demonstrated in practically every transformed cell. The *in vitro* transformations confirm the oncogenic potential of the hybrid virus.

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*TUMORS INDUCED IN HAMSTERS BY A STRAIN OF ADENOVIRUS TYPE 3: SHARING OF TUMOR ANTIGENS AND "NEOANTIGENS" WITH THOSE PRODUCED BY ADENOVIRUS TYPE 7 TUMORS**

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Recently, we observed tumors induced in hamsters by a strain of type 3 adenovirus which had been isolated from a child with acute respiratory illness. The type 3 inoculum designated as strain 15520 had been carried for seven passages in human diploid cells prior to injection of newborn hamsters.[1] A subcutaneous tumor appeared 275 days later at the site of injection in one of eight hamsters. When the

TABLE 1

ONCOGENICITY OF STRAINS OF ADENOVIRUS TYPES 3 AND 7

Virus Type	Virus Strain	Tissue culture passage history	Virus dose HEK $TCID_{50}$*	Postinoc. day tumor first noted	Hamsters with Tumors on Postinoc. Day Indicated 200	250	300	350	400
AD-3	15520	DT_7†	$10^{7.7}$	258	0/8‡	0/8	1/8	1/8	1/8
"	"	DT_7	$10^{7.7}$	151	1/17				
AD-7	Pinck.	HEK_5	NT	201	0/18	5/18	8/18	9/18	9/18
"	"	HEK_6	$10^{7.0}$	183	4/7	5/7			
"	"	HEK_5 KB_2	$10^{9.2}$	184	1/11	1/11	1/11	2/11	2/11
AD-7	C14500	HEK_4	$10^{7.8}$	209	0/38	7/38	10/38	16/38	17/38
AD-7	55142	DT_7	$10^{6.2}$	335	0/34	0/34	0/34	1/34	1/34
AD-7	19690	DT_4 KB_2	$10^{7.7}$	362	0/14	0/14	0/14	0/14	2/14
AD-7	Gomen	$HeLa_{11}$ KB_2	$10^{8.2}$	363	0/14	0/14	0/14	0/14	1/14
"	"	$HeLa_{11}$ KB_2	$10^{8.7}$	314	0/14	0/14	0/14	1/14	1/14

NT = Not titered.
* Titers obtained in HEK cultures observed for 28 days.
† Human diploid tissue.
‡ No. with tumors/no. on test.

same material was subsequently injected both subcutaneously and intraperitoneally, a tumor was observed intraperitoneally after 151 days in one of 17 hamsters. In its long incubation period prior to tumorigenesis, strain 15520 differed considerably from the prototype strains of adenovirus 12 and 18 which readily induce tumors in 1–4 months.[2, 3] On the other hand, it resembled five strains of adenovirus type 7 which have also required long periods before producing tumors in hamsters[4, 5] (see Table 1). Contrary to previous reports,[2, 3] the prototype (Gomen) strain of adenovirus type 7 also produced tumors. Since in most cases the infectivity titers of the types 3 and 7 inocula equaled or exceeded that of the types 12 and 18 preparations,[2–5] it appears that the former two serotypes are somewhat less potent producers of hamster tumors than the latter. The identities of the oncogenic 15520 strain of type 3 and of the several oncogenic strains of type 7 were confirmed by neutralization tests using types 3 and 7 prototype rabbit antisera. All strains gave specific reactions with their respective antisera.

The adenovirus types 3 and 7 tumor cells were easily transplanted to the sub-

TABLE 2

CROSS TITRATIONS OF POOLED SERA FROM ADENOVIRUS-TUMORED HAMSTERS VERSUS ADENOVIRUS-INDUCED HAMSTER TUMOR ANTIGEN POOLS

		Antigens: AD-3				AD-7				AD-12				AD-18			
Hamster antisera		2*	4	8	16	4	8	16	32	4	8	16	32	4	8	16	32
AD-3	20†	4	3	1	0	4	4	4	1	0				0			
	40	3	1	0	0	4	4	2	1								
	80	0	0	0		4	3	1	0								
AD-7	20	4	4	4	4	4	4	4	3	0				0			
	40	4	4	4	2	4	4	4	2								
	80	1	1	1	1	3	3	3	1								
	160	1	0	0	0	1	1	0	0								
AD-12	20	0				0				4	4	4	1	4	4	4	0
	40									4	4	4	0	4	4	4	0
	80									4	4	4	0	4	4	3	0
	160									3	3	0	0	4	4	1	0
AD-18	20	0				0				4	4	4	4	4	4	4	4
	40									4	4	4	3	4	4	4	4
	80									3	2	1	0	4	4	4	3
	160									0	0	0	0	4	4	4	1

Results are representative of several replicate tests.
* Reciprocal of antigen dilution horizontal column.
† Reciprocal of serum dilution vertical column.

