specific effects on LH in the case of ovarian steroids, and ACTH in the case of cortical steroids. Estrogen can stimulate ACTH secretion at the same time that it inhibits LH secretion.

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Further Tests in Hamsters for Oncogenic Quality of Ordinary Viruses and *Mycoplasma*, with Correlative Review.* (29789)

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The convincing evidence for primary role of viruses in neoplasia in animals together with the outstanding quality of ordinary nature of such viruses makes it expedient to examine the possible role of virus in carcinogenesis in man. During mid 1961, this laboratory undertook to examine the oncogenic potential for newborn hamsters of a wide variety of viruses which represent the major groups(1) of viruses of the human species and, in addition, some viruses of animal or avian origin with which man may have contact. In a first report(2), the findings in tests of 25 different viruses were recorded. In that report, the oncogenicity of types 12 and 18 adenovirus(3,4) was confirmed, the neoplastic potential for type 7 adenovirus was shown, and the appearance of tumor in 2 animals given Bryan strain Rous sarcoma was recorded. In a second report(5), the primary role of all of 3 newly isolated strains of type 7 adenovirus was shown and details relating to the virology, pathology and immunology of type 7 adenovirus carcinogenesis was presented. The present report records the failure of 25 additional virus or *Mycoplasma* strains to induce tumor when inoculated into newborn hamsters and discusses the relationship of oncogenicity to family, biophysical, biochemical and structural properties of the viruses studied.

Materials and methods. Virus preparations. Details relating to passage history, host tissue used to prepare virus for hamster inoculation, and the infectivity titer of each virus are presented in Table I. Certain of the viruses were freshly isolated from human specimens. Herpes simplex virus was obtained from Dr. E. H. Lennette; reovirus 1 and Coxsackie A10 and A21 from Dr. H. Wenner; arbovirus C strain Marituba from Dr.

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

History of virus or Mycoplasma			Virus or Mycoplasma used to inoculate hamsters		
Family and type	Strain	Passage history of seed used to prepare inoculum	Host tissue	Infectivity titration: Titer	Infectivity titration: Test system
Adenovirus 2	5175 (Fresh isolate)	HDCS 4	HDCS	$10^{-7.7}$ - $10^{-8.5}$/0.2 ml	HeLa
" 3	695 (Fresh isolate)	HEK 5	HEK	$10^{-4.5}$/0.2 ml	"
" 4	Curtis (Fresh isolate)	HEK 5/HDCS 2	HDCS	$10^{-6.5}$/0.2 ml	HEK
" 5	5646 (Fresh isolate)	HDCS 4	"	$10^{-7.0}$/0.2 ml	HDCS
" 6	8385 (Fresh isolate)	HEK 3	HEK	$10^{-6.7}$/0.2 ml	HeLa
Poxvirus					
Vaccinia	Infected calf lymph	Commercial vaccine	HDCS	$10^{-5.0}$/0.2 ml	HeLa
Herpesvirus					
Herpes simplex	McIntyre	PRK 4/HDCS 1	HEK	$10^{-4.5}$/0.2 ml	HEK
Reovirus I	106	HEp2-5/HEK 3	HEK	$10^{-6.5}$/0.2 ml	GMK
Picornavirus					
Enterovirus					
Poliovirus 1	Parker	MK (SV_{40}-free)	GMK	$10^{-7.3}$/0.2 ml	GMK
" 1	Mahoney	" "	"	$10^{-7.5}$/0.2 ml	"
" 2	MEF 1	" "	"	$10^{-7.7}$/0.2 ml	"
" 3	Saukett	" "	"	$10^{-7.5}$/0.2 ml	"
ECHO 25	5125 (Fresh isolate)	HDCS 2	HDCS	$10^{-5.0}$/0.2 ml	HDCS
Coxsackie A10	ET1085	Mouse 19/PHA 7	PHA	$10^{-4.0}$/0.2 ml	PHA
" A21	ETM-317	Mouse 17/PHA 5	"	$10^{-6.0}$/0.2 ml	"
EMC	B	NIH - unknown history/HuHt 10	HEK	$10^{-6.5}$/0.2 ml	HeLa
Arbovirus A	Chikungunya	Suckling mouse, i. cerebral 171	Suckling mouse brain (ICR/Ha)	$10^{-7.6}$/0.02 ml	Suckling mouse - i.c.
" B	Hawaiian dengue	" " " 119	" " " " "	$10^{-6.5}$/0.02 ml	" " "
" C	Marituba	" " " 18	" " " " "	$10^{-6.7}$/0.02 ml	" " "
" (unclass.)	Sicilian sandfly	" " " 36	" " " " "	$10^{-6.4}$/0.02 ml	" " "
Myxovirus					
Rubella	3429 (Fresh isolate)	GMK 17	GMK	$10^{-3.0}$/0.2 ml	GMK
Rabies	Flury	CETC 100 or >	CETC*	$10^{-4.6}$/0.02 ml	Newborn mouse - i.c.
Lymphocytic chorio.	Children's Hosp. of Phila.	Mouse brain - unknown passages	Mouse brain (ICR/Ha)	$10^{-4.7}$/0.03 ml	Adult mouse - i.c.
Mycoplasma	Pneumoniae (Eaton)	Agar 68/Broth 1	PPLO broth	$10^{-5.0}$ CFU/ml	PPLO agar
"	spp. (83810) (Fresh isolate)	HDCS 1	HDCS	$10^{-4.0}$ CFU/ml	" "

