description of another technique which resolves only one triglyceride fraction in serum (10).

The reasons for the appearance of, or increase in, the "saturated" fraction in serum as compared to plasma are not apparent. The difference could be explained by the release of platelet triglycerides into serum, which would not occur in plasma(11), or by enzymatic hydrogenation of the unsaturated bonds. However, the latter has been demonstrated only in the case of methyl elaidate (12). Studies are currently under way which we hope will resolve this problem.

Summary. A simple method is described for the fractionation of blood lipids by thin layer chromatography. Lipid patterns for different disorders of lipid metabolism were demonstrated. A difference was noted in the triglyceride fraction between plasma and serum.

1. Izmailov, N. A., Shraiber, M. S., Farmatsiya No. 3, 1938, 1.
2. Mangold, H. K., *J. Am. Oil Chem. Soc.,* 1961, v38, 708.
3. Weicker, H., *Klin. Wochenschr.,* 1959, v37, 763.
4. Abell, L. L., Levy, B. B., Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.
5. Fiske, B. H., Subbarow, Y., *ibid.,* 1925, v66, 375.
6. Van Handel, E., *Clin. Chem.,* 1961, v7, 249.
7. Van Handel, E., Zilversmit, D. B., *J. Lab. and Clin. Med.,* 1957, v50, 152.
8. Truter, E. V., *Thin Film Chromatography,* London, Cleaver-Hume Press Ltd., 1963, 155.
9. Sachs, B. A., Wolfman, L., unpublished observations.
10. Krell, K., Hashim, S. A., *J. Lipid Research,* 1963, v4, 407.
11. Marcus, A., personal communication.
12. Dhopeshwarkar, G. A., Mead, J. F., *J. Lipid Research,* 1962, v3, 238.

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Tests in Hamsters for Oncogenic Quality of Ordinary Viruses Including Adenovirus Type 7.* (29138)

A. J. GIRARDI, M. R. HILLEMAN AND R. E. ZWICKEY
Division of Virus and Cell Biology Research and Department of Pathology, Merck Institute for Therapeutic Research, West Point, Pa.

The oncogenic viruses of animal species exhibit ordinary properties of viruses, especially with regard to their structure, transmissibility, cell necrotizing capacity, and immunology. These attributes have provided support for the concept that ordinary viruses which infect the human species may, under appropriate circumstances, be responsible for certain malignancies in man.

In spite of extensive laboratory investigation, the direct attempts to recover viruses from human malignant tissue specimens have not succeeded in isolation of virus of proved etiologic relationship to neoplasia in man. Instead, the growing body of evidence has indicated that if viruses are responsible for human neoplasia, the virus may no longer be present in an infectious state, and only the significant genetic portion of the virus responsible for malignancy may remain. This has suggested the need for application of indirect approaches in human neoplasia such as the search for subviral components in malignant tissues and in examination of common viruses of human origin for their oncogenic potential in cell cultures and in animals.

The findings relating to tumor induction in newborn hamsters by SV_{40} virus(1,2) represented a breakthrough in studies of neoplasia in being the first demonstration of a malignant oncogenic quality for a virus of primate (monkey) origin. Even more striking were the findings of Trentin *et al*(3) and later Huebner *et al*(4) who showed an oncogenic capacity of human adenovirus types 12

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and 18 in the newborn hamster.

During mid 1961, we undertook to examine the oncogenic potential for newborn hamsters of a wide variety of viruses of human origin. Agents were selected for study which represented the major groups of viruses of the human species(5) and, in addition, some viruses of animal or avian origin with which man may have contact. These investigations are now in their third year and the findings to date with 25 separate agents are presented here. Types 12 and 18 adenovirus proved oncogenic for newborn hamsters in confirmation of previous reports by others(3,4). More important is the finding that a portion of hamsters inoculated with type 7 adenovirus developed sarcomas after a long incubation period. Type 7 adenovirus very commonly infects the human species and is a principal cause for epidemic acute respiratory diseases in man(6), especially in military populations. The findings in the studies are presented in detail and their significance is discussed.

Materials and methods. Experimental design and hamster study. Pregnant hamsters obtained from the Lakeview Hamster Colony, Newfield, N. J., were housed individually in isolation filter cages.† The newborn hamsters less than 24 hours of age were inoculated either subcutaneously or into the thoracic cavity with intent to expel virus into the lungs. Details pertaining to the inoculation of each particular virus are given in Table II. Control animals were given fluids from noninfected cell culture extracts from the same lot of cells used to prepare virus, or were inoculated with tissues of uninoculated mice, chicks or embryonated eggs of identical origin to those used for propagating the virus for hamster inoculation. The suckling hamsters were weaned at 18 days of age. They were housed in filter cages during the first 2 months following inoculation and then were transferred to conventional open-top cages. The animals were observed at least once each week‡ for development of palpable masses. Hamsters which died were routinely autopsied to determine the cause of death, but cannibalism sometimes made this impossible. Certain of the animals were sacrificed when the tumors had achieved sizable proportions. All animals alive at termination of the experiment were carefully examined at autopsy and tissues or organs which appeared abnormal were examined histopathologically. Tumor-bearing hamsters were bled at time of sacrifice and the excised tumor was prepared for histopathologic diagnosis. In certain instances, a portion of tumor was saved for transplantation to hamsters or for tests for detection of viral antigen. For histopathologic study, the tissues were fixed in 10% formalin and the paraffin sections were stained with hematoxylin and eosin. For transplantation purpose, the tissue was rapidly minced in Hanks' balanced salt solution (HBSS)(7) and implanted *via* trocar into the dorsal subcutaneous space of 1- to 3-month-old hamsters.

