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Immunological and Other Biological Characteristics of Pentons of Human Adenoviruses

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Comparative hemagglutination-enhancement (HE) tests demonstrated diversified patterns of antigenic specificities both in the fiber and vertex capsomer part of pentons of human adenovirus types 3, 11 (subgroup I), 9, 15 (II), 1, 2, 4, 5, 6 (III), and 12. All fibers contained a type-specific antigen. Subgroup II and III fibers, in addition, contained specificities both unique for each subgroup and also common to the two subgroups. Fibers of serotypes 4 and 12 displayed a somewhat deviating behavior. All vertex capsomers tested shared a group-specific part. This was the only antigenic specificity demonstrable for serotype 12. Maximal penton HE titers of all sera were reached in tests with incomplete hemagglutinin of type 11. In addition, maximal HE activity of sera against individual serotypes also was recorded against pentons of other members of the same subgroup. Antigen characteristics of vertex capsomers of type 4 indicated a closer relationship to subgroup I than to subgroup III. The toxin activity of pentons was more sensitive to trypsin treatment than their capacity to function as incomplete hemagglutinin. Homotypic antipenton sera, unabsorbed or absorbed with homotypic fibers to remove antibodies against this component, and, to a varying extent, also heterotypic antipenton sera could neutralize toxin activity. Antifiber sera could neutralize toxin activity of pentons carrying short fibers (10 nm, type 3) but not of those carrying long fibers (28 to 31 nm, type 2). It is concluded that toxin activity is carried by a specific part of vertex capsomers and that cell detachment can be brought about via a direct contact between this component and cell membranes. Fiber-mediated attachment does not seem to be necessary for this biological activity to become expressed.

The capsid of the adenovirus virion is composed of 240 nonvertex capsomers (hexons) and 12 vertex capsomers carrying projections [pentons (5, 29)]. Pentons are responsible for toxin (cell detachment) activity (1, 16, 21) and are composed of vertex capsomers, which have been described as both subgroup- (17, 29) and group-specific (6, 13, 27, 28), and fibers showing structural and immunological type and in some cases subgroup characteristics (5, 7, 10, 14*a*, 19, 25, 26, 29).

In the present study, the immunological complexity of fibers and pentons was studied in tests with sera against these two types of components from selected serotypes belonging to the three different subgroups. The tests employed were hemagglutination-enhancement (HE) and toxin neutralization (TN). A special interest was focused on the relationship between toxin activity and subunits of pentons.

MATERIALS AND METHODS

Preparation of virus material. The prototype strains of adenovirus types 1, 2, 4, 5, 6, 9, 11, and 15 were obtained from Arne Svedmyr, Central Bacteriological Laboratory of Stockholm City. Prior to arrival in this laboratory these strains were passaged on HeLa and KB cells between 14 and 22 times. In this laboratory, no serotype was passaged more than four times. An isolate of serotype 3 obtained from Torsten Jonsson, National Bacteriological Laboratory of Sweden, was used. The infectivity of this serotype was neutralized by hyperimmune sera against the prototype strain of adenovirus type 3. Virus preparations and sera against serotype 12 (Huie strain) were kindly provided by Jaro Ankerst, Department of Medical Microbiology, University of Lund.

Concentrated stock materials were prepared in monolayer cultures of either a human bone marrow cell line, MAS-A cells, or a human embryonic lung cell line, Lu 106, as has been described elsewhere (27). Serum was excluded at the last change of maintenance medium before harvest.

Purification of virus-specific structural components. Before separation of soluble components, virions and empty capsids were removed by three consecutive centrifugations at a performance index (Pi) of 5.3 (20,000 rev/min, rotor 40, Spinco Division, Beckman Instruments, Inc., Fullerton, Calif.; reference 14).

Purification of fibers of the various serotypes for

preparation of specific antisera included the following procedures.

Anion exchange chromatography on Sephadex A25 (Pharmacia Fine Chemicals, Uppsala, Sweden) was used on types 6 and 15 (7, 27). The same technique combined with exclusion chromatography on spherical agarose BioGel A15m (BioRad Laboratories, Richmond, Calif.) was used on types 2, 4, and 5 (24*a*, 25). Exclusion chromatography on Sephadex G200 was used on type 3 (9). All fiber preparations used as incomplete hemagglutinins in HE tests were purified either by zonal centrifugation or exclusion chromatography.

Monomers and polymers of pentons of the different adenovirus types studied, which were used for preparation of specific antisera, were isolated by the following procedures. Pentons of serotype 2 and pentons and dodecons of serotype 3 were prepared as previously described (9, 24*a*). Pentons of serotype 4 and aggregates of pentons of serotypes 5 and 6 were isoisolated by exclusion chromatography on Bio Gel A15m (27). Dodecons of type 9 were eluted from human O cells by receptor-destroying enzyme (10). The corresponding components of serotypes 11 and 15 were purified by rate zonal centrifugation (7, 8).