Abbreviations = Cell cultures: MK (primary rhesus or cynomolgus monkey kidney); GMK (primary grivet monkey kidney); HDCS (human diploid cell strains, Wistar); PHA (primary human amnion); PRK (primary rabbit kidney); HEK (primary human embryonic kidney); CETC (chick embryo cell culture); HuHt (stable human heart cell line, Girardi); CFU (colony forming units); ICR/Ha (strain of mouse).

*The chick embryo cell cultures were prepared from avian leucosis-free chick embryos.

J. Casals; Flury strain rabies from Dr. H. Koprowski; and *Mycoplasma pneumoniae* (Eaton agent) from Dr. L. Hayflick. The vaccinia virus was recovered from National Drug calf lymph smallpox vaccine and the polioviruses were standard vaccine production strains freed of SV_{40} agent. Strain 83810 of *Mycoplasma spp.* was recovered from a contaminated human diploid cell strain WI-26.‡ All other viruses were from the certified seed stocks routinely maintained in this laboratory. The passage history relates to the seed stock used to prepare the inoculum which was given to the hamsters. All viruses employed in the investigations were proved free of extraneous virus based on the results of exhaustive tests in cell cultures in which homologous specific neutralizing antisera were employed. All agents were identified in appropriate tests with reference antisera.

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 the 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, were given extracts of brains of uninoculated mice of identical origin to those used to prepare virus, or were given uninoculated *Mycoplasma* culture broth of the same lot used to prepare *Mycoplasma* 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. All animals alive at termination of the experiment were examined at autopsy and tissues and organs which appeared abnormal were examined histopathologically. For histopathologic study, the tissues were fixed in 10% formalin and the paraffin sections were stained with hematoxylin and eosin.

Results. Table II summarizes the findings to date. The period of observation ranged from 1 to 2 years for all agents, except for adenovirus 3 and 6 and reovirus 1 which are under observation at present, and for poliovirus 2 in which all animals died within a few days. No tumor was observed in any of the animals studied.

In certain instances, as noted in the Table, the virus dose was necessarily small since a greater amount of virus was lethal for the animals. It was of interest that the newborn hamsters were extremely sensitive to arbovirus A (Chikungunya) and C (Marituba) but not to B (Hawaiian dengue) or Sicilian sandfly fever virus.

