Further Observation on Typing Adenoviruses and a Description of Two Possible Additional Serotypes. (27626)

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Recent reports have described a hemagglutination-inhibition (HI) technic for typing adenoviruses and the use of this technic in the characterization of 4 previously unrecognized serotypes(1,2). This report describes 2 possible additional serotypes as well as 3 "prime" strains of previously known adenoviruses. Data are also presented on cross HI reactions among the viruses in the 2 subgroups containing all the recently recognized human adenoviruses.

The materials and methods employed were exactly the same as those used previously (1,2).

Results. In the course of typing adenoviruses recovered during a longitudinal study of children in a Washington, D.C. welfare institution(3), a number of strains were encountered which could not be identified by the HI technic as any of the 28 previously known human serotypes. Since these strains were suspected to be previously unrecognized adenoviruses, antisera were prepared against representative isolates. These antisera were then checked by HI against the previously known viruses. It was found that some of the isolates were "prime" strains of adenovirus types 16, 17, or 27. In other words, antisera against the isolates inhibited one of these prototype viruses to about the same extent as it did the homologous virus, but the latter was not inhibited by the prototype antiserum. Cross HI and neutralization reactions between 3 of these "prime" strains and the respective prototype strains are shown in Table I. It will be noted that the "prime" relationship was also observed in neutralization in 2 instances but not in the third. Surprisingly, the "prime" strains were recovered in the same time periods as conventional strains of the same serotype. In fact, the type 27 "prime" strain was recovered from a child 3 days after the isolation of a conventional strain of the same serotype from the same individual.

Several of the untypable isolates were found to fall into 2 serotypes which will be referred to as BP-6 and BP-7 respectively. As with the 4 other serotypes recovered for the first time from the same population(2), BP-6 and BP-7 isolates A) were obtained only from anal specimens, B) produced a cytopathic effect similar to that of types 9, 10, and 13 and different from that of types 1, 2, 3, and 5, and C) were less readily isolated than types 1, 2, 3, and 5.

The strain selected as the prototype of BP-6 was isolated from an anal specimen collected from a 35-month-old male Negro child on June 10, 1959. Strains of BP-6 were also isolated from the same population in 1958 and from children elsewhere in Washington, D. C. in 1959 and 1960.

The strain selected as the prototype of BP-7 was isolated from an anal specimen collected from an 18-month-old male Negro child on June 10, 1959. Strains of BP-7 virus were also isolated from other children in the institution in 1959 and from children elsewhere in Washington, D. C. in 1959 and 1960.

Some of the children yielding BP-6 and BP-7 isolates had minor illnesses of a variety of types. The data available were not sufficient to determine if the viruses were responsible for these illnesses.

The BP-6 and the BP-7 prototype strains were cross-checked in both neutralization and HI tests against each of the previously described human serotypes. The homologous neutralization titers of the prototype sera ranged from 1:80 to 1:320 or greater. The neutralization titers of the BP-6 and BP-7 sera were 1:160 and 1:320 respectively. All sera were used in neutralization tests at ini-

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TABLE I. Cross Hemagglutination-Inhibition (HI) and Neutralization (NT) Reactions between Prototype and "Prime" Strains of Adenovirus Types 16, 17, and 27.

Virus	Serum titers HI	NT	HI	NT
	Type 16 (prototype)		Type 16′ (Hunter)	
Type 16 (prototype)	160	≥320	40	≥320
Type 16′ (Hunter)	<10	<20	160	≥1280
	Type 17 (prototype)		Type 17′ (Little)	
Type 17 (prototype)	320	≥320	640	160
Type 17′ (Little)	<10	<20	320	≥320
	Type 27 (prototype)		Type 27′ (Glover)	
Type 27 (prototype)	1280	≥320	≥5120	≥320
Type 27′ (Glover)	<10	≥320	1280	≥320

tial dilutions of from 1:5 to 1:20 depending on the homologous titer. The homologous HI titers of the prototype sera were as described previously(2, also see below). The HI titer of both the BP-6 and the BP-7 sera was 1:1280. All sera were used in HI tests at initial dilutions of 1:10.

