Colony, Newfield, New Jersey, and the offspring were used within 24 hr of birth. The adult hamsters of both sexes were obtained from the same source.

Viruses. Details with respect to type, strain, passage history, and infectivity titer of virus preparations inoculated into hamsters in the thymectomy experiments are shown in Table I. The SV_{40} and Pinckney strain adenovirus 7 viruses used in the other experiments were the same as described in Table I except that another pool of SV_{40} virus ($10^{5.9}$ $TCID_{50}$/0.1 ml) was employed. The polyoma virus was obtained from Dr. W. P. Rowe; adenovirus 3 from Dr. R. J. Huebner; adenovirus 12 (original stool suspension) from Dr. S. Kibrick; reovirus 1 from Dr. H. Wenner; and *Mycoplasma orale* 1 (N-1 strain) from Dr. L. Hayflick. The Gomen strain of adenovirus 7 was obtained from the American Type Culture Collection at the tenth passage in HeLa cells. The remaining viruses were from seed stocks maintained in these laboratories.

Cell cultures. The cell cultures employed for virus propagation were prepared in these laboratories or were obtained from commercial sources. Conventional methods for cell propagation and maintenance were used. Uninfected cell culture fluids with passage histories similar to those of the corresponding viruses served as placebo as indicated in the text.

Thymectomized hamsters. Newborn hamsters were anesthetized by chilling briefly at −18 to −20° and were thymectomized as described by Roosa *et al.* (8). Approximately ⅔ of the animals of each litter were thymectomized and the remainder were either sham-thymectomized or were not treated surgically. Postoperative care was as described by Roosa *et al.* (8) except that tetracycline–HCl (0.25 mg/ml) was added to the drinking water for the entire observation period. The hamsters were sex-segregated at 3–4 weeks of age to preclude possible restoration of immunological responsiveness in parous females by fetal thymus during pregnancy (9). Mediastinal tissue was collected from all thymectomized animals for purpose of histological examination for residual thymus tissue, unless death and autolysis prevented this.

Virus inoculation and animal observation. Unless otherwise indicated in the text, the animals were inoculated subcutaneously in the scapular region with 0.2 ml of undiluted virus or control fluid. All animals were kept in filter cages for at least 2 months before transfer to open cages, and weaning was at

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TABLE I. Viruses and *Mycoplasma* Tested for Oncogenicity in Thymectomized and Control Hamsters.[a]

Family and type	Strain	Passage history	Infectivity titer: Test system	Infectivity titer: Titer ($TCID_{50}$) neg. log_{10}/0.1 ml
Polyoma	LID-1	ME 10	ME	5.5
SV_{40}	VA 45-54	GMK 5	GMK	7.3
Adenovirus 3	15520	HDCS7/HEK2	HEK	9.0
7	Pinckney	HEK5	HEK	7.5
12	Huie	HEK6	HEK	4.6
12	Huie	HEK6	HEK	7.5
1	Adenoid 71	HeLa5/HEK2	HEK	8.0
4	Curtis	HEK5/HDCS3/ HEK3	HEK	7.5
Influenza A	PR8	Unknown + EE7	EE	7.3
A2	5548	EE7	EE	6.3
B	50-537	GMK3/EE4	EE	5.2
Poliovirus 1	Mahoney	MK?/GMK1/ LLC-MK2	GMK	8.0
ECHO 25	5125	HDCS2/GMK4	GMK	5.5
Reovirus 1	J-423	DK2/HEK3	GMK	5.5
Vaccinia	Infected calf lymph	HDCS1/CE3	CE	6.7
Mycoplasma orale 1	N-1	Mycoplasma agar or broth 55	Mycoplasma agar	7.5–8.1 CFU/ml

[a] Cell cultures: ME = mouse embryo; GMK = primary grivet monkey kidney; HDCS = human diploid cell strain; HEK = primary human embryo; DK = dog kidney; CE = chick embryo; EE = embryonated hen egg; CFU = colony forming units.