Virus preparation. Details relating to passage history, host tissue used to prepare virus for hamster inoculation, and the infectivity titer for each virus are presented in Table I. The majority of strains were from virus stocks routinely maintained in this laboratory. The passage history relates to the seed stock used to prepare the virus inoculum. All the viruses employed were proved free of extraneous virus, except for measles virus cultured in chick embryo cells which contained RIF agent(8) and for adenovirus 2 which was found to contain a trace of SV_{40} virus one year after the hamsters were placed on test. This conclusion was based on the results of exhaustive tests in cell culture in which homologous specific neutralizing antisera were employed. All viruses were identified in appropriate tests with reference antisera. Certain of the viruses were freshly isolated in RIF-free(8) embryonated hens' eggs and chick embryo cell cultures, or in primary human embryonic cell cultures. The fresh isolation in this laboratory of Huie type 12 adenovirus was made in primary human embryonic kidney cell culture using the original

† Modification of basic design by Dr. L. Kraft, Yale University, for work with gastroenteritis in newborn mice.

‡ Animals given measles virus preparations were observed by Drs. H. Goldner and E. B. Buynak.

stool specimen of patient Huie kindly furnished by Dr. S. Kibrick. Type 12 Huie virus obtained from the American Type Culture Collection (ATCC) and freshly isolated type 12 and 18 adenovirus strains obtained from Dr. R. Chanock were included.

Adenovirus type 7 tumor antigens. Tumors which appeared in hamsters following inoculation of type 7 adenovirus were homogenized at 16,000 RPM in an Omnimix cup held in an icebath, employing sufficient diluent to give a 15% suspension. The homogenates were spun at 10,000 RPM for 20 minutes in the SS1 centrifuge and the supernates were examined for presence of complement-fixing (CF) antigen in tests with the paired acute and convalescent sera from the child Pinckney from whom the type 7 isolate was

TABLE I. Essential Information Relating to Viruses Tested for Oncogenic Potential in Newborn Hamsters.

Virus history			Virus used to inoculate hamsters		
Family and type	Strain	Passage history of seed	Host tissue	Infectivity titration	
				Titer	Test system
Adenovirus 1	AD 71 (Huebner)	HeLa 5/SA 1	SA	$10^{-6.5}$/0.1 ml	HeLa
" 2	IND 2 (Huebner)	Ad 1/HESk 2/MK 6/GMK 12	GMK	$10^{-4.7}$/0.1 ml	HeLa
" 7	Pinckney (Fresh isolate)	HEK 3/HDCS 2	HDCS	$10^{-5.5}$/0.2 ml	HEK
" 12 (a)	Huie (ATCC)	HEK/MK 2/KB/$HeLa_{12}$/KB 2	HEK	$10^{-6.0}$/0.2 ml	HEK
" 12 (b)	Huie (Fresh isolate)	HEK 1	HEK	$10^{-4.2}$/0.2 ml	HEK
" 12 (c)	9512 (Chanock)	HEK 2	HEK	$10^{-6.0}$/0.2 ml	HEK
" 18	14606 (Chanock)	HEK 3	HEK	$10^{-5.0}$/0.2 ml	HEK
Myxovirus					
influenza A 2	5548 (Fresh isolate)	Embryon. egg 3 **	Embryon. egg **	$10^{-7.0}$/0.2 ml	Embryon. egg **
parainfluenza 1	HA 2 C 35	MK 10/GMK 9	GMK	$10^{-6.7}$/0.2 ml	GMK
" 2	CA Greer	MK 4/GMK 12	GMK	$10^{-7.0}$/0.2 ml	GMK
" 3	HA 1 C 243	MK 6/MH 2/HeLa 5	GMK	$10^{-7.3}$/0.2 ml	HeLa
Respiratory syncytial	Long	Hu Ht 3/HEP 2/HeLa 1	HeLa	$10^{-3.2}$/0.2 ml	HeLa
Mumps	Johnson	Embryon. egg 4 **	Embryon. egg **	$10^{-6.0}$/0.1 ml	GMK
Measles (a)	Edmonston	PHA 36/HEK 1	HEK	$10^{-3.5}$/0.1 ml	HeLa
" (b)	Edmonston 749 D *	PHK 24/PHA 28/E 12/CETC 18	CETC	$10^{-1.5}$/0.1 ml	HeLa
" (c)	" " *	" " " " 18	CETC	$10^{-2.25}$/0.1 ml	HeLa
" (d)	" " *	" " " " 18	CETC	$10^{-2.50}$/0.1 ml	HeLa
Picornavirus					
Rhinovirus 11 (H) ***	7 (Fresh isolate)	HDCS 3	HDCS	$10^{-3.5}$/0.2 ml	HDCS
" 23 (H)	5007 (Fresh isolate)	HDCS 2	HDCS	$10^{-3.5}$/0.2 ml	HDCS
" 30 (M)	5582 (Fresh isolate	HDCS 8	HEK	$10^{-3.0}$/0.2 ml	HDCS
Enterovirus Poliovirus 3	Saukett	MK (Passage no. unknown)	MK	$10^{-6.5}$/0.1 ml	HeLa
Salivary gland virus	AD 169	MAF > 150	MAF	$10^{-0.5}$/0.2 ml	MAF
Varicella	Shaffer	PHA 4/HDCS 10	HDCS	Infectious	HDCS
Pneumonia virus of mice	PVM-15	Mouse (in vivo)	Mouse lung (ICR/Ha)	$10^{-4.0}$/0.05	Mouse - respiratory route
Avian leucosis complex					
Rous sarcoma	Bryan	Chick brain 66 (in vivo)	Chick brain	10^{-6} FFU/0.2	CETC
Visceral lymphomatosis	RPL-12 (7668) (Burmester)	Chick tumor, 85 X plants; Filtrates 16 pas.; CETC 1	CETC	Pos. RIF test	CETC
Resistance inducing factor	3073 S (Fresh isolate)	CETC 3	CETC	> $10^{-5.5}$ in RIF test	CETC

