The Serological Relationship of the Soluble Antigens of Adenovirus Type 19*

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SUMMARY

The relationship of group-specific (hexon) antigen, type-specific (fibre) antigen, soluble haemagglutinin (dodecon) and purified virus particles of adenovirus type 19 was studied by cross complement-fixation, haemagglutination-inhibition and neutralization with antisera prepared against purified antigens. The fibre antigen reacted as an indirect haemagglutinin, producing haemagglutination in the presence of selected heterologous adenovirus immune sera. Dodecon and fibre antigen were closely related, while hexon antigen showed no relationship to fibre and possibly a weak relationship to dodecon antigen. Neutralizing antibodies were found in antisera to hexon and dodecon, but not in fibre antisera.

INTRODUCTION

The structure of human adenoviruses and their soluble antigens has been elucidated for the three major subgroups (Valentine & Pereira, 1965; Norrby, 1966a; Gelderblom et al. 1967). However, the functional significance, the serological specificity and the relationship between the various substructures are less clearly defined. We hope to clarify the matter by making an integrated electron microscopic and serological study of the antigens of adenovirus type 19, as a representative of adenovirus group II (Rosen, 1960). To this end, antisera against purified viral components have been prepared and tested. The morphological study of these purified components has already been published (Gelderblom et al. 1967); the serological results are presented here. Some other serological and biological properties of the type-specific antigen are also reported. The antigens were designated according to the terminology proposed by Ginsberg et al. (1966) as hexon (group-specific antigen) and fibre (type-specific antigen). The soluble haemagglutinin we called ‘dodecon’ (Gelderblom et al. 1967). Penton antigen was not observed as a soluble antigen in type 19.

METHODS

Separation of antigens. Virus stocks, 50 times concentrated by the ‘cell packing’ method, were prepared in serum-free HeLa cell cultures. The separation procedure is outlined below. After DEAE-chromatography, no cell-specific antigen could be demonstrated in the eluates by complement-fixation. All purified antigens (except

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virus particles) were devoid of demonstrable infectivity. Crude concentrated virus was
dialysed against 0.01 M-phosphate buffer at pH 7.0 and passed through a DEAE-
cellulose column at this pH, elutions being made by increasing NaCl molarity. The
type-specific or fibre antigen passed unabsorbed through the column; all other viral
antigens (hexon, dodecon, virus particle) were eluted later with a great deal of over-
lapping (Fig. 1). This was much more pronounced than in earlier studies (Gelder-
blom, Wigand & Bauer, 1965), in which unconcentrated virus was used. DEAE fractions
were concentrated by pervaporation and simultaneous dialysis against saline. There-
after they were adjusted with solid CsCl to an optical refraction of nD = 1.365 and
centrifuged in an SW 39 rotor (Spinco L centrifuge) for 20 hr at 35,000 rev./min. Two
bands were visible in the tube, the lower containing the infectious virus, the upper
incomplete particles and other viral antigens. This second band could be separated
into a lower portion containing the bulk of incomplete particles, and an upper portion
containing the group-specific antigen and the soluble haemagglutinin. The infectious
virus was recentrifuged in CsCl. Virus assays were made first by haemagglutination
with rat erythrocytes, second by titration in HeLa cells, using fourfold dilutions and
reading the cytopathic end points 8 days after inoculation. Soluble haemagglutinin,
group-specific antigen and incomplete particles were separated by layering the serologi-
cally active material from the CsCl gradient on the top of a preformed sucrose gradient
(45 to 10%) and centrifuging for 4 hr at 35,000 rev./min. Thereafter, incomplete virus
particles were found in the lowest, dodecon in the middle and hexon antigen in the
top fractions of the gradient. Fractions from density gradients were collected through a
hole punctured in the bottom of the tubes. Further details about separation of anti-
gens may be found elsewhere (Wigand et al. 1966).

Serological tests. The performance of complement-fixation reaction, haemagglu-
tination-inhibition (HA-inhibition), and neutralization were described by Wigand
et al. (1965, 1966) and Bauer & Wigand (1963). Haemagglutination (HA) with fibre
antigen was tested by addition of 1% adenovirus 17 immune serum to the rat erythro-
cytes, instead of 0.5% normal rabbit serum, which was used for other antigens.