3–4 weeks of age. The animals were palpated weekly for tumors. Animals which died in the course of the experiments, as well as those which were sacrificed at the termination of experiments, were autopsied and examined for presence of tumor. The occurrence of tumors was confirmed by histopathologic examination. Tumor incidence was expressed as "percentage" of hamsters with tumor, *i.e.*, 100 times the ratio of the number of animals which developed tumor to the number of animals with or without tumor minus the number of nonspecific animal deaths. The percentages were corrected for nonspecific deaths according to standard life table procedures.

Serology. Tumors and sera from tumor-bearing test animals were examined for adenovirus 7 and SV_{40} T antigen and antibody by the microcomplement fixation (CF) test as described previously (10).

Results. Tests in thymectomized hamsters. Groups of hamsters thymectomized at less than 24 hr of age and appropriate control animals were given $10^{5.5}$ $TCID_{50}$ of *polyoma virus* subcutaneously in 0.1-ml amount at 7 days of age or $10^{6.5}$ $TCID_{50}$ of virus in 1.0 ml by the same route at 4 months of age, and were observed for development of tumor. As shown in Fig. 1, thymectomized animals inoculated at 7 days of age developed tumors earlier and in greater frequency (83%) than did sham-thymectomized (37%) or nonsurgically treated animals (10%). When hamsters were inoculated with virus at 4 months of age, the rates for tumor were 28, 13, and 7%, respectively, showing the enhancing effect of thymectomy but with lower tumor incidences than for the animals given virus when 7 days old. Histologic examination of approximately ¾ of the thymectomized animals showed that 10% were incompletely thymectomized. These animals were not excluded from the data since small amounts of residual thymus were detected as frequently among

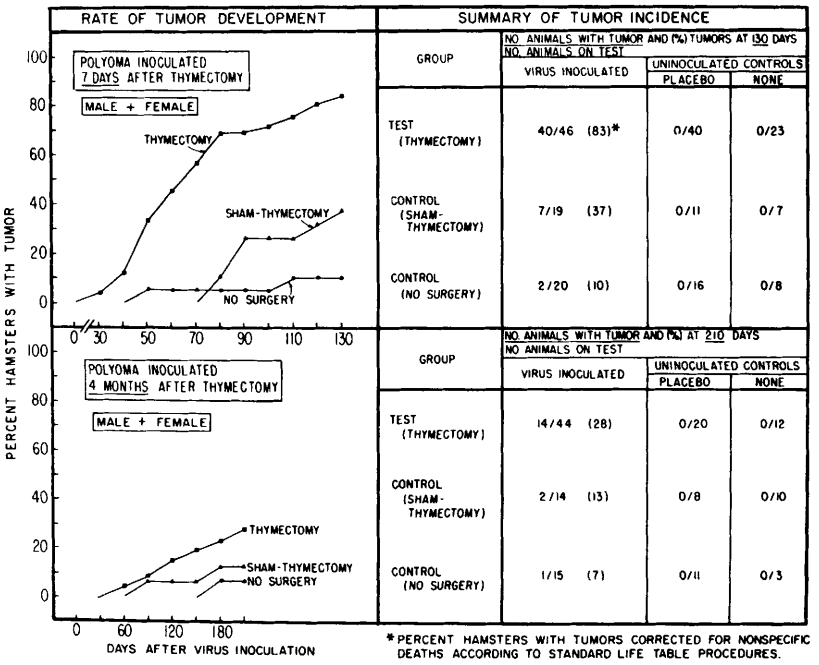

FIG. 1. Influence of thymectomy on incidence of tumors in hamsters inoculated subcutaneously with polyoma virus 7 days or 4 months after thymectomy.

animals which develop tumors as among those which did not.