Abbreviations: Cell cultures: SA (stable human amnion); HESk (primary human embryonic skin); HEK (primary human embryonic kidney); MK (primary rhesus or cynomolgus kidney); GMK (primary grivet monkey kidney); MH (stable monkey heart); Hu Ht (stable human heart); PHA (primary human amnion); CETC (chick embryo cell culture); HDCS (human diploid cell strains, Wistar); MAF (human embryo fibroblast, Microbiological Associates). Other: RIF (resistance inducing factor, *i.e.*, avian lymphomatosis or related virus); FFU (focus forming units of Rous sarcoma virus).

* Gave positive RIF test for presence of avian leucosis virus contaminant. ** RIF test negative (RIF-free materials). *** H strains propagate both in human and monkey kidney cells; M strains propagate only in monkey cells.

TABLE II. Results of Tests in Newborn Hamsters for Tumorigenic Capacity of Viruses.

Hamster inoculation				Result								
Virus	Route & volume	No. inoc.	No. weaned	No. survived according to time post-weaning, months								No. developed malignancy
				1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24	
Adenovirus 1	Subcut. 0.2 ml	42	26	26	24	20	17	9	4	3 T**		0
Control	" "	29	22	22	18	15	13	10	5	3 T		0
Adenovirus 2	" "	61	15	11	5	5	5	5	4	4	3 T	1
Control	" "	11	11	7	7	7	4	2	2	2	1 T	1
Adenovirus 7	" "	30	30	29	29	29	26	19				3
"	Intrathoracic 0.2 ml	35	21	21	18	7	5	4				1
Control	Subcut. 0.2 ml	31	31	29	27	27	26	25				0
"	Intrathoracic 0.2 ml	33	24	24	23	19	13	11				0
Adenovirus 12 (a)	Subcut. & intrathoracic *	39	24	23 T								21
Control	" "	20	20	20	17	15	13 T					0
Adenovirus 12 (b)	" "	14	13	11 T								9
Control	" "	18	17	17	16	15 T						0
Adenovirus 12 (c)	" "	27	20	20	19	16 T						12
Control	" "	20	20	20	17	15	13 T					0
Adenovirus 18	" "	32	25	25	22	18	17 T					8
Control	" "	26	23	23	22	17	15 T					0
Myxovirus												
Influenza A 2	" "	29	22	21	17	14	7 T					0
Control	" "	21	13	12	12	11	T					0
Parainfluenza 1	Subcut. 0.2 ml	26	15	15	13	13	10	2	1		T	0
" 2	" "	29	28	27	21	19	18	13	9	8	T	0
Control (1 & 2)	" "	29	28	24	24	24	19	17	13	8	7 T	0
Parainfluenza 3	" "	49	30	20	14	13	8	8	6	5	T	0
Control	" "	16	13	13	11	8	7	4	4	2	T	0
Respiratory syncytial	" "	32	18	18	18	17	17	15	11	8	7 T	1
Control	" "	49	23	22	22	22	21	17	13	7	4 T	0
Mumps	" "	24	23	17	16	15	12 T					0
Control	" "	36	34	34	29	27	22 T					0
Measles (a)	" "	66	42	39	30	23	22	18	11	3	T	0
Control	" "	71	36	34	32	26	25	23	16	1	T	0
Measles (b)	" "	60	32	24	21	19	17	13	11	7	4 T	0
Control	" "	78	58	49	36	29	25	22	16	10	8 T	0
Measles (c)	" "	79	51	42	39	34	30	18	13	12	T	0
Control	" "	100	65	59	49	43	33	23	13	7	T	1
Measles (d)	" "	68	49	47	42	41	38	34	25	20 T		0
Control	" "	100	71	40	31	25	21	16	12	10 T		0
Picornavirus												
Rhinovirus 11	" "	26	20	17	17	17	12	10	7	5	T	0
Control	" "	28	17	15	14	13	13	12	11	10	8 T	0
Rhinovirus 23	" "	36	29	29	24	24	22	20	19	13	11 T	0
Control	" "	31	30	29	27	27	27	21	16	13	10 T	1
Rhinovirus 30	" " & intrathoracic	37	31	30	28	28	26 T					
Control	" "	22	22	22	19	17	14 T					
Enterovirus												
Poliovirus 3	" "	30	30	28	24	23	18	10	7	7	3 T	0
Control		11	11	7	7	7	4	2	2	1	0 T	0
Salivary gland virus	" "	19	11	11	7	7	6	5	5	4	3 T	0
Control	" "	15	15	15	13	8	3	2	2	1	T	0
Varicella	" "	44	35	35	35	28	25	22	15	13 T		0
Control	" "	11	11	10	10	4	4	4	4	2 T		0
Pneumonia virus of mice	" "	31	31	30	26	23	23	21	16	10	T	0
Control	" "	24	9	7	6	6	6	4	3	2	T	0
Avian leucosis complex												
Rous sarcoma	" "	44	15	14	14	14	14	10	8	7	T	2
Control	" "	37	31	31	31	29	24	15	12	4	2 T	0
Visceral lymphomatosis	" "	38	28	18	14	13	7	4	3	2	T	0
Control	" "	22	13	11	8	8	3	2	1		T	0
Resistance inducing factor	Subcut. & intraperitoneal*	36	29	26	24	17	12 T					0
Control	" "	29	23	22	22	19	18 T					0