TABLE 3

CROSS TITRATIONS OF POOLED SERA FROM ADENOVIRUS-TUMORED HAMSTERS VERSUS TISSUE CULTURE-GROWN ADENOVIRUS NEOANTIGENS*

Hamster antisera		Antigens: AD-3 4†	AD-3 8	AD-3 16	AD-3 32	AD-7 4	AD-7 8	AD-7 16	AD-7 32	AD-12 4	AD-12 8	AD-12 16	AD-12 32	AD-18 4	AD-18 8	AD-18 16	AD-18 32
AD-3	20‡	4	3	2	2	4	4	3	3	0				0			
	40	3	1	0	0	4	4	2	1								
	80	2	1	0		3	2	1	0								
AD-7	20	4	4	4	4	4	4	4	4	0				0			
	40	4	4	4	4	4	4	4	4								
	80	4	4	4	4	4	4	4	4								
	160	4	4	4	3	4	4	4	4								
AD-12	20	0				0				4	4	4	4	4	4	4	4
	40									4	4	4	4	4	4	4	4
	80									4	4	3	2	4	4	4	3
	160									4	3	1	0	4	4	3	1
AD-18	20	0				0				4	3	1	0	4	4	4	4
	40									4	3	1	0	4	4	4	4
	80									3	2	0	0	4	3	1	1
	160									2	1	0	0	1	1	0	0

Results are representative of several replicate tests.
* 20 × concentrated KB cell packs harvested at 72 hr.
† Reciprocal of antigen dilution horizontal column.
‡ Reciprocal of serum dilution vertical column.

cutaneous and peritoneal tissues of both newborn and weanling hamsters; and as described previously by Larson *et al.*,[6] the hamsters carrying primary or transplanted type 7 tumors for several weeks frequently developed complement-fixing (CF) antibodies to antigens present in their homologous tumors. Table 2 shows the homologous and heterologous reactivities of pooled sera from hamsters carrying tumors induced by the various oncogenic adenoviruses. Hamsters carrying type 3 tumors developed CF antibodies less frequently and to lower titers than did those carrying type 7 tumors; however, the reactivity achieved was sufficiently strong to indicate a definite sharing of CF antigens in the tumors induced by types 3 and 7. There was no crossing with the tumor antigens shared by types 12 and 18,[7a] or with polyoma, SV40, Schmidt-Ruppin strain Rous sarcoma virus-induced hamster tumor antigens, F. Sa. 3 hamster tumor antigens, [7b,7c] or with other nonspecific reacting hamster tissue antigens.[5]

As shown in Table 3, nonvirion "neoantigens"[7–10a] produced in KB tissue cultures early after infection revealed a similar sharing of antigens between types 3 and 7. The neoantigens produced by several strains each of types 3 and 7 (including the prototypes) were compared by complement fixation tests. All of them gave almost identical reactions with the sera of hamsters carrying types 3 and 7 tumors. Hamsters carrying adenovirus type 7 tumors developed antibodies reactive to the early "cell-pack" antigens (neoantigens) found in tissue cultures infected with certain other adenoviruses. Table 4 shows that a standard pool of sera from hamsters carrying type 7 tumors reacted with neoantigen preparations of types 3, 4, 11, 14, 16, and 21, but not with neoantigen preparations of 10 other adenovirus serotypes, SV40, or polyoma,[8–10b] whereas the less highly reactive adenovirus type 3 hamster serum reacted only with the types 4 and 16 as well as with the types 3 and 7 neoantigens. There is recent evidence that purified type 21 is tumorigenic to newborn hamsters, but to date types 4, 11, 14, and 16 have not been reported to induce tumors in hamsters.[4, 5, 10c] As indicated by Pereira *et al.*,[11] type 31-induced tumors