The findings in similar tests in animals given $10^{7.6}$ $TCID_{50}$ of SV_{40} virus at 7 days of age are presented in Fig. 2. Definite enhancement of thymectomy on tumorigenesis was shown (%): thymectomized animals 80; sham-thymectomy, 42, and no surgery, 20. There was little, if any, difference in tumor rate between the sexes. Histologic examination of 97% of the thymectomized animals showed 11% with a small amount of thymus tissue and these animals were included in the summary of results since the majority of the animals with residual thymus developed tumors.

Additional groups of hamsters were thymectomized and inoculated with $10^{9.3}$ $TCID_{50}$ of adenovirus 3, $10^{7.8}$ $TCID_{50}$ of adenovirus 7 (Pinckney), or $10^{7.8}$ $TCID_{50}$ of adenovirus 12 within 24 hr of birth. Appropriate inoculum and surgery controls were included. The findings shown in Fig. 3 revealed a remarkable enhancing effect of thy-

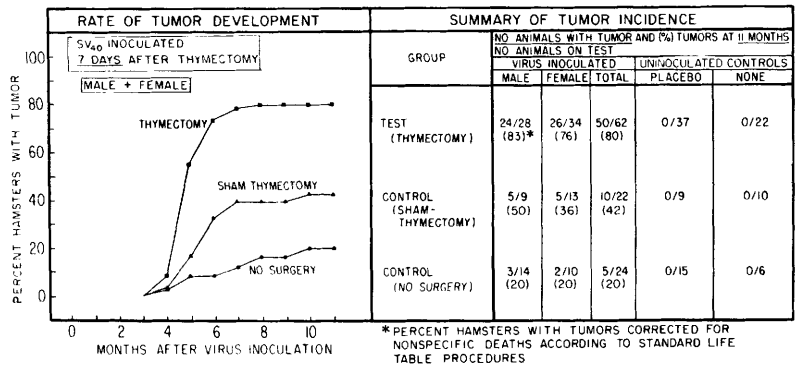

FIG. 2. Influence of thymectomy on incidence of tumors in hamsters inoculated subcutaneously with SV_{40} virus 7 days after thymectomy.

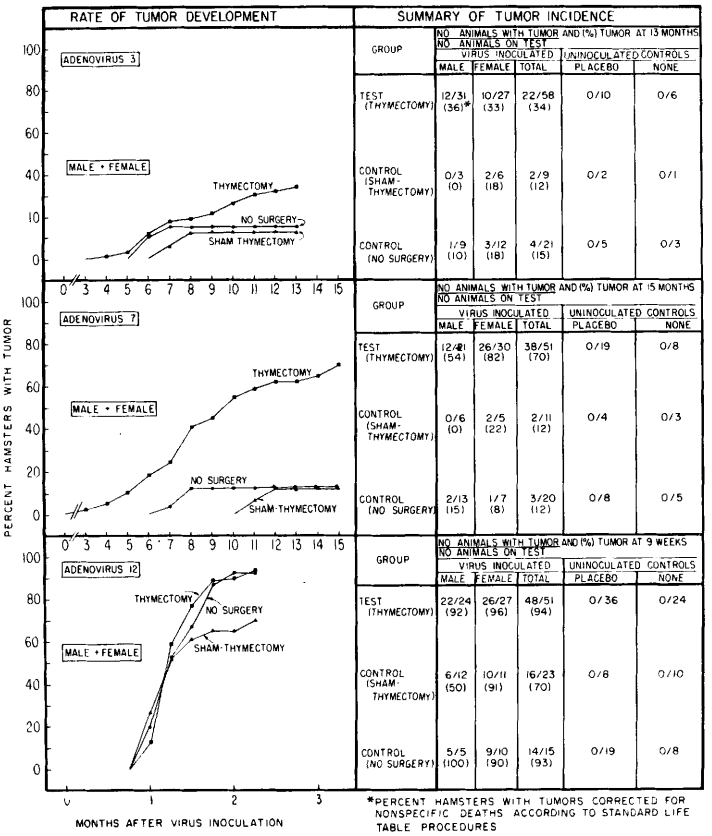

FIG. 3. Influence of thymectomy on tumor development in hamsters inoculated subcutaneously with adenovirus 3, 7, or 12 at time of surgery.

