intestinal wall was determined in an in vitro perfusion apparatus under experimental conditions in which (i) the potential difference across the wall was short-circuited, (ii) the DMO activity was kept equal on both sides of the gut wall, and (iii) the *p*H of both the mucosal and serosal Krebs buffer remained fixed at 7.40 ± 0.05 throughout the incubation. In experiments C1 and C2 (Table 1) the flux of DMO from the mucosal to the serosal fluid ($M_{m\text{-}s}^{DMO}$) exceeded the flux in the opposite direction ($M_{s\text{-}m}^{DMO}$) by 2.4- and 2.0-fold, respectively. Addition of glucose (200 mg/100 ml) to both solutions increased this difference (experiment D). However, in these experiments there was net movement of water from mucosal fluid to serosal fluid; hence the possibility existed that the inequality of the two unidirectional fluxes was due to solvent drag and not to transport mediated by a carrier. To eliminate this possibility, increasing amounts of mannitol were added to the mucosal buffer (experiments D1, D2, and D3) until net water movement was actually reversed (experiment D3). In this situation $M_{m\text{-}s}^{DMO}$ still was 4.2-fold greater than $M_{s\text{-}m}^{DMO}$. This finding provides unequivocal evidence that DMO is actively transported by mid-intestinal segments. Again, for comparison, the flux ratios of AIB under experimental conditions in which net water flux was essentially zero are shown in experiments E1 and E2. As is apparent, both the values for $M_{m\text{-}s}^{AIB}$ and the flux ratios obtained with this actively transported amino acid are quantitatively similar to those obtained with DMO.

Thus DMO, like AIB, is actively transported in the intestine. This finding is contrary to the currently held view that DMO is distributed across biologic membranes solely by passive diffusion. However, it should be emphasized that, to our knowledge, the only experimental substantiation of this concept is the data of Robin (*6*); these data show that passive distribution of DMO, in accordance with the existing *p*H gradient, occurs across the pericardial and coelomic membranes of the spiny dogfish and semiaquatic turtle. Of greater importance, however, is the present unequivocal demonstration that DMO is actively transported in the mammalian intestine.

Many substances are actively transported by both the intestinal mucosa and the plasma membrane of the muscle cell. One is AIB, which moves against an electrochemical gradient both in the intestine (*7*) and in skeletal muscle (*5*). Should DMO also be actively transported across the plasma membrane of the muscle cell, then the calculated intracellular *p*H would be falsely high if the direction of transport were into the cell or, conversely, falsely low if the direction of transport were out of the cell.

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Adenovirus Multiplication: Genetic Relatedness of Tumorigenic Human Adenovirus Types 7, 12, and 18

Abstract. *Hybridization measurements among the DNA's of six weakly tumorigenic strains of human adenovirus type 7 reveal that all six strains are very closely related (83- to 110-percent nucleotide sequence homology). The DNA homology between these six type 7 strains and nontumorigenic types 2 and 4 is 32 percent and 52 percent, respectively. The 10- to 20-percent homology between the type 7 DNA's and those of "potent" tumorigenic types 12 and 18 indicates a very low degree of genetic relatedness. These data indicate that, if the carcinogenic potential of adenovirus types 7, 12, and 18 lies in the possession of nucleotide sequences common to these three viruses, these regions represent a small portion of the total genome, approximately one to three viral cistrons.*

Several strains (*1*) of human adenovirus type 7 have been demonstrated (*2*) to be weakly tumorigenic, that is, they produce tumors in a small proportion of animals injected with large inocula and only after a considerable period of time. Accordingly, we are investigating the degree of genetic relatedness between these strains and those of highly tumorigenic human adenovirus types 12 and 18. The degree of genetic relatedness among the six type 7 strains and between these strains and highly oncogenic types 12 and 18 and nontumorigenic types 2 and 4 was determined by DNA homology measurements with the DNA-agar technique (*3*) as used in our laboratory (*4*). DNA homology here refers to the possession by DNA molecules of nucleotide sequences sufficiently similar to be detected as DNA-DNA hybrids.

Radioactive virus was prepared by the addition of P^{32} at the time of infection of continuously cultured KB cells (established human cell line) with adenovirus or by the addition of H^3-labeled thymidine at 17 to 19 hours after infection. After the virus was harvested and purified (*5*), its DNA was extracted according to the procedure of Green and Piña (*6*) with the modifications described by Lacy and Green (*4*). The radioactive DNA was sheared in a Raytheon 10-kcy sonic oscillator to yield DNA fragments having a molecular weight of about 7×10^5 (*7*). These labeled DNA fragments were then denatured and incubated with appropriate amounts of DNA-agar; the extent of hybridization was then determined (*3*). Unlabeled DNA was extracted in the same manner and, after heat-denaturation, was trapped in agar. The amount of DNA embedded was determined by measuring the ultraviolet absorbance at 260 mμ of a portion of the DNA-agar dissolved in hot 5*M* sodium perchlorate.