Pentons of the various serotypes used as incomplete hemagglutinins in HE tests were prepared as described above for fibers. None of the penton preparations used displayed HE after incubation with heterotypic fiber-specific antisera, indicating the absence of free fibers.

Hexons of serotype 2 were prepared by a procedure including anion-exchange chromatography on diethylaminoethyl-Sephadex A25, exclusion chromatography on Sephadex G200, and rate zonal centrifugation (27). Purification of hexons of serotype 3 was previously described (9).

Tests for biological activities. The techniques for hemagglutination, hemagglutination-inhibition (HI), HE antibody consumption (HEC), and HI inhibition antibody consumption tests were previously described (6, 11, 12, 24*a*, 27). The latter three types of tests indicate incomplete hemagglutinins, i.e., pentons or fibers or both, vertex capsomers, and the type-specific part of fibers, respectively. Complement fixation (CF) tests were performed with a modified Takatsy microdrop technique as previously described (28).

Titration and neutralization of toxin activity. The previously described technique (27) was used in most of the tests. The TN tests were performed by incubating 1 volume of twofold dilutions of serum with 9 volumes of toxin (dialyzed against maintenance medium), containing 8 arbitrary units per ml, for 2 hours at room temperature and overnight at 4 C. Of this mixture, 1 ml was inoculated into each of two tubes containing confluent monolayers of Lu 106 cells. The following toxin preparations were used: pentons of serotype 2 isolated either by zonal centrifugation or by exclusion chromatography on Bio Gel A15m (27) and pentons and dodecons of serotype 3 recovered after adsorption and elution of soluble components on monkey (*Cercopithecus aetiops*) red cells (6). To reduce the consumption of reagents and thus to be able to perform TN at low dilutions of sera, different microtechniques were developed and compared with the previously applied technique. One of these, which was used to determine toxin neutralization by sera at dilutions <20, was carried out as described above. The only differences were that this test involved 0.1 ml and was performed in disposable trays (Linbro FB-48; Flow Laboratories, Ltd, Irvine, Scotland). These trays were also used in a simplified technique for titration of toxin activity, essentially representing a modification of the test for inhibition of cell spreading developed by Sanderson (22). The latter technique was used in experiments concerning the kinetics of trypsin susceptibility. The virus material was titrated in serial twofold dilutions of 0.05 ml containing Hanks' medium with 10% calf serum, by a microtiter equipment for serological tests (Flow Laboratories Ltd.); 50,000 Lu 106 cells in 0.05 ml of the above mentioned medium was added to all cups, after which these were sealed by adhesive covers. The trays were then incubated in a 5% CO_2 air atmosphere for 16 to 18 hr. The test was considered to be positive if more than half of the number of cells remained rounded up at that time. The results appeared to be rather insensitive to variations in the number of cells employed. The titers obtained corresponded to titers of cell detachment determined in tubes with confluent monolayers of Lu 106 cells.

Determination of trypsin sensitivity. Pentons of serotype 2 isolated by exclusion chromatography on Bio Gel A15m (25) and concentrated by forced dialysis against polyethylene glycol were, prior to the enzyme treatment, dialyzed against a buffer of *p*H 7.8 (at 37 C) containing 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, 0.1 M NaCl, and 0.01 M $CaCl_2$. After incubation in a water bath at 37 C with trypsin (2 × crystallized, Fluka AG, Buchs, SG, Switzerland) at various concentrations for different times, samples were placed in an ice bath and a double amount of soy bean trypsin inhibitor (5 × crystals lized; Nutritional Biochemicals Corp., Cleveland, Ohio) was added.

Preparation of hyperimmune sera. Rabbits were inoculated intramuscularly with 4 ml of virus material mixed with Freund's complete adjuvant. An intravenous booster of 1 ml was given 4 to 5 weeks later, and the animals were exsanguinated after another week.

Absorption of hyperimmune sera. Sera to be absorbed were mixed with antigen in amounts calculated to be four to five times higher than that required to combine with all specific antibodies. After incubation at room temperature for 2 hr and overnight at 4 C, the complexes of antigen-antibody formed were removed by centrifugation at Pi 16.7 (SW39, 35,000 rev/min) for 2 hr. After absorptions with fibers, it was found necessary to separate antigen-antibody complexes from free antibodies by exclusion chromatography on Sephadex G200. Fractions containing isolated immunoglobulin G were concentrated by ultrafiltration and adjusted to the original volume. The low HA activity noted in most sera after absorption with antigen was eliminated by adsorption with preparations of packed rat or green monkey erythrocytes (24*a*).