mectomy on tumor induction by the moderately oncogenic adenovirus 7 agent (70 versus 12%), and moderate enhancement for weakly oncogenic type 3 virus (34 versus 12–15%). Adenovirus 12, at this dose level, was so highly oncogenic even in nonthymectomized animals that no enhancement could be shown even if it did occur. Likewise, in similar tests with a low dose of adenovirus 12 ($10^{4.9}$ $TCID_{50}$), thymectomy did not result in enhancement of tumorigenesis. Thus, 0/32 (0%) of thymectomized animals, 2/12 (15%) of sham-thymectomized animals, and 1/6 (6%) nonsurgically treated hamsters developed tumors. There was no remarkable difference in tumor incidence according to sex; the uninoculated control animals remained free of tumor. The presence of residual thymus tissue in the thymectomized animals was so infrequent, as in the above experiments, as to have no substantial effect on the results.

The nine viruses and 1 mycoplasma shown in Table II were inoculated into newborn thymectomized or nonthymectomized hamsters. Thymectomized and nonthymectomized animals in similar number given placebo were included for control purpose but the data are not presented in Table II since they were mostly negative. The infectivity titers of the viruses used are shown in Table I (influenza A virus diluted 1:10 before inoculation) and the rates of development of tumor in the animals which received virus are presented in Table II. Tumor occurrence was very rare in the animals which were inoculated with virus (10 animals) as well as in control groups (5 animals). None of the tumors appeared to be causally related to the agent given. As shown in Table III, all the

TABLE II. Tests for Oncogenic Potential of Ordinary Viruses and Mycoplasma in Thymectomized and Nonthymectomized Newborn Hamsters.

Agent inoculated	Treatment of hamsters	No. of animals surviving with time (months) and occurrence of tumor[a]								No. of animals with tumor
		3	6	12	16	17	18	20	27	
Adenovirus type 1	Thymectomy	54	50	41	33	27[a]				1
	Nonthymectomy	30	27	22	16	16				0
4	Thymectomy	61	60	43[a]	35	34[a]	26	20	4[b]	4
	Nonthymectomy	45	45	40	34	30	24	17[a]	2	1
Influenza type A	Thymectomy	32	29	25	16	16				0
	Nonthymectomy	28	28	23	11	7				0
A2	Thymectomy	65	57	25	10					0
	Nonthymectomy	47	44	16	10					0
B	Thymectomy	61	56	50	28	23				0
	Nonthymectomy	43	43	39	21	16				0
Poliovirus type 1	Thymectomy	57	54	29	11	8				0
	Nonthymectomy	44	41	21	11	9				0
ECHO type 25	Thymectomy	52	50	41	22	18	14			0
	Nonthymectomy	35	34	25	15[a]	13	8			1
Reovirus type 1	Thymectomy	56	55	40	30	26[a]				1
	Nonthymectomy	46	43	36	21	18[a]				1
Vaccinia	Thymectomy	53	48	15	7	7	6			0
	Nonthymectomy	40	32	11	4	4	4			0
M. orale type 1	Thymectomy	52	42	23	13[a]	8	6	5		1
	Nonthymectomy	38	31	21	10	8	4	2		0

[a] One animal developed tumor.
[b] Two animals developed tumor.

tumors occurred late in life (337–797 days), at which age spontaneous tumors commonly appear in hamsters. Five of the 15 tumors occurred in animals which had received placebo or were not inoculated at all. The tumors were of diverse histopathologic type; and only 2 occurred near the site of injection (adenovirus 4, 797 days; *M. orale*, 439 days). The larger number of animals with tumor given adenovirus 4 reflected the longer period of observation as compared with animals given other agents. In these tests, 42 to 81% of the thymectomized animals were examined histologically and 0 to 3% of the animals were found to have residual thymus tissue.