The heterologous neutralizations observed were as follows. There was reciprocal cross-neutralization between type 15 and BP-6 viruses. However, the degree of cross-neutralization varied greatly depending on the virus dosage employed (Table II). All virus dilutions shown in Table II met the criteria for satisfactory dosage in "procedure 2" described by Rowe, *et al.*(4). It was also observed that a serum for the Hunter virus (type 16′) neutralized BP-6 virus to a titer of 1:160. The homologous titer of this serum was 1:1280 or greater.

BP-7 serum neutralized type 18 virus to a titer of 1:320 and type 13 virus to a titer of 1:40. The relationship between BP-7 and type 18 virus is shown in Table III. Although type 18 serum failed to neutralize BP-7 virus it did cause a marked delay in the progression of its cytopathic effect. Slight differences were noted in the type of cytopathic effect produced in unstained rhesus kidney cultures by the prototype strain of BP-7 as compared with the prototype strain of type 18. The former virus caused less clumping of affected cells and the average size of these cells appeared to be larger than those affected by type 18.

TABLE II. Cross Neutralization Reactions between Type 15 and BP-6 Viruses.

Serotype	Dilution	Serum titers Type 15	BP-6
Type 15	1:10	40	<20
	1:30	160	160
BP-6	1:1	<20	160
	1:3	40	≥320

TABLE III. Cross Hemagglutination-Inhibition (HI) and Neutralization (NT) Reactions between Type 18 and BP-7 Viruses.

Virus	Serum titers Type 18 HI	NT	BP-7 HI	NT
Type 18	—*	80	—*	320
BP-7	<10	<5†	1280	320

* Not done because type 18 does not hemagglutinate.
† See text.

No cross HI reactions were observed with either BP-6 or BP-7 and any of the human prototype viruses.

With both BP-6 and BP-7 viruses a significant (4-fold) rise in both neutralizing and HI antibody titer was demonstrated in one or more children yielding isolates. Children with BP-6 isolates had significant neutralizing antibody rises against type 15 virus when the latter was used at a dilution of 1:30 or greater but not when it was used at a dilution of 1:10. No neutralizing antibody rises were noted in the same children when Hunter virus was used. No HI rises were noted in children with BP-6 isolates when type 15 virus was used as the antigen.

BP-6 and BP-7 viruses were found to have the same titer in rhesus kidney cell cultures after treatment with 20% ethyl ether for 18 hours at 4°C as did control samples similarly treated with 0.85% NaCl.

Using BP-6 and BP-7 viruses as antigens a significant rise in complement-fixing antibody titer was demonstrated in paired sera from 3 individuals each of whom was known to

have been infected with a different previously described adenovirus.

Neither BP-6 nor BP-7 virus could be passed serially in rhesus kidney cultures and their cytopathic effect as seen in unstained rhesus kidney cultures was distinctly different from that of the chimpanzee and monkey adenoviruses described by Rowe, *et al.*(4).

Both BP-6 and BP-7 viruses agglutinate rat erythrocytes completely and hence fall into hemagglutination "group 2"(1). The cross reactions which have been observed within "group 1" and "group 2" are shown in Tables IV and V respectively. No cross reactions have been detected among viruses in different hemagglutination groups. It will be noted that two major reciprocal cross reactions occur with the newly recognized viruses in "group 2." Types 10 and 19 are identical by HI, and there is marked crossing between types 15 and 22.

Discussion. The existence of "prime" strains creates a problem in the identification of adenovirus isolates. Unlike the "prime" strains of type 7 described previously(4) the "prime" strains referred to in this report are not identical with the prototypes by HI. A practical solution might be to prepare typing sera with "prime" strains rather than with the prototypes.

BP-6 virus appears to have some relationship to type 15 virus by neutralization, but none by HI. In view of the many viruses which show a relationship to type 15 by neutralization(2) it is proposed that BP-6 virus be considered a distinct serotype until the significance of these relationships is determined.

BP-7 serum neutralized type 18 virus to

TABLE IV. Hemagglutination-Inhibition Cross Reactions among Adenoviruses of Group 1.

Virus \ Serum	T 3	T 7	T 11	T 14	T 16	T 20	T 21	T 25	T 28
	Hemagglutination-inhibition titers								
Type 3	*5120*	—*	—	—	—	—	—	—	—
" 7	—	*2560*	20	10	20	—	—	—	—
" 11	—	10	*80*	—	—	—	—	—	—
" 14	—	160	40	*320*	—	—	—	—	—
" 16	—	—	—	—	*320*	—	—	—	—
" 20	—	—	—	—	—	*80*	—	—	—
" 21	—	—	—	—	—	—	*160*	—	—
" 25	—	—	—	—	—	—	—	*80*	—
" 28	—	—	—	—	—	—	—	—	*80*

* — = <10.