Specificity of hyperimmune sera. The type specificity of sera was controlled in HI tests. Sera against fibers were negative both in HE tests with pentons of serotype 11 and with group-specific hexons in CF tests. Penton antisera displayed no reactivity in CF tests with group-specific hexons, with the exception of sera against aggregates of pentons of serotypes 5 and 6 in which moderate CF activity was noted. Hexon antisera were negative both in HE tests with pentons of serotype 11 and in HI tests with soluble components. However, these antisera displayed HI by homologous purified virions (14). Hexons but not host cell components reacted in CF tests with hexon sera.

RESULTS

Fiber HE by sera against serotype 12 and selected members of subgroups II and III of adenoviruses. Fibers of adenoviruses belonging to subgroups II and III contain antigenic specificities besides the type-specific antigen (7, 13, 17), which allow their demonstration as incomplete hemagglutinins in HE tests. This test was used to determine the immunological relationship between fibers. Serotypes 9 and 15 (subgroup II), 1, 2, 4, 5, 6 (subgroup III), and 12 were studied. Two different sera against each serotype included were tested, and good agreement between the results was noted. No HE activity of any of the fiber preparations was demonstrable in tests with sera against fibers of adenovirus type 3 (subgroup I). Sera against members of other subgroups displayed varying HE titers in tests with heterotypic fibers, except those of subgroup I. This finding was repeatedly encountered, and a distinct pattern of immunological reactivity with heterotypic fibers, in the following referred to as "titer profile," could be established for each antiserum.

Besides the reactivity shared between members of subgroups II and III, a distinct subgroup-specific preference was prevalent within subgroup II (Fig. 1). All sera against serotype 12 and members of subgroup III displayed a distinct preference in the relation to fibers of serotypes 1, 2, 5, and 6. However, somewhat paradoxically, the sera against the latter components displayed comparatively low HE titers with fibers of serotypes 4 and 12 similar to those obtained with fibers belonging to subgroup II (Fig. 1). Antigen specificities of subgroup nature were confirmed to occur within both subgroups II and III by absorption experiments, in which sera against serotypes 5 and 9 were absorbed with fibers of types 2 and 15.

Penton HE by sera against monomers or polymers of pentons of selected adenovirus types. In view of the complex immunological composition of the fiber presented above and the occurrence of both type- (2, 3, 9, 15, 30) and group-specific (2, 3, 9, 15, 18, 29, 30) antigenic specificities of the hexon, also the vertex capsomer might be expected to display an immunological diversification extending beyond the group-

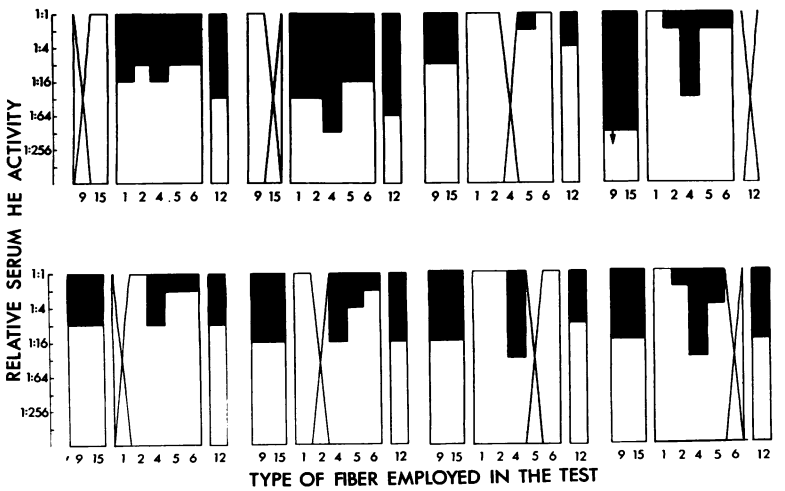

FIG. 1. *Hemagglutination enhancement (HE) activity of sera against fibers of serotype 12 and selected members of subgroups II and III in tests with fibers of different serotypes (denoted along bottom part of columns). Crossed columns mark the serotype origin of antibodies examined in individual sets of HE tests. Upper row: sera against type 9 dodecons, type 15 fibers, type 4 fibers, and type 12 crude virus material. Lower row: sera against serotype 1 crude virus material, serotype 2 fibers, serotype 5 fibers, and serotype 6 fibers. The HE titer of sera in tests with different fibers is expressed in a relative fashion. The unfilled part of a column gives the ratio of serum HE activity with a specific fiber over the maximal titer obtained with any heterotypic fiber.*

specific reactivity so far demonstrated (6, 13, 27, 28). HE of pentons by different penton antisera appeared to represent the most sensitive system available for mapping relationships between vertex capsomers of different serotypes. Types 3, 11 (subgroup I), 9, and 15 (subgroup II) and 1, 2, 4, 5, and 6 (subgroup III) were studied. Unfortunately, pentons of serotype 12 could not be included in this study owing to the fact that they occur spontaneously only in small amounts (*9a*). Two different sera against each type of pentons included were studied, and, generally, a good agreement between the titer profiles of these sera were noted. All sera displayed HE with every heterotypic penton preparation.