Preparation of immune sera. Each of the following antigens was used to immunize
two rabbits: crude virus prepared in serum-free human-thyroid cultures, purified virus
particles, hexon antigen, fibre antigen, and dodecon (soluble haemagglutinin). The
antigens used for immunization (except hexon) were separated from one another in
the first purification steps, as described above. If necessary, the purification was re-
petd until the antigens were serologically pure, when the following tests were nega-
tive: group complement fixing reactivity and direct HA for fibre antigen, type com-
plement fixing reactivity and HA for hexon antigen, group complement fixing reactivity
and indirect HA after absorption with rat erythrocytes (see below) for dodecon, HA
with human blood cells and indirect HA after absorption with rat erythrocytes for
complete and incomplete virus particles. Thereafter the last purification step (DEAE
chromatography for fibre and hexon antigen, sucrose gradient centrifugation for
dodecon, CsCl gradient centrifugation for virus particles) was repeated before the
products were considered sufficiently pure to be used for immunization.

Hexon antigen was purified differently. After DEAE-chromatography it was
absorbed with rat erythrocytes (eight absorption cycles) to remove virus particles,
empty capsids and dodecon. Thereafter the DEAE-chromatography was repeated. The
materials used had complement-fixation titres of approximately 1/64 for hexon and
Relationship of adenovirus soluble antigens

1/32 for fibre antigen. The HA titres for both dodecon and virus particles were 1/160. Four ml. of the purified antigens were inoculated intravenously in two doses into each of two rabbits on two consecutive days. After 1 month a booster injection with 2 ml. antigen was given, and immune sera were collected 8 to 10 days later. The use of adjuvants was purposely avoided to prevent the antigenic action of possible minor contaminants in the immunizing materials being reinforced. In addition to these component antisera, 'routine' adenovirus rabbit immune sera (Wigand et al. 1965) were tested in some experiments.

Serum absorption. For absorption of component antisera, undiluted serum was mixed with the respective antigen containing 2 to 3 times the estimated amount of antigen which would react by complement fixation with the antibody in the serum. After 16 hr storage at 4 °, the mixture was centrifuged for 20 min. at 4000 rev./min. and the supernatant fluid was absorbed with rat erythrocytes to remove excess dodecon antigen or nonspecific serum haemagglutinins, before being used in HA-inhibition tests.

RESULTS

Preparation and properties of the fibre antigen

A type-specific antigen was obtained by DEAE-chromatography at both pH 7.0 and 5.2. Most of it passed through the column without being adsorbed (Fig. 1, upper part) and was present in relatively low titre. It did not react with human adenovirus convalescent sera or with the majority of heterologous rabbit immune sera. The complement fixing reaction with antiserum against fibre antigen showed (Fig. 1, top) that a small portion of this antigen eluted later, thus partially overlapping with the other antigens. It is of interest that this type-specific antigen acted as an indirect haemagglutinin. It agglutinated rat erythrocytes in the presence of some heterotypic adenovirus immune sera, but not in the presence of normal rabbit serum. The HA pattern was often incomplete; in some tests it was apparent only after shaking and resettling of the rat erythrocytes. In comparison with adenoviruses of group III, in which indirect haemagglutinins were first demonstrated (Rosen, 1960), only a few selected immune sera could be used in group II (see below), while many were reactive in group III (Bauer & Wigand, 1963).

The fibre antigen of type 19 was not adsorbed to any measurable extent to rat erythrocytes. Even after three adsorption cycles of the antigen to 5% rat erythrocytes at both 4 ° and 37 °, alone or with the addition of an appropriate heterologous immune serum, the titre of HA remaining in the supernatant was not diminished (Table 1). The indirect haemagglutinins of group I and III viruses also do not adsorb to the respective erythrocytes (Pereira & de Figueiredo, 1962; Bauer & Wigand, 1963; Norrby, 1966b). In contrast to type 19, however, the indirect haemagglutinin of type 3 (group I) was adsorbed when heterologous immune serum was added (Norrby, 1966b). It is noteworthy that in type 3 the indirect haemagglutinin appears to be penton and not fibre antigen (Norrby, 1966b). The different adsorption properties of direct and indirect haemagglutinin of type 19 enables the demonstration of fibre antigen to be made in the presence of dodecon and virus-associated haemagglutinin, since the latter two can be absorbed out with rat erythrocytes.
Table 1. Treatment of the indirect haemagglutinin of adenovirus 19 with rat erythrocytes

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Adenovirus 17 immune serum</th>
<th>HA titre after 3 adsorptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Not added</td>
<td>128</td>
</tr>
<tr>
<td>37</td>
<td>Not added</td>
<td>256</td>
</tr>
<tr>
<td>4</td>
<td>Added</td>
<td>128</td>
</tr>
<tr>
<td>37</td>
<td>Added</td>
<td>128</td>
</tr>
<tr>
<td>Control (no adsorption)</td>
<td></td>
<td>128</td>
</tr>
</tbody>
</table>

Fig. 1. DEAE chromatography of concentrated adenovirus 19, pH 5.2, elution by increasing molarity of NaCl (0 to 0.65 M) from fractions nos 9 to 20. △——△, Infectivity; •——•, direct haemagglutination; ○——○, indirect haemagglutination (from fraction 10 after absorption with red blood cells); □——□, complement fixation with heterologous rabbit immune serum (group reactivity); ■——■, complement fixation with homologous rabbit immune serum (type reactivity); △——△, complement fixation with fibre antiserum (qualitatively) (type reactivity).