TABLE V. Hemagglutination-Inhibition Cross Reactions among Adenoviruses of Group 2.

Virus \ Serum	T 8	T 9	T 10	T 13	T 15	T 17	T 19	T 22	T 23	T 24	T 26	T 27	BP-6	BP-7
	Hemagglutination-inhibition titers													
Type 8	*1280*	160	—*	—	—	10	—	—	—	—	—	—	—	—
" 9	80	*2560*	—	—	—	10	—	—	—	—	—	—	—	—
" 10	—	40	*640*	—	—	—	80	—	—	—	—	—	—	—
" 13	—	—	—	*1280*	—	10	—	—	—	—	20	—	—	—
" 15	—	—	—	—	*640*	—	—	160	—	—	—	—	—	—
" 17	—	—	—	—	—	*320*	—	—	—	—	—	—	—	—
" 19	—	40	640	—	—	—	*160*	—	—	—	—	—	—	—
" 22	—	—	—	—	40	—	—	*1280*	—	—	—	—	—	—
" 23	—	—	—	—	—	—	—	—	*2560*	—	—	—	—	—
" 24	—	—	—	—	—	—	—	—	—	*160*	—	—	—	—
" 26	—	—	—	—	—	—	—	—	—	—	*5120*	—	—	—
" 27	—	—	—	—	—	—	—	—	—	—	—	*320*	—	—
BP-6	—	—	—	—	—	—	—	—	—	—	—	—	*1280*	—
BP-7	—	—	—	—	—	—	—	—	—	—	—	—	—	*1280*

* — = <10.

the same titer as the homologous virus and hence might be considered a "prime" strain of type 18. Since type 18 virus is not known to hemagglutinate, it cannot be checked in this direction by HI. In view of its hemagglutinating properties, it is proposed that BP-7 virus be considered a distinct serotype until its relationship to type 18 can be evaluated by further studies.

BP-6 and BP-7 viruses were not tested against the "type 19" virus described by Zhdanov and Dreizin(5) since it has been shown(6) that this virus is identical with type 21 described by Bell, *et al.*(7).

The data presented here and that described previously(1,2) indicate that neutralization and HI tests do not always show parallel relationships among adenoviruses. Which, if either, of these two types of tests yields the more significant information is presently unknown. It is obvious, however, that if one of the methods is used exclusively, some similarities and differences will not be detected.

Summary. "Prime" strains of adenovirus types 16, 17, and 27 and 2 possible newly recognized adenoviruses are described. Data are presented on cross HI reactions among members of 2 adenovirus subgroups.

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Effect of Varying Doses of Progesterone on Implantation in the Ovariectomized Hamster.* (27627)

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Implantation takes place without delay in females ovariectomized in the first 4 days of gestation and given 2 or 3 mg of progesterone daily from time of ovariectomy to time of autopsy on the 7-9th day(1,2). The present experiment was designed to study the effect of small doses of progesterone on implantation in the castrate hamster, and to determine if very small doses could induce delay such as that described for the rat by Chambon(3).

Materials and methods. Virgin females weighing 80 to 130 g in which several normal cycles had been followed were used. Females were placed overnight with males of known fertility, or, for animals raised under reverse light and dark conditions (LD), actual matings were observed. Presence of sperm was confirmed by vaginal smears. For this paper, day 1 of pregnancy was the day in which sperm were detected or the day following the observed matings in the LD colony. Ovariectomies were performed on day 2 and synthetic U.S.P. progesterone administered from time of ovariectomy to day of autopsy, day 6, 7 or 9, when conceptual swellings are easily visible. All injections were administered in ¼ cc of corn oil subcutaneously. The dose of progesterone was decreased progressively from 2 mg to .0312 mg.

Each animal was isolated after ovariectomy, maintained on Purina Laboratory Rat Chow and water, weighed and examined daily for the appearance of the vaginal phenomena (4).

At autopsy the entire tract was removed, pinned out on aluminum foil supported by cardboard, and fixed in AFA (3 parts 95% alcohol, 1 part commercial formalin, 1 part

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