* Subcut. 0.2 ml, intrathoracic 0.05 ml, intraperitoneal 0.2 ml. ** T = Experiment terminated.

Control material consisted of uninoculated materials, otherwise identical to the virus preparation. Poliovirus controls received nothing.

derived, with convalescent serum from a patient with adenovirus infection in a type 4 and 7 epidemic of respiratory disease, and with the sera from 3 tumor-bearing and 2 nontumor-bearing animals which had received type 7 adenovirus. The CF procedure was the same as previously employed in this laboratory(9) and the tests were kindly performed by Drs. V. Hamparian and B. Leagus.

Results. Tumor development. Table II presents the findings with the viruses tested for tumorigenic quality in newborn hamsters. On occasion, there was a considerable mortality during the suckling period which occurred both in virus-inoculated and in control animals. Death, in certain instances, might have been due to virus infection as for adenovirus 2, *e.g.*, but appeared in most instances to be of nonspecific cause. After weaning there was a gradual reduction of the number of animals resulting from death of undetermined but presumably natural causes. The numbers of animals which survived, according to 3-month intervals, are as shown in the Table. Inspection of these figures permits a determination of how many animals were at risk for development of malignancy in each of the groups at each time period. Hamsters which developed tumors (Table III) were sacrificed for gross and histopathologic examination. All experiments were terminated by the end of 24 months following

TABLE III. Description of Tumors Which Occurred in Hamsters.

Hamster inoculation		Result			
Virus or control	Route	Animal no.	Day first detected	Site	Histopathologic type
Adenovirus 2 (SV_{40} contam.)	Subcut.	1	424	Subcut.	Fibrosarcoma
Control	"	2	582	Intestine	Liposarcoma
Adenovirus 7	Intrathoracic	1	231	Subcut. (Ventral)	Undifferentiated sarcoma
"	Subcut.	2	266	Subcut.	" "
"	"	3	286	"	" "
"	"	4	405	"	" "
Adenovirus 12 (a) (Huie - ATCC)	Subcut. & intrathoracic	1-21	Range 26-75	14 Subcut. 4 Subcut. & intrathoracic 2 Intrathoracic 1 Kidney	All " "
Adenovirus 12 (b) (Huie - fresh isolate)	Subcut. & intrathoracic	1-9	Range 36-88	3 Subcut. 1 Subcut. & intrathoracic 5 Intrathoracic	All " "
Adenovirus 12 (c) (Chanock - fresh isolate)	Subcut. & intrathoracic	1-12	Range 82-216	11 Subcut. 1 Intestinal	All " "
Adenovirus 18 (Chanock - fresh isolate)	Subcut. & intrathoracic	1-8	Range 70-280	5 Subcut. 2 Intrathoracic 1 Intestinal	All " "
Respiratory syncytial	Subcut.	1	456	Intestine	Leiomyosarcoma
Measles					
Control	"	1	692	Kidney	Adrenal cortical carcinoma
Rhinovirus 23					
Control	"	1	690	Intestine	Reticulum cell sarcoma
Rous sarcoma	"	1	504	Lung	Osteogenic sarcoma
	"	2	538	Liver	Malignant lymphoma, probably a reticulum cell sarcoma.

virus inoculation.

Among the total group of 1434 animals which survived to weaning, 61 developed malignancy. Three of the tumor-bearing animals were in the control groups; 1 received adenovirus 2; 4 received adenovirus 7; 42 received adenovirus 12; 8 received adenovirus 18; 1 received respiratory syncytial virus; and 2 received Rous sarcoma virus.

Details relating to time of appearance and kind of tumor are shown in Table III. Except for adenovirus 2, all adenovirus-induced tumors were undifferentiated sarcomas. The adenovirus 12 and 18 tumors generally appeared much earlier than did the adenovirus 7 tumors. The other tumors were of a wide diversity of histomorphologic types and occurred infrequently in any particular animal group.

Adenovirus type 7 tumors. All 4 primary tumors were undifferentiated sarcomas. Tumor 1 (Table III) appeared on day 231 following intrathoracic inoculation of virus; the tumor occurred subcutaneously but was ventrally located at the site of virus inoculation. The 3 other tumors which appeared on days 266, 286 and 405, respectively, were at the site of subcutaneous inoculation and were dorsally located. The tumors were collected 13, 6 and 8 days, respectively, following their first detection and they weighed 8.5, 4.0 and 2.9 g in the order shown.