Tests in hamsters of various ages. Hamsters aged <24 hr, 1 month, 3 months, or 12.5 to 14 months were given $10^{6.2}$ $TCID_{50}$ of SV_{40} virus, $10^{7.8}$ $TCID_{50}$ of adenovirus 7 (Pinckney) virus, or placebo, as shown in Table IV. The animals were observed for development of tumor for 12 months thereafter. Thirty of 33 hamsters which received SV_{40} virus when newborn developed tumor within 1 year. Importantly, 1 animal inoculated at 1 month, 1 at 3 months, and 1 at 1 year developed tumor also. None of the placebo control animals developed tumors at the site of inoculation. The occurrence of nonspecific deaths in animals given virus at 12.5 to 14 months of age was especially great due to aging and rates for tumor incidence were not possible to assess. The fact that tumor occurred in 1 animal which was inoculated at approximately 1 year of age and which survived for an additional year appeared to be of special significance in showing the effect of aging on oncogenicity. The 3 tumors which occurred in animals given virus at 1 month of age or older were all fibrosarcomas and all occurred at the site of injection. A CF test for SV_{40} T antigen was done on the tumor from the animal inoculated at 12.5 to 14 months of age and a titer of 1:16 was obtained. The serum from the animal inocu-

TABLE III. Description of Tumors in Thymectomized and Nonthymectomized Hamsters Inoculated with Virus or Mycoplasma or Held as Controls.

Inoculum	Surgical treatment of hamsters	Tumor description		
		Day detected	Gross description (site, no.)	Histopathologic type
Adenovirus type 1	Thymectomy	505	3 Ventral subcutaneous masses	Autolyzed
4	Thymectomy	337	Multiple, ventral and cervical subcutaneous, liver, spleen, and kidney masses	Reticulum sarcoma
	Thymectomy	595	Multiple, intraperitoneal and lung masses	Sarcoma with areas suggestive of osteosarcoma or chondrosarcoma but not resembling adenovirus tumors
	Thymectomy	730	Intraperitoneal	Undifferentiated sarcoma
	Thymectomy	797	Subcutaneous adjacent to cheek pouch	Encapsulated aggregate of cells suggestive of extramedullary hematopoiesis (not typical of adenovirus-induced neoplasm)
	Sham-thymectomy	570	Intrathoracic	Reticulum cell sarcoma
None	Thymectomy	404	Intraperitoneal	Resembles undifferentiated sarcoma
ECHO type 25	Sham-thymectomy	397	Multiple, ventral subcutaneous, lung and thoracic wall masses	Undifferentiated sarcoma
Reovirus type 1	Thymectomy	493	Subcutaneous mass on leg	Chondroma
	Sham-thymectomy	493	Spleen	Hemangioma
Placebo	Thymectomy	511	Liver and spleen	Reticulum sarcoma
	Thymectomy	519	Ventral and eyelid subcutaneous masses	Undifferentiated sarcoma
	None	378	Intraperitoneal	Leiomyosarcoma
M. orale type 1	Thymectomy	439	Small subcutaneous mass in scapular region	Undifferentiated sarcoma
Placebo	Sham-thymectomy	355	Subcutaneous mass in thoracic region	Fibrosarcoma

lated at 3 months of age had antibody to SV_{40} T antigen (1:80) while the sera from the other 2 animals failed to show such antibody ($<$1:10). As expected, 3 of 20 (15%) of hamsters given adenovirus 7 virus when newborn developed tumor at the injection site. None of the animals injected at older age and none of the placebo controls developed tu-

TABLE IV. Effect of Age of Hamster on Induction of Tumors (at the injection site) Following Inoculation with SV_{40} or Adenovirus 7 Virus.