TABLE 4

SPECIFICITY AND SHARING OF TISSUE CULTURE-GROWN NEOANTIGENS

Neoantigens[a]		Standard Pooled Antisera Used at 4–8 Units: Human adenovirus group-specific	Hamster ad 3	Hamster ad 7	Hamster ad 12	Hamster ad 18	Hamster SV40
Adenovirus type[b]	1	128[c]	0[d]	0	0	0	0
	2	128	0	0	0	0	0
	3	128[e]	8	32	0	0	0
	4	256[e]	8	32	0	0	0
	5	128	0	0	0	0	0
	7	256[e]	32	128	0	0	0
	9	128[e]	0	0	0	0	0
	11	128[e]	0	16	0	0	0
	12	128	0	0	128[e]	8	0
	13	128[e]	0	0	0	0	0
	14	256[e]	0	64	0	0	0
	15	128[e]	0	0	0	0	0
	16	128[e]	8[e]	128[e]	0	0	0
	17	128[e]	0	0	0	0	0
	18	128[e]	0	0	64[e]	64[e]	0
	21	128	0	8	0	0	0
	31	128[e]	0	0	64[e]	8	0
SV40[f]		NT	0	0	0	0	8
Polyoma[g]		NT	0	0	0	0	0

NT = Not tested.
[a] 20× concentrated cell packs harvested at 72 hr.
[b] KB tissue culture.
[c] Reciprocal of antigen dilution.
[d] No complement fixation with antigen dilution of 1:4.
[e] No end point at the highest antigen dilution tested.
[f] Monkey kidney primary tissue culture.
[g] Mouse embryo primary tissue culture.

contained antigens shared with those found in types 12 and 18 tumors. Similarly, the type 31 neoantigens crossed with those of types 12 and 18 (Table 4). Thus, insofar as their nonvirion neoantigenic properties are concerned, the tumors induced by adenoviruses 3 and 7, on the one hand, and by 12, 18, and 31, on the other, appear to fall into two distinct categories.

Unlike the hamsters carrying type 12 tumors,[7a] none of the hamsters carrying type 3 or type 7 tumors developed virus-neutralizing antibodies at a 1:10 dilution, and CF tests of tumored hamster sera for antibodies to the type-specific "C" antigen of type 7 were also negative. Only one of 27 hamsters carrying adenovirus type 7 (Pinckney strain)-induced tumors developed CF antibody to the purified virion of adenovirus type 7.

Cells from adenovirus types 3- and 7-induced primary and transplanted hamster tumors were established in tissue culture[12] following which "packed-cell suspensions"[8] from serially passed subcultures were tested for tumor antigens versus their respective positive hamster sera. While tumor antigens were demonstrated in one line of type 3 cells after three tissue culture passages and as early as the primary tissue culture in one line of type 7 cells, several other tissue culture lines derived from types 3 and 7 tumors had little or no complement-fixing antigen when carried as far as the 9th and 13th subculture.

Several tumor cell tissue cultures were transplanted to newborn and weanling hamsters. One tissue culture line of type 3 cells having no demonstrable complement-fixing tumor antigen in the 10th subculture was passed four more times and transplanted to newborn hamsters which developed tumors having detectable complement-fixing antigens versus type 7 hamster antisera. When these tumors were transplanted to weanling hamsters, several developed high-titered specific serum antibodies to the tumor and neoantigens of both types 3 and 7.

Discussion.—The sharing of neoantigens between adenovirus types 3, 4, 7, 11, 14, 16, and 21 was not wholly unexpected since serological[13–23] and other common properties have been described previously for these serotypes.[20, 21, 24–29] Types 12, 18, and 31, which share neoantigens different from those of the former group, also have other properties in common; most notably, they show crossing in neutralization tests and produce little or no hemagglutinin.[11, 22, 23, 30] The neoantigens are cellular products apparently not incorporated in the virion particle. Appearing in the nuclei of cells early after infection before virion antigens are produced,[31, 32] they very likely have a function in the genesis of virus. It is not wholly clear whether or not they are coded for by newly expressed cell genes, or by viral genes. However, the distribution of neoantigen content according to other properties which are clearly expressions of the viral genome tends to support the latter as the determining factor in the production of neoantigens.[10a]

The oncogenic properties of types 3 and 7 may prove to be particularly important in their possible relationship to human cancer because, unlike types 12 and 18, these two serotypes are known to cause numerous febrile respiratory illnesses in children and in military recruits.