Tumor 3, which was excised 6 days after its appearance, was transplanted subcutaneously *via* trocar to 5 adult hamsters. All developed masses between 13 and 20 days later and these were diagnosed as undifferentiated sarcomas. The first of the transplant tumors was transplanted further into adult hamsters with the appearance of subcutaneous undifferentiated sarcomas at the inoculation site approximately 13 days later.

Grossly, the tumors were soft, whitish, amorphous masses and generally were nonadherent. Cut surfaces presented a homogeneous dull whitish appearance. Some of them revealed central necrosis and hemorrhage.

Fig. 1 shows the histopathologic appearance of primary tumor 1 and Fig. 2 shows a section of a transplant of primary tumor 3.

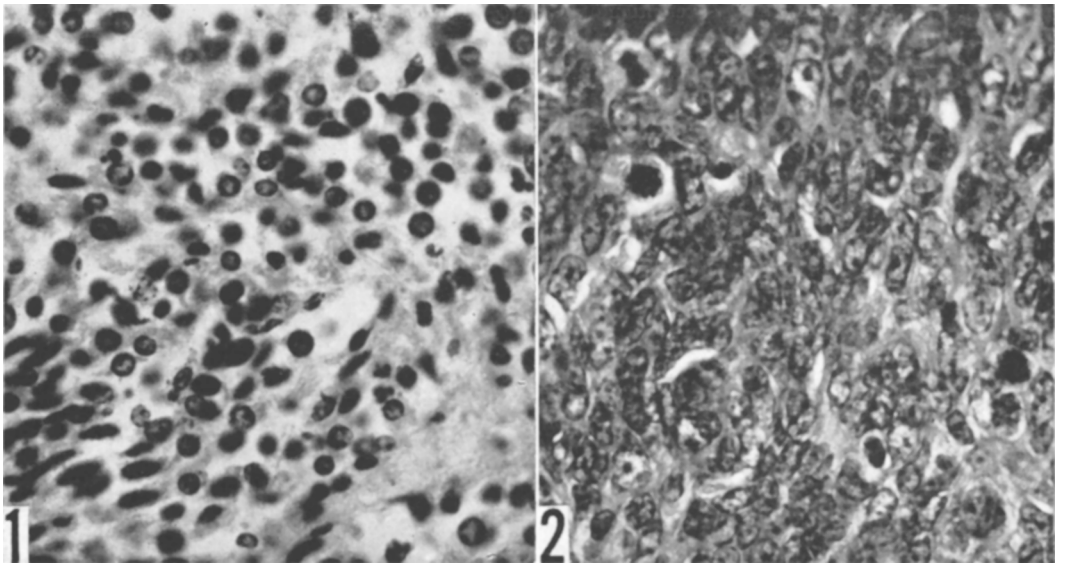

FIG. 1. Section of primary subcutaneous tumor which appeared on day 231 following intrathoracic inoculation of type 7 adenovirus. The tumor is an undifferentiated sarcoma with considerable nuclear variation. H & E stain. Mag. 500 ×.

FIG. 2. Section of first transplant of sarcomatous adenovirus 7 tumor No. 3 showing undifferentiated character and mitoses. H & E stain. Mag. 500 ×.

All adenovirus type 7 primary tumors as well as transplant tumors were undifferentiated sarcomas composed of pleomorphic cells with scanty cytoplasm and poorly defined cell walls. The nuclei were round, oval, or elongated and often vesicular. Mitoses, sometimes bizarre, were occasionally observed. Focal necrosis and hemorrhage were evident in several of the tumors. In certain tumors, there were small areas with characteristics suggestive of lymphoma.

Tests were carried out to examine for presence of viral CF antigen in the type 7 adenovirus tumors, such as were found by Huebner *et al*(15) in types 12 and 18 adenovirus tumors. In the tests, the tumor antigen was used undiluted and serial dilutions of sera were tested. The findings presented in Table IV are interpreted as follows: All 3 sera from tumor-bearing animals contained antibody capable of reacting with adenovirus type 7 tumor antigen. Such antibody was present in greatest amount in serum from hamster 3. Extract of tumor 4 contained sufficient antigen to permit positive test results with all 3 hamster sera. Tumor 2 extract was weak antigenically and gave positive results only in tests with the high titer serum of hamster 3. Tumor 3 extract was highly anticomplementary and this might have been due to presence of a large amount of adenovirus antigen combined *in situ* with the high titer serum antibody of hamster 3. Transplant tumor 3 did not contain sufficient antigen to permit positive test results with any of the sera. The high titer serum from hamster 3 reacted with homologous type 7 adenovirus but not with heterologous type 4 adenovirus antigen prepared in ordinary cell culture. None of the other hamster sera were of sufficient titer to give positive results with the adenovirus cell culture antigens. None of the hamster tumor antigens gave positive results in tests with positive sera from cases of adenovirus infection in human beings. None of the normal control antigens or sera from nontumor-bearing hamsters gave positive serologic results.

None of the hamster sera neutralized either SV_{40} or polyoma virus. Tests are currently in progress to detect infectious virus in extracts of type 7 adenovirus tumors.