Inoculum	Hamsters		Tumor incidence according to time after inoculation (months)				
	Age when inoculated	No. inoculated	1	3	6	9	12
SV_{40} virus	<24 hr	51	0/49[a]	0/46	20/37	27/33	30/33
Placebo	<24 hr	30	0/27	0/25	0/21	0/14	0/10
SV_{40} virus	1 month	36	0/36	0/36	0/36	0/31	1/27
Placebo	1 month	18	0/18	0/18	0/17	0/17	0/15
SV_{40} virus	3 months	36	0/36	0/36	0/29	1/24	1/17
Placebo	3 months	19	0/19	0/14	0/10	0/9	0/9
SV_{40} virus	12.5–14 months	33	0/28	0/19	0/5	0/3	1/1
Placebo	12.5–14 months	16	0/16	0/11	0/6	0/4	0/4
Adenovirus 7	<24 hr	33	0/26	0/26	0/25	3/25	3/20
Placebo	<24 hr	14	0/13	0/13	0/13	0/13	0/13
Adenovirus 7	1 month	42	0/42	0/42	0/36	0/32	0/27
Placebo	1 month	26	0/26	0/25	0/24	0/23	0/18
Adenovirus 7	3 months	43	0/43	0/43	0/39	0/31	0/22
Placebo	3 months	24	0/24	0/24	0/19	0/16	0/11
Adenovirus 7	12.5–14 months	30	0/28	0/24	0/16	0/16	0/4
Placebo	12.5–14 months	22	0/18	0/14	0/12	0/8	0/4

[a] No. with tumor/total no. of animals.

mors at the site of inoculation.

Influence of passage history of adenovirus 7 virus on oncogenicity. The occurrence of tumors following inoculation (0.2 ml subcutaneously in the scapular region and 0.05 ml in the thoracic cavity) of newborn hamsters with Pinckney and Gomen strain adenovirus 7 preparations which had different cell culture passage histories is summarized in Fig. 4. Both strains of adenovirus type 7 were oncogenic to approximately the same degree when tested at equivalent dose levels regardless of the number of times they had been passed in cell culture or the kind of cell cultures used. A fresh isolate of Pinckney virus which had been passed only 3 times in primary HEK cell cultures induced tumors at approximately the same rate (14.1%) as when passage of the virus had been continued in an established cell line (KB) for a total of 14 cell culture passages (8–15.3%). Gomen strain virus preparations which had been passed a total of 14 times either in established cell lines (HeLa, KB) only or the last 3 passages in primary HEK cells resulted in tumor incidences of 26.4% and 9–18.7%, respectively.

All the tumors considered to be of adenovirus 7 etiology occurred at the sites of inoculation, were histologically characteristic of adenovirus-induced tumors, and, in most cases, the animals possessed either specific T antigen in the tumors or T antibody in their sera.

Discussion. The early studies (1) of the effect of thymectomy on host immunologic competence and on resultant viral oncogenesis suggested that this procedure might provide a more sensitive system for testing of oncogenic potential of viruses not previously found to be oncogenic. The present studies confirmed the previous demonstration by others (11–13) that thymectomy enhanced the oncogenicity of the known cancer-inducing viruses polyoma, SV_{40} and adenovirus 7. In addition, it was shown that the tumorigenicity of weakly oncogenic adenovirus 3 could also be enhanced by thymectomy. Adenovirus 12 at high virus dose was so highly oncogenic in newborn nonthymectomized hamsters as to preclude demonstration of any enhancing effect whereas, at low dose, there was no enhancement. Although Girardi and Roosa (14) did not observe increased