From the results of the homology measurements (Table 1), all six strains of adenovirus type 7 studied are close to 100-percent related. The 83- to 110-percent homology observed with the various strains is not considered different from 100 percent in view of the experimental error (5 to 15 per-

Table 1. Homology between DNA's of six strains of human adenovirus type 7. Agar containing DNA of one of the adenovirus type 7 strains (0.2 to 0.3 g containing 2 to 8 μg DNA) was incubated in 0.2 to 0.3 ml of 0.3*M* NaCl, 0.03*M* sodium citrate at 60°C for 16 hours with P^{32}- or H^3-labeled fragments of denatured DNA of another strain. Reciprocal reactions were run within each group except in groups 7 and 8. The ratio of trapped to labeled DNA was 10 : 1 or greater except in two reactions in group 4 where it was 7 : 1. SD, standard deviation.

Group No.	Hybrid pair of adenovirus types	Labeled DNA bound relative to homologous reaction	
		(% ± SD)	No. of reactions
1	7(a); 7(Gomen)*	90 ± 3	4
2	7(Pinckney); 7(Gomen)	96 ± 9	9
3	7(C14500); 7(Gomen)	110 ± 4	4
4	7(Champagne); 7(Gomen)	83 ± 3	4
5	7(Grider); 7(Gomen)	101 ± 8	4
6	7(a); 7(Pinckney)	97 ± 8	6
7	7(a); 7(C14500)	94 ± 1	2
8	7(Pinckney); 7(C14500)	104 ± 8	2

* Seed virus for the Gomen (prototype) and "a" strains were obtained from the American Type Culture Collection. Recent data from R. Huebner's laboratory (*2*) indicate that these strains are also carcinogenic. Thus far, in our laboratory, we have induced tumors with purified preparations of the 7(Pinckney), 7(C14500), and 7(a) strains of virus. The Pinckney strain (obtained from A. J. Girardi and R. Huebner), the C14500 strain (obtained from B. Forsyth), and the Champagne and Grider strains (obtained from A. J. Girardi) have been reported to be carcinogenic (*2*).

Table 2. Homology between DNA's of six strains of adenovirus type 7 and nontumorigenic adenovirus types 2 and 4. Agar containing DNA of one of the adenovirus types (0.2 to 0.3 g containing 2 to 15 μg DNA) was incubated in 0.2 to 0.3 ml of a mixture of 0.3*M* NaCl, 0.03*M* sodium citrate at 60°C for 16 hours with H^3-labeled fragments of denatured DNA of another type. Reciprocal reactions were run within each group. The ratio of trapped to labeled DNA was 10 : 1 or greater, except in two reactions in group 1 where it was 7 : 1, two reactions in group 7 where it was 7 : 1 and 6 : 1, and two reactions in group 9 where it was 4 : 1.

Group No.	Hybrid pair of adenovirus types	Labeled DNA bound relative to homologous reaction	
		(% ± SD)	No. of reactions
1	7(Gomen); 2*	27 ± 8	16
2	7(a); 2	35 ± 7	10
3	7(Pinckney); 2	31 ± 9	10
4	7(C14500); 2	34 ± 3	4
5	7(Champagne); 2	30 ± 12	8
6	7(Grider); 2	33 ± 1	2
7	7(Gomen); 4	55 ± 13	12
8	7(a); 4	50 ± 5	4
9	7(Pinckney); 4	53 ± 12	8
10	7(C14500); 4	41 ± 4	4
11	7(Champagne); 4	62 ± 5	4
12	7(Grider); 4	52 ± 17	3

*Seed cultures of adenovirus type 2 (38-2) and type 4 (68578-D11) were obtained from R. Huebner and R. Chanock, respectively. Origin of the type 7 strains as described at end of Table 1. These viruses were then cultivated, isolated and purified and their DNA's were extracted as previously described (*4, 6*).

Table 3. Homology between DNA's of six strains of human adenovirus type 7 and tumorigenic adenovirus types 12 and 18. Agar containing DNA of one of the adenovirus types (0.2 to 0.3 g containing 1.3 to 11.7 μg of DNA) was incubated in 0.2 to 0.3 ml of 0.3*M* NaCl, 0.03*M* sodium citrate at 60°C for 16 hours with H^3-labeled fragments of denatured DNA of another type. Reciprocal reactions were run within each group except in group 11. The ratio of trapped to labeled DNA was 10 : 1 or greater, except in one reaction in group 4, where it was 8 : 1, two reactions in group 7 where it was 9 : 1, one reaction in group 8 where it was 8 : 1, five reactions in group 9 where it was 5 : 1 to 9 :1, two reactions in group 10 where it was 7 : 1.