Discussion. Hamsters from the Lakeview Colony generally show a remarkably low incidence of spontaneous malignancies during

TABLE IV. Findings in Complement Fixation Tests with Tumors and Sera from Hamsters Injected with Pinckney Strain of Type 7 Adenovirus.

Serum	Serum titers obtained in tests with antigen:						
	Extract of hamster tumor number:					Cell culture, viral antigen	
	4	2	3	Tr. 3 *	Normal **	Type 7 (Pinckney)	Type 4 (RI-67)
Human:							
Pinckney acute	0***	0	Indeterminate due to anticomplementary activity of antigen.	0	0	0	0
" convalesc. (type 7)	0	0		0	0	80	80
Dan. convalesc. (Type 4 or 7 ?)	0	0		0	0	80	80
Tumor-bearing hamster no.:							
1	40	0		0	0	0	0
2	160	0		0	0	0	0
3	> 320	20		0	0	40	0
Nontumor-bearing hamster							
A	0	0		0	0	0	0
B	0	0		0	0	0	0
Anticomplementary antigen	0	0	Positive	0	0	0	0

* Transplant of tumor 3. ** Normal hamster tissue (muscle-skin). *** 0 = titer $< 1:10$ or $< 1:20$.
**** Type 7 isolate from an adult military recruit.

the first 18 to 24 months of life(1,10). This quality, which seems to apply to hamsters in general, has made the hamster an especially valuable host in studies to measure the oncogenic potential of viruses *in vivo*. As shown by Fortner *et al*(11-13), hamsters may develop in later life a variety of spontaneous transplantable tumors originating mainly in the gastrointestinal tract, the adrenogenital systems, the reticuloendothelial system, and in cells giving rise to malignant melanoma. Although certain of these neoplasms have appeared in untreated animals, they have been noted most frequently following injection of substances such as bile or bile salts, following the feeding of an iodine deficient diet, or following a drastic hormonal alteration such as castration. Contagious tumors of hamsters (14) and the occurrence of multiple types of tumor in a single animal(13) have been reported. These suggest that a virus may be involved in certain of the malignancies observed.

The adenovirus types 12 and 18 tumors resembled those described by Trentin *et al* (3) and by Huebner *et al*(4) as to histology and time of appearance. The type 7 tumors gave the same gross and histopathologic appearance, but they usually appeared much later (231-405 days) than either the type 12 (26-216 days) or type 18 (70-280 days) malignancies, even though the numbers of infectious doses of virus given were roughly the same.

Trentin *et al*(3) and Huebner *et al*(4) failed to show tumor appearance in hamsters given types 7 or 7a virus. The animals were observed for less than 6 months, usually less than 90 days, and this time period is far less than the minimum 231-day interval needed in our studies to evolve type 7 adenovirus tumors. These workers also failed to show tumorigenesis in tests with nearly all the other known adenoviruses, although Huebner *et al*(4) did report suggestive evidence for adenovirus M-1 of monkeys. The finding of a long latent period for induction of adenovirus 7 tumor indicates that lack of oncogenic quality for adenoviruses cannot be concluded unless the animals are observed for at least a year post-virus and suggests that all the adenoviruses should be reexamined under these conditions.

The etiologic role of adenovirus type 7 in tumorigenesis in the present study appears to be conclusive even though the proportion

of animals which developed tumors to date has been rather small. Far more extensive investigations using 3 freshly isolated strains of type 7 adenovirus are currently in progress. Supporting evidence for the role of the type 7 virus in tumorigenesis was given by the demonstration of specific CF antigen and antibody in tumor and in sera from the affected animals and in the specific reaction of tumor-bearing hamster serum with type 7 but not with type 4 adenovirus antigen. Huebner *et al*(15) demonstrated similar CF reactions with their hamster adenovirus 12 and 18 systems. Large tumors tended to show greatest amount of CF antigen and many of the largest tumors were anticomplementary, presumably due to *in vivo* antigen-antibody reactions. As found by Huebner (personal communication) the tumor antigens failed to react with the serum from human beings convalescent from infection with homologous virus. Such antigens were believed by Huebner *et al*(15) to be of the soluble type-specific ("C-soluble") variety such as is produced in cell cultures during viral propagation.

It is recorded, additionally, that the Pinckney strain of type 7 adenovirus was a recent isolate which had never been passed in other than primary or serial passage strains of human cells and was free from detectable cytopathic agents including SV_{40}. The hamsters were injected with Pinckney type 7 adenovirus before types 12 and 18 adenovirus were brought into the laboratory and the animals which received type 7 virus were housed in a separate room from those used for animals given types 12 or 18 adenovirus, SV_{40} or polyoma viruses. The type 7 adenovirus tumors occurred at the site of virus inoculation. The gross and histomorphologic appearance of type 7 tumors was similar to those induced by types 12 and 18 adenovirus and, based on literature survey, spontaneous undifferentiated sarcoma occurs very infrequently in hamsters if at all. The subcutaneous tumors of hamsters which follow SV_{40} or polyoma virus inoculation are fibrosarcomas and these are quite distinct from the adenovirus tumors. The failure of the sera from the tumor-bearing hamsters to neutralize SV_{40} or polyoma virus lends further support to the significance of adenovirus 7 in the tumors observed.

The significance of the fibrosarcoma which appeared in a single hamster which received type 2 adenovirus cannot be stated. It might have been due to the trace of SV_{40} virus which, when given in larger amount(16) causes induction of fibrosarcomas in hamsters. It should be noted, additionally, that fibrosarcomas may appear spontaneously in hamsters(12).