INOCULUM			TUMOR OCCURRANCE AT INOC. SITE			
ADENOVIRUS TYPE 7 STRAIN	VIRUS PASSAGE HISTORY	NEG LOG_{10} $TCID_{50}$ VIRUS INOCULATED	NUMBER ANIMALS WEANED	NO. TUMORS AND (%) AT 14 MO NO. ON TEST	TIME OF FIRST TUMOR DETECTION (DAYS) RANGE	AVERAGE
PINCKNEY	HEK 3*	7 9	59	3/8** (14 1)	145 - 209	180 6
PLACEBO	"	NONE	47	0/14 (0 0)		
PINCKNEY	HEK3/KBII	8 7	59	6/12 (15 3)	140 - 275	199 6
PINCKNEY	"	8 7	69	5/32 (8 0)	127 - 330	233 4
PLACEBO	"	NONE	51	0/31 (0 0)		
GOMEN	HeLa 10/KB4	9 4	61	7/13 (26 4)	161 - 386	266 8
CONTROL	KB4	NONE	53	0/25 (0 0)	333	333
PLACEBO	HeLa 10/KBI/HEK3	8 9	65	7/21 (18 7)	203 - 391	257 1
GOMEN	"	7 9	55	4/19 (9 0)	216 - 338	304
PLACEBO	KBI/HEK3	NONE	52	0/16 (0 0)		

* HEK = PRIMARY HUMAN EMBRYONIC KIDNEY CELLS, KB = KB MALIGNANT LINE CELLS, HeLa = MALIGNANT LINE CELLS

** CUMULATIVE NO. ANIMALS WITH TUMOR / TOTAL NO. ANIMALS WITH OR WITHOUT TUMOR MINUS NONSPECIFIC DEATHS

ANIMALS WITH TUMORS ADJUSTED FOR NONSPECIFIC DEATHS BY STANDARD LIFE TABLE PROCEDURES

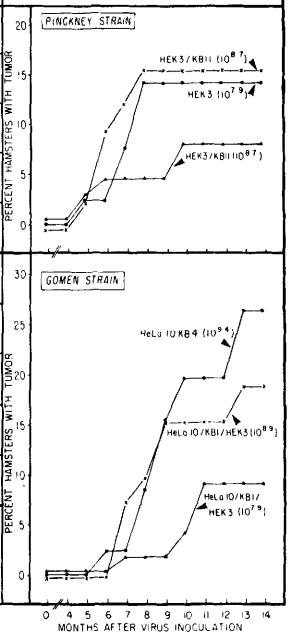

FIG. 4. Oncogenicity for newborn hamsters of Pinckney and Gomen type 7 adenovirus strains with different cell culture passage histories.

oncogenicity in thymectomized and irradiated hamsters when SV_{40} virus was inoculated within 24 hr of birth, the present studies did demonstrate marked enhancement by delaying inoculation of the virus until the animals were 7 days old. It is apparent that demonstration of enhancement of viral tumorigenesis is readily influenced by factors such as the susceptibility of the host species, virus dose, and time of virus inoculation.

Application of the thymectomized hamster system to assay for possible cancer-inducing capacity of adenovirus types 1 and 4, influenza types A, A2, and B, poliovirus 1, ECHO 25, reovirus 1 and vaccinia viruses as well as *M. orale* 1 showed no single example of oncogenic capability of these agents. Influenza viruses were included because they may act as cocarcinogens (15) and because they have been reported to induce transformation of mouse cells *in vitro* (16). Poliovirus 1 and vaccinia were tested because of their reported cocarcinogenic properties (15). Reovirus 1 was tested because it has an unusual association with the mitotic apparatus of the host cell during division (17), because of the relationship of this agent to wound tumor virus of plants (18), because of its reported isolation from Burkitt's lymphoma (19), and because of the association of murine reovirus infection with mouse lymphoma (20). *M. orale* 1, strain N1, was selected for test because this particular strain was isolated directly from leukemic bone marrow (21) and because numerous isolations of mycoplasmas from leukemic patients have been reported (22, 23). ECHO 25 was included as a representative of an enterovirus. Adenovirus type 1 was tested because it has been isolated from human tumors (24, 25), because tumors have been detected in hamsters following neonatal inoculation with the virus (26), and because the agent transforms rat cells *in vitro* (27). Adenovirus type 4 was selected for testing because of its use in human vaccines (28), and since it is the only one of the 31 adenovirus types of man not shown to date to be associated with oncogenicity *in vivo* or *in vitro*. The few tumors which did occur following adenovirus 4 injection (see Tables II and III) were not at the inoculation site, were of the spontaneous type seen

in uninoculated hamsters, and were of no significantly greater frequency in test animals than in controls.