The titer profiles of sera against the four serotypes representing subgroups I and II were, as a whole, similar (Fig. 2). However, some subgroup-specific preference was suggested, in particular, in tests with sera against subgroup I members. The titers obtained with pentons of serotype 11 were equivalent to the maximal titers in all sera tested with exception for sera against serotype 2, whereas type 3 pentons generally displayed lower titers by a factor of two to four. The HE titers of sera against members of subgroups I and II noted for pentons of serotypes 4 and 6 approached the values observed with serotypes from subgroup I. In tests with pentons of type 5, somewhat lower serum titers were recorded, although they still exceeded the low titers, corresponding to the group-specific activity, obtained with type 1 and 2 pentons. The high titers observed when the latter components were incubated with serotype 15 antisera represented the single exception to this simplified general description.

Among the sera against pentons of serotypes belonging to subgroup III, the sera against type 4 differed from the remaining antisera. The titer profile of type 4 antisera displayed a close correlation to the titers obtained with sera against serotype 3. The sera against the remaining members of subgroup III displayed a subgroup-specific

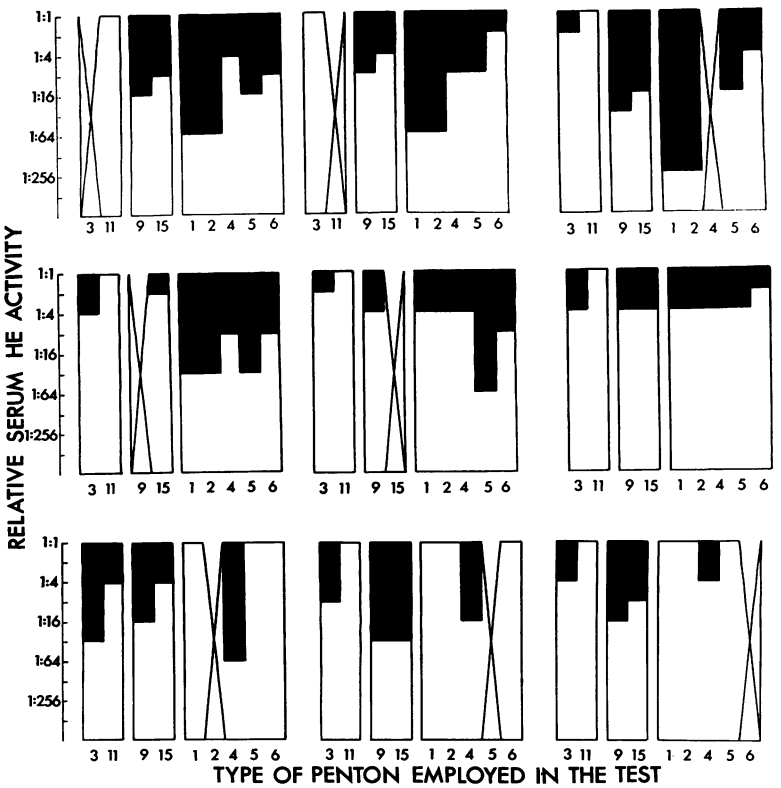

FIG. 2. *Hemagglutination enhancement (HE) activity of sera against monomers or polymers of pentons of selected serotypes in tests with different heterotypic pentons. Top row: sera against serotypes 3, 11, and 4. Middle row: sera against serotypes 9 and 15 and crude virus material of type 12. Bottom row: sera against serotypes 2, 5, and 6. Concerning arrangement of staple diagram see Fig. 1.*

"preference," since all HE titers in subgroup-specific relations were on a level with or, in the case of serotype 2, higher than the titers obtained with pentons of serotype 11. HE titers noted for pentons of serotype 3 were intermediate between the subgroup-specific titers and the comparatively low titers obtained with pentons of serotypes belonging to subgroup II. In this respect, sera against type 2 also represented an exception, since the lowest titers were obtained with pentons of serotypes 3 and 4. Conversely, type 2 pentons were responsible for the minimal HE titers recorded in tests of antisera against both serotypes 3 and 4. The absence of any marked variation in HE titers of the antisera against type 12 contrasted with the behavior of antisera against all other types tested.