Induction of tumors in hamsters by viruses which commonly infect man has considerable theoretical interest from the standpoint of human neoplasia. The fact that type 7 virus is widespread in the population and causes epidemic acute respiratory illness(6,18) lends special interest to this particular type. It also provides a theoretical basis, at least, for preference of killed adenovirus vaccines(18) over purposely induced live virus infections (19,20), assuming, of course, that the nucleic acid of "killed" virus is not itself active.

Types 12 and 18 adenovirus are unique among adenovirus types by virtue of their failure to cause hemagglutination while type 7 virus does affect agglutination of rhesus monkey erythrocytes(21). Adenoviruses show certain resemblances to the frank tumor-inducing viruses of the papovavirus(22) group (rabbit papilloma, mouse polyoma, monkey SV_{40} and human wart). Thus, all are DNA viruses, are of cubic symmetry with icosahedral structure, are devoid of an envelope, are ether resistant, are acid stable and multiply in the cell nucleus(5,21,22). Green and Pina(23) have reported that adenovirus types 12 and 18 share with the polyoma and Shope papilloma papovaviruses the quality of low guanine-cytosine (48-49%) as compared with adenovirus types 2 and 4 (56-57%). They noted that the guanine-cytosine base composition of the tumorigenic viruses is closer to that of mammalian cell DNA (42-44%), the adeno 12 and 18 possibly having evolved from nontumorigenic adenoviruses by deletion of a piece of DNA rich in guanine and cytosine. These workers sug-

gested that the tumorigenic and nontumorigenic adenoviruses may be quite unrelated genetically. While it is possible that tumorigenic adenovirus and papovavirus DNA may exhibit homology for host cell DNA(23), cognizance must be taken of the basic differences between these agents and the evidence which indicates common genetic origins for all the adenoviruses. Thus, the adenoviruses elaborate a common group-specific complement-fixing antigen which has not been found present in the papovaviruses(5). Additionally, the papovaviruses are 45 mμ in diameter with 42(22) or possibly 92(24) capsomeres. Adenoviruses characterized to date are of about 70 mμ in diameter and have 256 capsomeres.

Influenza A 2, parainfluenza 1, 2, 3, mumps, measles, rhinoviruses, salivary gland virus, varicella and pneumonia virus of mice were all clearly nononcogenic under the present conditions of test. The failure of measles virus to induce tumors is of interest in view of the findings of Nichols *et al*(25) and Fjelde *et al*(26) that measles apparently causes breakage of chromosomes in human cells, and in the purported association of increase in lymphatic leukemia in childhood following measles epidemics. Also, the findings of lack of oncogenic effect of mumps and varicella viruses is worthy of note in relation to clinical reports (cf 27) of a possible association between infection with these agents and the appearance of malignancies in man. The intestinal leiomyosarcoma which occurred in a single hamster given respiratory syncytial virus was of doubtful significance in view of the low incidence of tumors and the observation(11,12) of spontaneous occurrence of such malignancy in hamsters.

Various strains of chicken sarcoma virus have been shown to produce sarcomas in rats, mice, guinea pigs, monkeys and hamsters and also sarcoma-like, though regressive, lesions in rabbits(28-31). Using the Schmidt-Ruppin variant of Rous chicken sarcoma, Ahlstrom *et al*(28) produced tumors in hamsters injected when newborn or at 2 months of age with chicken tumor extract presumed to be cell-free. The Mill Hill strain of chicken sarcoma was not oncogenic for these animals (30). Munroe and Windle(31) induced pleomorphic giant cell sarcomas in hamsters given a strain of chicken sarcoma obtained from Dr. Zilber in Moscow. The appearance of 2 sarcomas, although different, in hamsters injected with Bryan's strain of Rous sarcoma in the present study suggests that this strain might also be oncogenic. Neither the RPL-12 strain of avian visceral lymphomatosis nor the resistance-inducing factor, presumed to be an avian leucosis agent(32), presented any evidence for inducing malignancy, even though such agents are highly oncogenic for chickens. Resistance-inducing factor, present in measles virus grown in chick tissue, also failed to show oncogenic activity.

Among the viruses reported, tumor induction was definitely centered on adenovirus types 7, 12 and 18. Additional investigations are presently in progress to measure the oncogenic potential for hamsters of adenovirus 4 and 5, additional strains of adenovirus 7, arbovirus groups A, B and C and Sicilian sandfly fever virus, rubella, rabies, herpes simplex, poliovirus 1, 2 and 3, ECHO 25, Coxsackie A10 and A21, ECHO 25, lymphocytic choriomeningitis and encephalomyocarditis viruses, and 2 strains of *Mycoplasma pneumoniae*. These experiments have been in progress for 7 to 12 months without the appearance to date of tumors. McLeod and Ham(33) recently recorded failure for poliovirus 1, 2 and 3, Coxsackie A9, 16 and 21, ECHO 9, vaccinia, varicella, measles, adenovirus 3 and canine adenovirus to cause malignancy in hamsters during an incubation period of 95 to 450 days.