The results presented here confirm and extend previous attempts in this laboratory (29) and others (13, 28, 30) to detect oncogenic potential of "ordinary" viruses in normal newborn hamsters or in thymectomized hamsters. One may question the usefulness of thymectomy for testing for oncogenic potential of viruses since the effect is most evident where oncogenicity is already high and where thymectomy is not needed to demonstrate the effect. Oncogenic potentiation by thymectomy of weakly oncogenic agents or low doses of highly oncogenic viruses is very poor and if this be true of even less oncogenic viruses, then tests in thymectomized animals really present no advantage.

It is well known (4–6) that for most oncogenic viruses there is drastic reduction in tumor incidence with the advance in age of the host animal at the time the virus is given. This is presumed to be due in part at least to enhanced resistance which accompanies maturation of the immune mechanisms. The question is raised as to whether the process might be reversed leading to increase in tumor incidence in animals given virus at advanced age, at a time when the functions of the immune mechanisms are declining (3). In the present studies with weakly oncogenic adenovirus 7 virus, no tumors occurred in hamsters given virus beyond the newborn period. By contrast, 1 of 36 hamsters which was given SV_{40} virus at 3 months of age and which survived for 9 months thereafter developed tumor. Additionally, 1 of the 33 hamsters which was given virus at approximately 1 year of age and which survived for an additional year, developed a tumor. These results extend the previously reported (4) maximum susceptible age of hamsters to SV_{40} tumorigenesis from 4 months to 1 year. The rate of attrition due to aging in 1-year-old hamsters is so great as to require extremely large numbers of animals initially to obtain incidence rates. The fact, however, that tumor can occur when virus is given as late as 3 to approximately 12 months of age indicates that cancer may not always be a phenomenon of infection in early life. Instead, there might be continuous low level susceptibility throughout adulthood or there may be increased susceptibility with advanced age when immunity is waning.

Earliest tests for tumor induction in hamsters by the weakly oncogenic adenovirus 7 agent were negative (31, 32). The viruses employed had all been passed many times in cell culture. The later findings in which positive tumor induction was obtained (33, 34) employed viruses which have been passed only a few times in cell culture. The results in the present study showed no apparent difference in the oncogenicity of the Pinckney and Gomen strains of adenovirus whether passed 3 or 14 times in continuous cell lines or primary cell cultures. Instead, the virus dose and the long observation period were the important determining factors.

Summary. Thymectomy of newborn hamsters enhanced the tumorigenicity of polyoma virus given 1 week or 4 months after surgery, of SV_{40} virus given 1 week after surgery, and of adenovirus 3 and 7 injected immediately after thymectomy. There was no effect on tumor incidence following adenovirus 12 given in high or low dose immediately after surgery. Adenovirus types 1 and 4, influenza types A, A2, and B, ECHO 25, poliovirus 1, reovirus 1, vaccinia, and *M. orale* 1 failed to induce tumor when inoculated into newborn thymectomized and nonsurgically treated hamsters. The influence of age on host susceptibility to viral oncogenesis was studied by inoculating adenovirus 7 and SV_{40} virus into newborn, 1-, 3-, and 12.5–14-month-old hamsters. Adenovirus 7 induced tumors in the newborn group alone; by contrast, SV_{40} virus induced tumors in all age groups. The influence of virus strain and cell culture passage on oncogenicity was studied with the Pinckney and Gomen strains of adenovirus 7. These latter factors did not affect the induction of tumors in newborn hamsters; instead, the amount of virus given was the most important determinant. The significance of the findings in relation to detection of oncogenic viruses are discussed.

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