There was a gradual reduction in number of animals resulting from death of undetermined but presumably natural causes. The number of animals which survived according to 3-month intervals is shown in the Table. Inspection of the figures permits a determination of how many animals were at risk in each of the groups for each time period.

Discussion. The findings in tests in hamsters such as recorded here, provide scarcely more than insight and guideline for further studies as they relate to tumorigenicity of viruses for such distantly related species as man. Such optimal tests as would be carried out in the human species itself must necessarily be excluded.

A summary of our findings(2,5,6) to date, to survey the oncogenic potential of viruses and *Mycoplasma* in newborn hamsters is presented in Table III. The observations in the hamsters are correlated with the family relationships of the agents studied and with their structural, biochemical and biophysical properties(1,7). Tumorigenic capacity among the viruses of man was limited quite strictly to

‡ This PPLO failed to type in complement-fixation tests with antisera against PPLO of human origin performed by Dr. Norman Somerson, of Nat. Inst. Health.

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

certain of the adenovirus types such as type 7 which is a principal cause for acute respiratory illness in human beings(8). It is of some interest that the adenoviruses, except for size, bear remarkable biochemical, biophysical and morphologic similarity to the highly tumorigenic papovavirus group(9), *viz.*, SV_{40}, polyoma, and papilloma. Vaccinia virus was not oncogenic even though members of this same family, *viz.*, rabbit myxoma