Absorption of sera against pentons of serotype 4 by heterotypic pentons. A number of biological characteristics (13, 14, 23, 26, 28) and also the results presented above seem to indicate that serotype 4 appeared to be more closely related to members of subgroup I than to subgroup III. To establish the immunological relationships on the vertex capsomer level between this and other serotypes, the sera against pentons of serotype 4 were absorbed with the following preparations: pentons of serotypes 11, i.e., preparations of soluble components from which dodecons have been removed by erythrocyte adsorption (8); pentons of serotypes 15 and 2 isolated by anion-exchange chromatography (7) and exclusion chromatography (25), respectively; and dimers of pentons of serotype 6 obtained after zonal centrifugation (27). The remaining HE activity was tested with pentons of all serotypes under study. These absorptions had to be carried out at different dilutions of serum on account of the large span between the minimal and maximal HE titers obtained with different pentons (Table 1).

It was found that absorption with pentons of serotype 2 did not affect the titer of any heterotypic serotype except type 1. This titer was reduced but not altogether removed. Absorption with both serotypes 6 and 15 eliminated the HE antibody reacting with types belonging to the same subgroup. Type 15 removed in addition the HE reactivity with pentons of types 1 and 2, whereas serotype 6 reduced the HE activity of subgroup I pentons and eliminated the HE antibody reacting with the remaining serotypes. Absorption with serotype 11 pentons eliminated all demonstrable HE activity with the serotypes tested. It should be noted, however, that changes in the HE titers obtained with pentons of serotypes 1 and 2 could not have been detected at the high serum dilutions used for absorption with pentons of serotypes 6 and 11.

TABLE 1. *Remaining HE activity (units per 0.4 ml) in a serum against pentons of adenovirus type 4 after absorption with different heterotypic pentons*

HE activity against pentons of		Serum sample absorbed with pentons of types				
Type	Subgroup	0	11 pentons	15 pentons	2 pentons	6 dimers of pentons
3	I	20,480	<320	10,240	20,480	2,560
11	II	40,960	*<320*[a]	40,960	40,960	5,120
9	II	640	<320	<40	640	<160
15	II	1,280	<320	*<40*	1,280	<160
1	III	128	<320	<40	64	<160
2	III	128	<320	<40	*<8*	<160
5	III	2,560	<320	1,280	2,560	<160
6	III	5,120	<320	2,560	5,120	*<160*

[a] Values for corresponding peptons are in italics.

Studies on the significance of different penton structures for the toxin activity were conducted.

Trypsin digestion of pentons of adenovirus type 2. The previously reported relative resistance of pentons of serotype 2 to trypsin treatment (27) was further studied in two kinds of experiments in which (i) the relative concentrations of trypsin needed to reduce the toxin activity and the capacity of vertex capsomers to react with HE antibodies were compared and (ii) inactivation rates of these two biological properties of type 2 pentons were determined (Fig. 3).

Digestion with trypsin, in a concentration of 0.1 μg/ml for 2 hr at 37 C, of type 2 pentons prepared by exclusion chromatography on Bio Gel A15m was sufficient to reduce the toxin activity, whereas a hundred times higher concentration of enzyme was needed to bring about a reduction of the HE antibody combining activity.

A trypsin concentration of 30 μg/ml was found to destroy the toxin activity in less than 1 min, whereas the penton HE activity was not significantly reduced until after 30 min. The HEC activity (not included in Fig. 3), indicating vertex capsomers isolated or as part of pentons, was reduced to the same extent as the HE activity. HE tests with an antiserum against type 5 fibers demonstrated that the trypsin treatment caused a gradual release of free fibers.

Effect of homotypic and heterotypic antisera on toxin activity of adenovirus types 2 and 3. The active principle of the toxin activity was suggested to reside in a part of vertex capsomers (Fig. 3). Since a group-specific reactivity of the vertex capsomer can participate in the HE reaction, it was considered of interest to determine