Group No.	Hybrid pair of adenovirus types	Labeled DNA bound relative to homologous reaction	
		(% ± SD)	No. of reactions
1	7(Gomen); 12*	18 ± 4	7
2	7(a); 12	16 ± 2	5
3	7(Pinckney); 12	17 ± 5	8
4	7(C14500); 12	17 ± 2	5
5	7(Champagne); 12	11 ± 3	6
6	7(Grider); 12	9 ± 3	6
7	7(Gomen); 18	16 ± 5	7
8	7(a); 18	17 ± 7	14
9	7(Pinckney); 18	14 ± 3	10
10	7(C14500); 18	20 ± 3	4
11	7(Champagne); 18	18 ± 5	2
12	7(Grider); 18	13 ± 5	3

* Seed virus for adenovirus types 12 and 18 were obtained from R. Huebner. Origin of type 7 strains as described in Table 1. These viruses were cultivated, isolated, and purified and their DNA's were extracted as previously described (*4, 6*).

cent) in the hybridization procedure. In further support of this close relation, no differences are noted among the strains of type 7 in their ability to hybridize with each adenovirus type (Tables 2 and 3).

Results of homology measurements (Table 2) between the type 7 strains and type 2 indicate that they are about 32 percent related, whereas the 52 percent homology observed with type 4 indicates a closer genetic relationship between adenovirus types 7 and 4 than between types 7 and 2.

Only 10- to 20-percent hybridization was observed between all six strains of adenovirus type 7 and adenovirus types 12 and 18 (Table 3). This surprisingly low degree of nucleotide sequence homology suggests that "weakly oncogenic" adenovirus type 7 and "highly oncogenic" types 12 and 18 are quite unrelated genetically; that is, they differ in 80 to 90 percent of their nucleotide sequences.

Highly tumorigenic types 12 and 18 are very closely related genetically and possess about 80-percent DNA homology (*4*). Specific nucleotide sequences held in common by these two viruses may be responsible for tumor induction. However, what proportion of the total genome is associated with carcinogenesis is not known. In view of these considerations, the low degree of homology observed between the DNA's of type 7 and those of types 12 and 18 does not eliminate the possible common possession of a "carcinogenic nucleotide sequence or sequences" between types 7, 12, and 18. Adenovirus DNA has a molecular weight of 23 million (*6*) and may contain about 25 cistrons; that is, sufficient genetic information is present to code for the synthesis of at least 25 polypeptides. The 10 to 20 percent of nucleotide sequences of type 7, held in common with types 12 and 18, would thus represent two to six viral cistrons, which may code both for the common characteristics of the adenoviruses and for the tumorigenic capacity of types 7, 12, and 18. Alternatively, the viral cistrons responsible for tumorigenicity in adenovirus type 7 could be different from those of types 12 and 18.

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Rabbit 19S Antibodies with Allotypic Specificities of the a-Locus Group

Abstract. *Activity of bacteriophage T4 neutralizing antiserums obtained 8 days after a primary immunization of rabbits with purified phage T4 is due to antibodies of the 19S (IgM) type. This neutralizing activity could be partially inhibited by treatment with excess antiserum directed against the a-locus group allotypic specificity carried by the whole rabbit serum.*

Todd (*1*) presented evidence that rabbit 19*S* macroglobulin—IgM (*2*)—carried allotypic specificities controlled at both the *a* and *b* loci (*3*). The 7*S* γ-globulin (IgG) has specificities of both locus groups; these group specificities are distributed in such a way that the "light" chains (L or B) of IgG have specificities only of the *b*-group (A4, A5, A6) and the "heavy" chains (H or A) of IgG probably have specificities only of the *a*-group (A1, A2, A3) (*4*). Such a separation of group specificities is not surprising, as the two loci, *a* and *b*, are not closely linked (*5*). One might expect IgM to have the *b*-group specificities since L or B chains are probably common to the different types of rabbit immunoglobulins as seems to be the case with human and horse immunoglobulins (*6*). But since the *a*-group specificities are carried by the A or H chain (*4*), a portion of which is not common in antigenicity (*7*) between IgG and IgM, it is surprising that the two types of rabbit immunoglobulins both have the *a*-group allotypy. Feinstein *et al.* (*8*) confirmed the existence of *b*-group specificities on IgM, but could come to no conclusions about the existence of *a*-group specificities. One of us (*9*) found some evidence for the *b*-group specificity but failed to find *a*-group specificity on a single sample of IgM. We now have confirmed and extended the findings of Todd (*1*) by showing that bacteriophage T4-neutralizing antibodies of the 19*S* type have allotypic specificities of the *a*-group.