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

Hamster inoculation				Result							
Virus or control	Route, volume and dilution *	No. inoc.	No. weaned	No. survived according to time post-weaning, months: 1-3	4-6	7-9	10-12	13-15	16-18	24	No. developed malignancy
Adenovirus 2	Subcut., 0.2 ml	58	41	38	28	24	15	13	7	4	0
" "	Intrathor., 0.2 ml	83	24	21	17	9	8	7	5	4	0
Control	Subcut., 0.2 ml	57	47	45	39	32	22	15	14	6	0
"	Intrathor., 0.2 ml	53	40	40	39	39	33	20	13	4	0
Adenovirus 3	Subcut., 0.2 ml and	66	57	54	22	18	(on test)				0
Control	Intrathor., 0.05 ml	30	19	19	4	3	(on test)				0
Adenovirus 4	Subcut., 0.2 ml	17	17	17	16	16	14	9	5	2	0
" "	Intrathor., 0.2 ml	34	16	16	15	15	15	13	7	3	0
Control	Subcut., 0.2 ml	34	26	25	23	19	12	10	10	4	0
"	Intrathor., 0.2 ml	39	31	31	31	31	29	16	9	1	0
Adenovirus 5	Subcut., 0.2 ml	27	15	15	14	13	13	10	9	2	0
" "	Intrathor., 0.2 ml	42	11	11	11	9	9	7	5	2	0
Control	Subcut., 0.2 ml	30	11	5	5	4	4	4	4	2	0
"	Intrathor., 0.2 ml	39	29	28	25	23	20	19	16	7	0
Adenovirus 6	Subcut., 0.2 ml and Intrathor. 0.05 ml	84	69	68	36	21	(on test)				0
Control	"	12	5	4	2	2	(on test)				0
Poxvirus											
Vaccinia	Subcut., 0.2 ml	38	35	33	32	27	9	5	1 T.		0
"	" " 10^{-1}	54	37	37	34	26	20	15	5 T.		0
Control	Subcut., 0.2 ml	61	49	47	38	29	18	15	12 T.		0
Herpesvirus											
Herpes simplex	Subcut., 0.2 ml and Intraper., 0.1 ml 10^{-6}	25	0	-	-	-	-	-	-		0
" "	" " $10^{-6.5}$	65	19	19	18	16	11	9	T.		0
" "	" " $10^{-7.0}$	13	11	11	11	11	8	4	T.		0
Control	Intraper., 0.1 ml	37	29	29	27	25	19	15	T.		0
Reovirus 1	Subcut., 0.2 ml	82	52	43	35	(On test)					0
Control	" "	59	35	32	31	(On test)					0
Picornavirus											
Enterovirus											
Poliovirus 1 (Parker)	Subcut., 0.2 ml	63	43	40	39	35	22	19 T.			0
" 1 (Mahoney)	" "	66	62	56	52	40	36	33 T.			0
" 2	" "	51	0	-	-	-	-	-	-		0
" 3	" "	63	49	47	45	29	19	13	T.		0
Control	" "	43	33	30	30	25	23	17	T.		0
ECHO 25	" "	51	49	43	39	27	17	15 T.			0
Control	" "	44	43	36	32	30	22	19 T.			0
Coxsackie A10	Subcut., 0.2 ml $10^{-3.5}$	67	0	-	-	-	-	-	-		0
" " "	" " 10^{-4}	91	29	29	28	24	16	13 T.			0
" A21	" " 10^{-1}	66	48	45	42	35	25	23 T.			0
Control	Subcut., 0.2 ml	44	33	31	29	23	19	18 T.			0
EMC	Subcut., 0.2 ml 10^{-8}	49	0	-	-	-	-	-	-		0
"	" " 10^{-9}	101	59	51	40	29	23	20	T.		0
Control	Subcut., 0.2 ml	22	22	22	19	17	14	14	T.		0
Arbovirus type A	Subcut., 0.2 ml 10^{-7}	26	0	-	-	-	-	-	-		0
" " "	" " 10^{-8}	105	6	6	6	3	1	T.			0
" " B	" " undil.	67	9	8	6	6	6	5	4 T.		0
" " "	" " 10^{-2}	73	62	62	57	50	35	31	T.		0
" " C	" " 10^{-8}	48	0	-	-	-	-	-	-		0
" " "	" " 10^{-9}	93	53	53	50	37	29	24	17 T.		0
" Sicilian sandfly	" " undil.	87	2	1	1	1	1	1	T.		0
" "	" " 10^{-1}	45	19	12	11	10	T.				0
Control	Subcut., 0.2 ml	91	91	90	83	75	50	30	23 T.		0
Myxovirus											
Rubella	Subcut., 0.2 ml	82	72	70	63	42	35	26	13 T.		0
Control	" "	73	62	62	55	44	35	31	18 T.		0
Rabies	Subcut., 0.2 ml 10^{-3}	18	4	3	3	2	1	1	T.		0
"	" " $10^{-3.5}$	190	25	23	19	16	13	12	T.		0
Control	Subcut., 0.2 ml	41	39	37	33	31	22	14	T.		0
Lymphocytic Chorio.	Subcut., 0.2 ml 10^{-2}	22	0	-	-	-	-	-	-		0
" "	" " 10^{-4}	195	28	21	19	18	12	6	T.		0
Control	Subcut., 0.2 ml	28	28	27	24	21	15	10	T.		0
Mycoplasma pneumoniae	Subcut., 0.2 ml	20	18	18	17	11	11	T.			0
" "	Intrathor., 0.05 ml	37	23	23	21	13	10	7	T.		0
Control	Subcut., 0.2 ml and Intrathor., 0.05 ml	25	18	14	12	11	8	T.			0
Mycoplasma spp. (83810)	Subcut., 0.2 ml	59	56	54	51	45	32	27	22 T.		0
" "	Intrathor., 0.05 ml	70	64	61	51	47	39	34	T.		0
Control	Subcut., 0.2 ml	63	41	36	35	32	19	19	T.		0
"	Intrathor., 0.05 ml	33	30	29	26	24	17	12	T.		0

*Undiluted unless indicated otherwise. T = experiment terminated.
Control material consisted of uninoculated materials, otherwise identical to the virus preparation.

and Yaba agent(10,11) may induce malignant or benign tumors in animals and poxvirus may act as a co-carcinogen together with a primary carcinogen(12). The herpesvirus-like group agents were nononcogenic even though herpes simplex virus may cause marked chromosomal changes in cells(13). Reovirus 1 has not produced tumor to date but remains of special interest because of its purported morphologic and serologic similarity to wound tumor virus of plants(14). Picornaviruses were nonproductive of tumor though certain of the group are recognized as capable of co-carcinogenic activity(15). The arboviruses were nononcogenic though West Nile virus(16) may act as a co-carcinogen. Myxoviruses, which ordinarily infect man, were nononcogenic though agents such as in-

TABLE III. Summary. Present and Previous Findings in Tests in This Laboratory of Oncogenic Potential for Hamsters of Viruses and *Mycoplasma*. Correlation with other properties (refs. 1, 7).