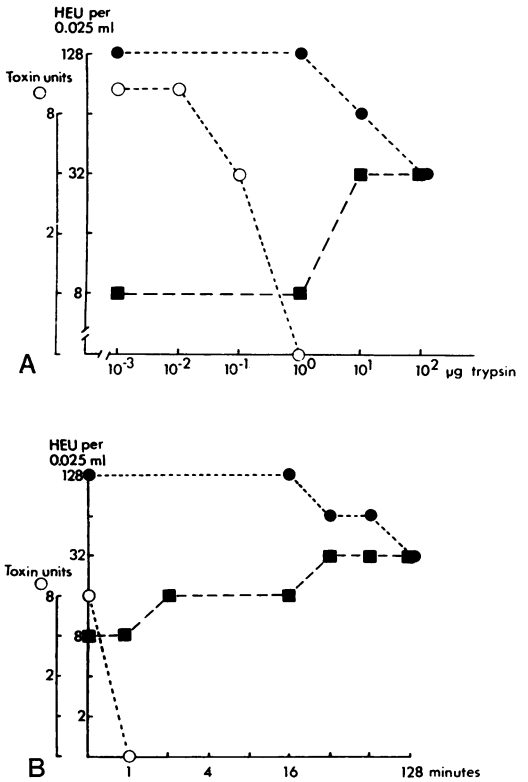

FIG. 3. *Effect of trypsin on biological activities of pentons of adenovirus type 2. (A) Effect of incubation with various concentrations of trypsin for 2 hr at 37 C on the following activities of the penton:* (○) *toxin activity;* (●) *HE with an antiadenovirus type 11 dodecon serum, i.e., indicating penton incomplete HA;* (■) *HE with an antiadenovirus type 5 fiber serum, i.e., indicating fiber incomplete HA. (B) Inactivation rate of different penton activities in the presence of 30 µg/ml of trypsin. Symbols are described under A.*

the effect of some of the penton antisera (Fig. 2) on the toxin activity.

All sera tested, specific for monomers or polymers of pentons, displayed neutralization of the toxin activity carried by pentons of types 3 and 2 (Tables 2 and 3). To be able to compare the relative neutralizing capacity of sera against different adenovirus types, the ratio between the titers obtained in HE and TN tests was determined. A wide range of ratios was noted. The highest ratio observed, 530, was that between the titers in HE and TN tests with toxin of serotype 3 by a serum against serotype 4 pentons. The ratios recorded for toxins of both adenovirus types 2 and 3 with antisera against serotypes belonging to subgroup II ranged between approximately 50 and 170.

A considerable capacity to neutralize the toxin activity of both serotypes 2 and 3 was present in antisera against serotypes of subgroups I and III with the exception of type 4. All these serotypes displayed toxin activity (1, 6, 16, 21, 27). The ratios of HE to TN titers found were comparatively low, ranging between approximately 5 and 20. It should be noted that the homotypic ratio was also included in this range.

The specificity of the TN results was also examined in tests with homotypic hexon antisera. These sera displayed a CF titer with group-specific hexons of 640 units per 0.025 ml. No neutralization of toxin activity could be detected. Toxin neutralization tests with homotypic antifiber sera revealed an interesting discrepancy between serotypes 2 and 3. The TN titers of the sera against serotype 3 fibers and pentons were of the same magnitude, whereas two different sera against serotype 2 fibers (HI titers about 6,400 units/0.4 ml) did not neutralize 8 units of the homotypic toxin. This observation corroborated recent findings by Pettersson et al. (19). However, it was noted in this laboratory that serial dilutions of toxin of serotype 2 in the presence of large amounts of homotypic antifiber antibodies repeatedly caused a twofold reduction of the titer as compared to the control.

Effect of absorption by homotypic fibers on toxin-neutralizing activity of sera against adenovirus type 3 pentons. The relative effect of antibodies against the fiber and vertex capsomer part of pentons in the TN activity of adenovirus type 3 by homotypic antipenton sera could not be evaluated from the data presented above. For this reason, an experiment was performed in which antipenton sera absorbed with homologous fibers were compared to the same untreated serum with regard to toxin-neutralizing capacity. The HI titer was reduced by a factor exceeding 100 after absorption, whereas approximately half of all toxin-neutralizing activity remained (Table 4). This indicates that antibodies against the vertex capsomer part of the penton alone can neutralize the toxin activity of pentons of serotype 3.

DISCUSSION

A large variety of antigenic specificities displaying relatively complex relationships appear to occur in hexons (2, 3, 9, 14, 30) and also, as demonstrated above, in pentons of different adenovirus types. Techniques employed to demonstrate these differences were antibody absorption tests and, in the case of pentons, also determination of HE antibody profiles of specific sera. It should be mentioned that the reliability of results derived from this kind of experiments will be dependent upon the requirement (i) that no antigenic specificities are selectively

TABLE 2. *Capacity of antisera against various structural components of different serotypes to neutralize the toxin activity of type 3 pentons*