Infections and illnesses due to type 3 have been found wherever studies have been made. Surveys for serum antibodies to type 3 carried out in various parts of the United States, Western Europe, Russia, Japan, Australia, and Formosa indicated that 40–50 per cent of children and 50–70 per cent or more of adults had neutralizing antibodies to type 3.[33–44]

In the Washington, D. C., area where contemporary serologic surveys confirm a high prevalence for neutralizing antibodies to type 3,[1, 45] we have evidence that this high prevalence may be the result of relatively recent events. A survey of sera taken between 1950 and 1953 from the same population showed that only 20 per cent of the children and adults (to age 35) had neutralizing antibodies to type 3.[46] It was shortly after this (1954) that outbreaks of pharyngoconjunctival fever due to type 3 were observed not only in various parts of the United States and Canada but also in many European countries and Australia, Japan, and Formosa as well.[43, 46–53] Outbreaks of pharyngoconjunctival fever have not been reported in the United States since 1960; this is not unexpected since the high prevalence of neutralizing antibodies reported for all the areas surveyed would preclude epidemic occurrences of type 3. However, continuing studies of adenovirus prevalence among the clinic patients attending the Washington, D. C., Children's Hospital show that type 3 is the most frequent adenovirus cause of febrile respiratory illnesses.[1] Prevalences of antibodies to adenovirus types 7, 12, and 18, insofar as they are known, appear to be quite low in those childhood populations which reveal high rates of adenovirus 3 neutralizing antibodies.[1, 37, 40, 43] Should the adenoviruses which are oncogenic in hamsters, rats, and mice also manifest oncogenic activity in man, then type 3 would have to be regarded as the prime suspect, if only because, among the known oncogenic adenoviruses, type 3 in recent years is the only serotype which has had an opportunity to infect most humans at an early age. However, it should be stated that as yet no evidence has been presented which suggests that any of the adenoviruses are oncogenic in man.

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*THEORY OF THE FLOW OF ACTION CURRENTS IN ISOLATED MYELINATED NERVE FIBERS, IV**

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We continue the presentation of the theory of the isolated fiber.[1] To facilitate cross references the number of the figures in preceding communications will be preceded by the corresponding reference. For example, 1*c*, Figure 3 is Figure 3 in reference 1*c*.

Technique of Interpretation of Electrical Recordings.—The diagrams in Figures 1–3 illustrate the physical meaning of equations (1), (2), and (4) of reference 1*b*. They present instantaneous distributions of action currents (external and membrane) and of external action potential, $-V_e$, in the Ringer pools and in the recording gaps at selected instants during the propagation of an impulse along the isolated segment of fiber.

The diagrams consider situations that arise when the amplifier is in position *IV* (1*b*, Fig. 1) and node N_1 is located in the central pool (*I–V*), or at the center of the first gap (*VI*). According to the uncertainty principle[1d] the distributions of potential given in the diagrams in general do not apply to the situations which arise when the amplifier is placed in positions *I* or *II*, but they do apply to those particular instances in which, owing to the presence of suitable external shunts, the impulse propagates itself in approximately the same ways when the amplifier is in positions *I*, *II*, or *IV*. As was explained in ref. 1*c* (Fig. 3), after active zone *a* has reached a certain position, the impulse executes successive jumps, whereby additional active zones (*b*,*c* or b_1,b_2,*c*) are created in rapid succession and, since the active zones coexist for significant periods of time, the external longitudinal current flows in two or more directions in each recording gap.

The diagrams indicating the flow of action currents are self-explanatory; they are intended to indicate only the direction of flow; the magnitude of the longitudinal flow of current at any given point of the recording gaps is measured by the slope that the distribution of external potential has at that point. On the other hand, since the distributions of potential are only approximate, their didactic value can be increased by assuming that, when their ordinate values are measured from suitable base lines (labeled $i_m = 0$ in Fig. 1, *Ia*), the $-V_e$ curves also give the distributions of the density of the flow of membrane current in the still inactive segments of fiber, in which an outward flow is taking place. The assumption is not far from the truth, because in those segments the distributions of potential must be nearly exponential.

The diagrams of the distributions of external potential $-V_e$ can easily be read if the following circumstances are taken into account. Since in the Ringer pools the flows of external action cur-