Nucleic acid type	Ether and/or chloroform sensitivity	pH 3.0 lability	Other quality	Morphology (according to Almeida)(ref. 7)	Family & species	Type	Strain	Maximum time on test (Months)	Oncogenicity
DNA	Stable	Stable	70 mμ	Simple, cubic, nuclear	Adenovirus	1	AD 71	21	No
					"	2	5175	24	"
					"	3	695	9 (on test)	(No to date)
					"	4	Curtis	24	No
					"	5	5646	"	"
					"	6	8385	9 (on test)	(No to date)
					"	7	Pinckney	-	Yes
					"	"	Grider	-	"
					"	"	Champagne	-	"
					"	12	Huie and 9512	-	"
					"	18	14606	-	"
"	"	"	40-50 mμ	" " "	Papovavirus	-	SV_{40}	-	"
"	Labile	Labile	200 x 300 mμ Heat stable	Complex, cytoplasmic (Brick)	Poxvirus	-	Vaccinia	18	No
"	"	"	180 mμ Heat labile	Compound, cubic, nuclear (Sphere)	Herpesvirus-like				
					Herpes simplex	-	McIntyre	16	"
					Varicella	-	Shaffer	21	"
					Cytomegalovirus	-	AD 169	23	"
RNA	Stable	Stable	Heat stable	Simple, cubic, cytoplasmic	Reovirus	1	106	6 (on test)	(No to date)
"	"	"	" "	" " "	Picornavirus				
					Enterovirus				
					Poliovirus	1	Parker	14	No
					"	1	Mahoney	"	"
					"	3	Saukett	24	"
					ECHO	25	5125	14	"
					Coxsackie	A10	ET 1085	"	"
						A21	ETM-317	"	"
		Labile	-		Rhinovirus	11	7	23	"
					"	23	5007	"	"
					"	30	5582	12	"
		-	-		Encephalomyocarditis	-	B	16	"
"	Labile	Labile	30-50 mμ	Compound, cubic, cytoplasmic	Arbovirus	A	Chikungunya	14	"
						B	Hawaiian dengue	17	"
						C	Marituba	16	"
						Unclassif.	Sicilian sandfly	17	"
"	"	"	100-120 mμ	Compound, helical, cytoplasmic	Myxovirus				
					Influenza	A2	5548	12	"
					Parainfluenza	1	HA2 C35	22	"
						2	CA Greer	"	"
						3	HA1 C243	"	"
					Respiratory syncytial	-	Long		"
					Mumps	-	Johnson	12	"
					Measles	-	Edmonston - virulent	22	"
					"	-	" 749D atten.	23	"
					Rubella	-	3429	18	"
					Rabies	-	Flury	16	"
					Avian leucosis*				
					Rous sarcoma	-	Bryan	22	Yes (?)
					Visceral lymphomatosis	-	RPL-12	"	No
					Resistance inducing factor	-	30738	12	"
-	-	-	-	-	Pneumonia virus of mice	-	PVM-15	22	"
-	-	-	-	-	Lymphocytic choriomeningitis	-	CHOP	16	"
-	-	-	-	-	*Mycoplasma pneumoniae*	-	Eaton	15	"
					" spp.	-	83810	16	"

*? Myxovirus group.

fluenza may act as a co-carcinogen(17), though measles virus may cause extensive chromosome breakage(18) and though the Myxoviruses of man exhibit apparent biologic kinship to such highly oncogenic animal viruses as the avian leucosis, mouse leukemia or mouse mammary carcinoma groups. Pneumonia virus of mice, lymphocytic choriomeningitis and *Mycoplasma* showed no oncogenic quality in hamsters.