Antiserum directed against allotypic specificities were prepared (*10*) and were shown by ring test to be monospecific for the particular allotypic activity. Bacteriophage-neutralizing antiserums were prepared by the single injection of about 10^{10} bacteriophage-T4 plaque-forming units per rabbit; 8 days later the rabbits were bled from the marginal vein of the ear. The serums were stored frozen until used. High-titer neutralizing antiserum to T4 was obtained by continuing the immunization for 3 months, about 10^{10} phage being injected at monthly intervals; serums were obtained 10 days after the final injection. Phage-neutralizing activity was assayed by the kinetic method (*11*), and results were recorded as first-order rate constants of neutralization. After more than 90 percent phage neutralization had occurred phage survival was generally not linear (if the log of the number surviving was plotted against time); the values of neutralization constants given were obtained from the initial, linear region in these cases. The neutralizing activity of IgM against T4 was destroyed by reduction of the globulin with 0.1*M* or 0.2*M* 2-mercaptoethanol (1 hour at 37°C) before the addition of bacteriophage and the start of the neutralization assay (*12*). Residual activity was determined in the presence of the reducing agent; no effect of the mercaptoethanol was noted with high-titer (7*S*) antiserum to T4 assayed in the above manner.

Sucrose-gradient sedimentation was performed as described by Vaerman *et al.* (*12*), except that 5 percent sodium chloride was replaced by 0.1*M* phosphate, *p*H 7.5. Samples were centrifuged in the cold at 35,000 rev/min (Spinco Model L, SW-39 head) for 14 or 16 hours. The β-galactosidase activity of a crude extract of induced *Escherichia coli* K12 was used as a sedimentation-velocity marker; the enzyme, as detected by its activity with *o*-nitrophenyl-β-D-galactoside, has a reported sedimentation value of about 16*S* (*14*).

The effect of the antiserum with allotypic specificity on the neutralizing activity of the antiserum to T4 was assayed by addition of 0.005 ml of 8-day antiserum to T4 to 1 ml of either normal rabbit serum or rabbit antiserum with allotypic specificity. This mixture was incubated at 37°C for ½ or 1 hour and then was held in the refrigerator overnight. The next day the total mixture was brought to 37°C, and the phage was added (about 2×10^5 plaque-forming units). Phage survival was followed up to 750 minutes, and the first-order rate constant of neutralization was calculated. The results of such assays are given in Table 1. The homologous antiserum to allotypic specificity significantly inhibited the activity of the antiserum to phage in every case, the inhibition being from 40 to 90 percent. The one antiserum to *b*-group tried (antiserum to A4 with serum A002) also resulted in a large inhibition (78 percent) of the antiserum to T4.

The effect was specific (Table 1); antiserum directed against an allotypic specificity lacking in the antiserum to T4 gave no comparable inhibition. The neutralizing values in such cases were within the experimental error of that found when antiserum to T4 was mixed with normal rabbit serum, although some small effect could not be ruled out by these experiments. Also (Table 1) the addition of a normal serum with the A1 allotypic specificity to the mixture of antiserum to A1 and A1-negative antiserum to T4 was without effect. This control was included to test for possible indirect effects of the reaction of antiserum to allotypic specificity and IgG (that could not react with T4) such that 19*S* antibody would be inhibited. However, no such indirect action was observed. No explanation is attempted for the variability of the degree of the inhibition by antiserum directed against allotypic specificity on the 19*S* neutralizing antibodies. However, the final three values in Table 1 were obtained with rabbits immunized with a fresh purified lysate of phage T4, and this same lysate was used to assay these three antiserums for activity against T4.

To show that the phage neutralizing antibody of these early antiserums was actually 19*S* antibody (IgM), use was made of several properties of IgM. Thus the early antiserums were assayed with and without mercaptoethanol treatment (*12*). By this assay all antiserums to T4 were 80 to 90 percent, or more, sensitive to the reducing agent, as expected of IgM antibody. Sucrose-gradient sedimentation separations were carried out on serums A002 and A003 as a direct measurement of the 19*S* antibody of each serum. Again the phage-neutralizing activity of both serums was only in the 19*S* peak—slightly faster in sedimentation velocity than that of β-galactosidase and far