Summary. Studies were conducted to measure the *in vivo* oncogenic potential of a variety of "ordinary" viruses. Adenovirus 1, 2, 7, 12 and 18, influenza A2, parainfluenza 1, 2 and 3, respiratory syncytial, mumps, measles, rhinovirus 11, 23 and 30, poliovirus 3, salivary gland virus, varicella, pneumonia virus of mice, Rous sarcoma, visceral lymphomatosis and resistance-inducing factor (avian leucosis) viruses were tested for ability to induce tumors in hamsters inoculated when newborn. A majority of animals given

adenovirus types 12 or 18 developed tumors. Additionally, a portion of animals which received type 7 adenovirus developed sarcomas. Evidence is presented for the significance of type 7 adenovirus in tumor development. Two animals given Bryan's Rous sarcoma virus also developed sarcomas. None of the animals which received any of the other viruses presented significant evidence for induction of malignancy. Studies of additional viruses are in progress.

ADDENDUM: Since the time the present manuscript was submitted, type 7 Pickney virus was found to belong to adenovirus hemagglutinin group 1 in that it agglutinated rhesus but not rat erythrocytes. The strain was identified serologically with antisera to various type 7 adenoviruses and there was no serologic crossing with type 12 or 18 adenovirus. In a repeat experiment, 7 of 60 hamsters inoculated with type 7 Pinckney virus developed tumors to date. A portion of newborn hamsters inoculated with two additional type 7 adenoviruses, Grider and Champagne, have developed palpable masses resembling tumor, suggesting that tumorigenesis may be a generic quality of type 7 adenoviruses.

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1. Girardi, A. J., Sweet, B. H., Slotnick, V. B., Hilleman, M. R., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 649.

2. Eddy, B. E., Borman, G. S., Grubbs, G. E., Young, R. D., *Virology,* 1962, v17, 65.

3. Trentin, J. J., Yabe, Y., Taylor, G., *Science,* 1962, v137, 835.

4. Huebner, R. J., Rowe, W. P., Lane, W. T., *Proc. Nat. Acad. Sci.,* 1962, v48, 2051.

5. Hamparian, V. V., Hilleman, M. R., Ketler, A., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 1040.

6. van der Veen, J., *Am. Rev. Resp. Dis.,* 1963, v88, (no. 3 part 2), 167.

7. Melnick, J. L., *Diagnostic Procedures for Virus and Rickettsial Diseases,* Am. Pub Health Assn., New York, 2nd ed., 1956, 97.

8. Rubin, H., *Bact. Rev.,* 1962, v26, 1.

9. Hamparian, V. V., Ketler, A., Hilleman, M. R., Reilly, C. M., McClelland, L., Cornfeld, D., Stokes, J., Jr., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 717.

10. Girardi, A. J., Hilleman, M. R., Zwickey, R. E., *ibid.,* 1962, v111, 84.

11. Fortner, J. G., Mahy, A. G., Schrodt, G. R., *Cancer Res.,* 1961, v21, 161.

12. Fortner, J. G., Mahy, A. G., Cotran, R. A., *ibid.,* 1961, v21, 199.

13. Fortner, J. G., *ibid.,* 1961, v21, 1491.

14. Brindley, D. C., Banfield, W. G., *J. Nat. Cancer Inst.,* 1961, v26, 949.

15. Huebner, R. J., Rowe, W. P., Turner, H. C., Lane, W. T., *Proc. Nat. Acad. Sci.,* 1963, v50, 379.

16. Girardi, A. J., Sweet, B. H., Hilleman, M. R., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 662.

17. Klein, M., *J. Nat. Cancer Inst.,* 1961, v26, 1381.

18. Hilleman, M. R., *Am. Rev. Resp. Dis.,* 1963, v87, 165.

19. Hitchcock, G., Tyrrell, D. A. J., Bynoe, M. L., *J. Hyg.,* Camb., 1961, v58, 277.

20. Couch, R. B., Chanock, R. M., Cate, T. R., Lang, D. J., Knight, V., Huebner, R. J., *Am. Rev. Resp. Dis.,* 1963, v88 (no. 3 part 2), 394.

21. Ginsberg, H. S., *Virology,* 1962, v18, 312.

22. Melnick, J. L., *Science,* 1962, v135, 1128.

23. Green, M., Pina, M., *Proc. Nat. Acad. Sci.,* 1963, v50, 44.

24. Mattern, C. F. T., *Science,* 1962, v137, 612.

25. Nichols, W. W., Levan, A., Hall, B., Ostergren, G., *Hereditas,* 1962, v48, 367.

26. Fjelde, A., Holtermann, O. A., *Life Sci.,* 1962, No. 12, 683.

27. Hilleman, M. R., *Viruses, Nucleic Acids and Cancer,* 17th Annual Symposium on Fundamental Cancer Research, Houston, Texas, Williams & Wilkins, Baltimore, 1963, 580.

28. Ahlstrom, C. G., Forsby, N., *J. Exp. Med.,* 1962, v115, 839.

29. Ahlstrom, C. G., Bergman, S., Ehrenberg, B., *Acta Path. et Microbiol. Scand.,* 1963, v58, 177.

30. Ahlstrom, C. G., Jonsson, N., *ibid.,* 1962, v54, 136.

31. Munroe, J. S., Windle, W. F., *Science,* 1963, v140, 1415.

32. Friesen, B., Rubin, H., *Virology,* 1961, v15, 387.

33. McLeod, D. L., Ham, A. W., *Canad. Med. Assn. J.,* 1963, v89, 799.

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