There is no definitive information at present to relate any known virus to neoplasia in man. As recently reviewed(19,20), such agents as rubella, mumps, vaccinia, herpes simplex, herpes zoster, cytomegalovirus, wart, and lymphogranuloma venereum viruses have been rendered suspect because of clinical data which would suggest some sequential relationship between virus infection and tumor appearance. More substantial but still nondefinitive data have been recorded in the isolation of mongoloid hamster virus from tissues of patients with cancer(21), the recovery of a herpes-like agent(22) and a reovirus(23) from Burkitt lymphoma, the recovery of type 1 adenovirus from human tumor(24,25), the reported isolation of unidentified viruses from human malignant specimens(26-29) or in the demonstration of virus-like structures in tissues of patients with cancer(30-33).

The failure by McLeod and Ham(34) to induce tumor in hamsters inoculated with Picornaviruses (poliovirus 1, 2 and 3, Coxsackie A9, 16 and 21, ECHO 9), vaccinia, varicella, measles, adenovirus 3 or canine adenovirus during an incubation period of 95 to 450 days is in accord with the present results.

Summary. Previous and present findings demonstrated the failure of tumor induction in newborn hamsters by adenovirus 1, 2, 3 (tentative), 4, 5, and 6 (tentative), vaccinia, herpes simplex, varicella, cytomegalovirus, reovirus 1 (tentative), poliovirus 1 and 3, ECHO 25, Coxsackie A10 and A21, rhinovirus 11, 23 and 30, encephalomyocarditis, chikungunya, Hawaiian dengue, Marituba, Sicilian sandfly, influenza A2, parainfluenza 1, 2 and 3, respiratory syncytial, mumps, measles (virulent and attenuated), rubella, rabies, visceral lymphomatosis (including RPL-12 and RIF agent), pneumonia virus of mice and lymphocytic choriomeningitis viruses and two *Mycoplasma species* including Eaton agent. Adenovirus 7 was moderately and adenovirus 12 and 18 were highly oncogenic. Tumors occurred in 2 animals which received Bryan strain Rous sarcoma. The findings are discussed in relation to the familial, biophysical, biochemical and structural properties of the viruses studied and with respect to the evidences which suggest a possible role of virus in human neoplasia.

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Arbovirus Complement Fixing Antibodies in Sera of Wildlife of West Central Utah.* (29790)

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During the routine serological examination of wildlife sera for complement fixing (CF) antibodies(1-3) using *Rickettsia rickettsii*, *Coxiella burnetii*, *Pasteurella pestis* and psittacosis antigens, certain species were found to produce multiple and suspected false positive reactions. In attempts to determine the cause of these reactions, the serum samples were tested with several other CF antigens, including western equine encephalomyelitis (WEE), eastern equine encephalomyelitis (EEE), Venezuelan equine encephalomyelitis (VEE) and St. Louis equine encephalomyelitis (SLE) antigens.

The antigens used in these tests were obtained commercially from Lederle and Markhan Laboratories. The tests utilized 2 exact units of complement (fresh whole guinea pig serum), 2 units of antigen, as determined by block titration, and 2 units of commercial hemolysin. A 2-4°C overnight incubation was used for this combination of serum, antigen and complement. This incubation was followed by addition of sensitized sheep red blood cells and another incubation period of one hour in a 37°C waterbath. Hemolysis of 50% or less was considered indicative of a positive sample at a serum dilution of 1:16 or greater. Appropriate controls, including known positive and negative serum samples, were used in each test.

Known outbreaks of WEE have occurred recently in Utah(4), and SLE reportedly has a distribution which includes this area(5). Evidence of EEE virus has been reported as far west as Texas(6), and several reports have indicated the possible existence of VEE

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