Sera against			TN activity against type 3 pentons[a]	Homotypic HI[b]	HE of type 11 penton[b]	HE of type 3 penton[b]	HE titer with type 3 pentons/ TN titer
Type	Component	Subgroup					
3	Pentons	I	640–1,280	12,800	25,600	12,800[c]	13
3	Dodecons		320–450	25,600	5,120	—[d]	—
3	Fibers		450–900	51,200	<10	—	—
3	Hexons		<10	32	<10	—	—
11	Dodecons		450–640	12,800	—	5,120	9
9	Dodecons	II	8	12,800	5,120	640	80
15	Dodecons		8–22	25,600	5,120	2,560	170
4	Pentons	III	32–45	10,240	40,960	20,480	530
2	Pentons		22–32	3,200	2,560	160	6
6	Dimers of pentons		225–256	20,480	20,480	5,120	21

[a] Expressed as units per 1.0 ml.
[b] Expressed as units per 0.4 ml.
[c] Determined by use of serum absorbed with homotypic fibers.
[d] No test was performed.

TABLE 3. *Capacity of antisera against various structural components of different serotypes to neutralize the toxin activity of type 2 pentons*

Sera against			TN activity against type 2 pentons[a]	Homotypic HI[b]	HE of type 2 pentons[b]	HE titers with type 2 pentons/TN titer
Type	Component	Subgroup				
3	Pentons	I	64	12,800	640	10
11	Dodecons		16–22	12,800	80	4
9	Dodecons	II	8–22	12,800	1,280	85
15	Dodecons		45–64	25,600	2,560	47
4	Pentons	III	4	10,240	320	80
2	Pentons		900–1,280	3,200	—[c]	—
2	Fibers		<10	6,400	—	—
2	Hexons		<10	<10	—	—
5	Dimers of pentons		225–252	2,560	2,560	11
6	Dimers of pentons		1,280–1,800	20,480	20,480	13

[a] Expressed as units per 1.0 ml.
[b] Expressed as units per 0.4 ml.
[c] No test was performed.

TABLE 4. *Capacity of antiserum against type 3 pentons to neutralize homotypic toxin activity before and after absorption with homotypic fiber*

Absorbed with homotypic fibers	TN activity against type 3 pentons[a]	Homotypic HI[b]	HE of type 11 penton[b]	HE of type 3 penton[b]
−	1,280	64,000	68,000	—[c]
+	450–900	500	68,000	34,000

[a] Expressed as units per 1.0 ml.
[b] Expressed as units per 0.4 ml.
[c] Not demonstrable due to the high amount of HI antibodies present.

destroyed by purification procedures used (this is of importance both for antigen prepared for immunization and for absorption), and (ii) that the animals used for immunization respond in a reproducible fashion to all antigenic specificities present. The fact that both crude virus material and purified components—used either for immunization or absorption—gave similar antibody "profiles" in different rabbits suggests that neither one of these two possible restrictions have any major significance on the outcome of the present experiments. An analysis of the results obtained reveals that certain groupings and preferences on both the fiber and the vertex

capsomer levels can be deduced from the characteristic HE titer profiles of sera against individual serotypes.

(i) The fibers of members of subgroups II and III can be demonstrated as incomplete hemagglutinin in the presence of sera against members of both these two subgroups, thus indicating the occurrence of common antigen specificities.

(ii) Interestingly enough, a distinct subgroup-specific preference was demonstrated among the fibers of subgroup II, although the lengths of the former were only 12 to 13 nm (7, 10). Within subgroup III, the 23- to 31-nm long (14*a*) fibers of serotypes 1, 2, 5, and 6 displayed strong relations. The remaining member of this subgroup, serotype 4 with 17- to 18-nm long fibers and the 28- to 31-nm long fibers of serotype 12, reacted only on the level of the common antigen specificity for subgroups II and III, with the fiber antisera of these four types. However, fiber preparations of serotypes 1, 2, 5, and 6 displayed maximal titers in antisera against both types 4 and 12. This discrepancy might be explained if it is assumed that the fibers of serotypes 4 and 12 formed comparatively inefficient hemagglutinins after combination with heterotypic antibodies. Alternatively, these fibers might contain antigenic sites which are immunogenic in vivo but less prone to form hemagglutinating complexes with immunoglobulin G antibodies in vitro.

(iii) Fibers of all adenovirus types tested carried a type-specific antigen responsible for the reaction with HI antibodies (5, 8, 10, 12, 24*a*). This was the only antigenic specificity demonstrated for the 10-nm long fibers of serotypes 3, 11, 16 (5, 8, 12), members of subgroup I.

The relationships between vertex capsomers can be summarized as follows.

(i) The vertex capsomer contained a group-specific part. Serotype 12 alone displayed only this antigenic specificity.

(ii) On the vertex capsomer level, certain special features in the relationship between serotypes belonging to different subgroups according to Rosen (20) could be demonstrated.

First, generally high titers were observed with vertex capsomers from subgroup I, in particular with serotype 11. This may partly be due to the fact that the vertex capsomer of this serotype probably contains the major portion of the antigenic variants among the serotypes tested, and partly the fact that pentons of serotype 11 can form unusually stable hemagglutinin. It should be mentioned that the sensitivity to temperature variations (6, 24, 24*a*) might represent one reason for the somewhat lower titers obtained with pentons of serotype 3.

Second, the HE profile obtained with pentons of serotype 4 was characteristic for subgroup I. This is not unexpected, since type 4, also with regard to a number of other biological characteristics, appears to be associated with this subgroup (23, 28).

Third, compared to the remaining serotypes the relationship between the vertex capsomers of serotype 2 and the types belonging to subgroup I plus type 4 was relatively weak. In contrast to this, a somewhat unexpected strong relation on the vertex capsomer level between serotypes 2 and 15 was noted.

(iii) Subgroup specificity was clearly observed in the vertex capsomers of serotypes belonging to subgroup I. A similar specificity was also demonstrated within the other subgroups, although the maximal HE titers never (except with sera against type 2) exceeded those obtained with serotype 11 pentons. It is of some interest to note that antiserum against serotype 4 reacted with certain members of subgroup III, even after all subgroup II specific activity was removed by absorption with pentons. This suggests that there exists a vertex capsomer specificity not carried by subgroup II members which relates type 4 to members of subgroup I and also to two members (types 5 and 6) of subgroup III.

(iv) Type specificity of the vertex capsomers was not demonstrated, but could not be excluded on the basis of the indicator system used.

Since the maximal titers of the sera tested were obtained in HE tests with pentons of serotype 11, this test system can be generally employed for demonstrating antibodies against vertex capsomers. The sensitivity of this test appears to exceed complement fixation by a factor of the order of magnitude of 20 to 100 (4; Wadell, *unpublished data*).

Toxin activity has been demonstrated in preparations of serotypes 3 and 11 and 1, 2, 5, and 6 among the serotypes studied. The distribution of this activity could not be correlated to any relationship revealed by the HE tests. Neutralization of the toxin activity of adenoviruses has previously been described both to be (1), and not to be type-specific (21). Three different mechanisms possibly can be responsible for TN: mechanism a, direct interaction between antibodies and the toxin-carrying part of pentons; mechanism b, sterical interference caused by antibodies combining with antigenic determinants (either on vertex capsomers or on fibers) adjacent to the toxin-active part of vertex capsomers; and mechanism c, changes of this active part due to

allosteric effects resulting from the combination between antibodies and pentons.

Our data clearly demonstrate the occurrence of a heterotypic TN. Specific antisera against all the serotypes studied inhibited the toxin activity of both serotypes 2 and 3. This neutralization did not appear to indicate any distinct qualitative difference between toxins of these two serotypes, representing subgroup III and I. The toxin activity in preparations of subgroup III members, however, somewhat exceeded that of subgroup I members when preparations of equal HE titers were compared. A certain degree of neutralization of toxins of both types 2 and 3 was also noted with sera against pentons of serotypes 4, 9, and 15, in preparations of which no toxin activity has been demonstrated. This might indicate either that neutralization of heterotypic toxin preparations represented a more sensitive technique for indication of toxin present in too low concentrations to be detected in direct tests (mechanism a) or, alternatively, that the effect is mediated via mechanisms b or c presented above. The possibility that either one of the latter two mechanisms can have significance is suggested by the observation that specific sera against the 10-nm long fibers of serotype 3 (5) displayed a marked capacity to bring about homotypic toxin neutralization. Almost no such effect could be demonstrated when antisera against fibers of serotype 2, measuring 28 to 31 mm (14a), were assayed against homotypic pentons. Immunoglobulin G antibodies by their size preferably should be capable of causing sterical interference in the first system. However, no results are available which allow the exclusion of mechanism c.

A number of the findings discussed above suggest that a fiber-mediated attachment of vertex capsomers to cell membranes is not needed for a toxin activity to be expressed. In particular, the absence of any neutralizing activity of anti-adenovirus type 2 fiber sera on homotypic toxin activity should be mentioned. It can furthermore be mentioned that preincubation of cells with fibers of type 2 in amounts sufficient to block all receptors on cells does not block the toxin activity of pentons of the same serotype (Wadell, *unpublished data*). Thus free vertex capsomers would be expected to carry toxin activity. This was recently demonstrated by use of type 2 vertex capsomers isolated from pyridine-treated material in a parallel study by Pettersson and Höglund (Virology, *in press*).

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