**Type-specificity of the fibre antigen**

The serological specificity of the fibre antigen was studied in three ways (Table 2). Various adenovirus immune sera were tested for complement-fixing antibodies against it. The same sera were tested for HA-inhibiting antibodies against fibre and, in com-
Relationship of adenovirus soluble antigens to dodecon antigen. Finally the sera were tested for their capacity to evoke HA with fibre antigen. Sera of adenoviruses outside group II did not react in any of these tests (inhibition of HA caused by fibre antigen was not attempted with these sera). A large number of group II sera was also devoid of activity. However, in addition to type 19 sera, type 10 immune sera also reacted in complement-fixation and HA-inhibition tests, reflecting the known serologic relationship between types 10 and 19 (Bell, Rota & McComb, 1960). Furthermore, three of four type 17 antisera reacted in the complement-fixing reaction. At the same time, these sera and, in addition, one type 9 serum, were the only ones which induced HA by fibre antigen. With all other sera of group II, negative or (with four sera) irregularly positive results were obtained.

Table 2. Specificity of the type-specific (fibre) antigen of adenovirus 19

<table>
<thead>
<tr>
<th>Rabbit immune sera*</th>
<th>Complement-fixation</th>
<th>Inhibition of HA caused by Dodecon antigen</th>
<th>Induction of fibre antigen to produce HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (types 3, 7, 11, 14, 16, 21)</td>
<td>- †</td>
<td>- †</td>
<td>N.D. †</td>
</tr>
<tr>
<td>Group III (types 1, 2, 4, 5, 6)</td>
<td>-</td>
<td>-</td>
<td>N.D.</td>
</tr>
<tr>
<td>Group IV (types 12, 18)</td>
<td>-</td>
<td>-</td>
<td>N.D.</td>
</tr>
<tr>
<td>Group II Types 8, 9, 15, 13, 15, 20§, 22, 23, 24, 26, 27, 28§, 29, 30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Type 9</td>
<td>±</td>
<td>20</td>
<td>+</td>
</tr>
<tr>
<td>Type 10</td>
<td>+</td>
<td>640</td>
<td>80</td>
</tr>
<tr>
<td>Type 17</td>
<td>+</td>
<td>20</td>
<td>+</td>
</tr>
<tr>
<td>Type 25§</td>
<td>+</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Type 19</td>
<td>+</td>
<td>5120</td>
<td>640</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2560</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2560</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2560</td>
<td>160</td>
</tr>
</tbody>
</table>

* Two immune sera of each type were tested, except types 17 and 19 for which 4 sera were tested.
† Serum dilution 1/10 for complement-fixation and 1/20 for HA-inhibition.
‡ Not determined.
§ Types 20, 25 and 28 are considered to be atypical members of group II adenoviruses (Wigand, 1968).

Table 3. Effect of heating on dodecon and fibre antigens

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HA-titre</th>
<th>Fibre antigen (HA with Adeno 17 immune serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1024</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>1 hr, 55°</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>1 hr, 60°</td>
<td>2</td>
<td>16</td>
</tr>
</tbody>
</table>

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Serological properties of dodecon, virus particle and empty viral capsids

Dodecon used as an antigen in complement-fixation tests did not react with human convalescent sera, but did react with type 19 immune sera as a type-specific antigen. It was also positive in tests with those heterologous rabbit immune sera which reacted with fibre antigen (Table 2), but not with others. The complement-fixation titre of dodecon was approximately 100-fold less than the HA titre. Eight to sixteen HA units of purified virus showed only group-specific complement fixation, but when 50 HA units were used, there was also a reaction with fibre antisera. Empty capsids or incomplete virus particles were prepared in a sucrose gradient from the lower portion of the HA band of the CsCl gradient. These particles were devoid of infectivity. Like the complete virus particles they agglutinated rat erythrocytes but not human blood cells, which are agglutinated by dodecon (Bauer, Wigand & Adam, 1964). The HA was inhibited by component antisera in the same way. In complement-fixation tests they exhibited group but not type-specific activity with the quantity of antigen used.

Table 4. Complement fixation. Cross-reactions of adenovirus 19 component antisera

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Hexon</th>
<th>Fibre</th>
<th>Dodecon</th>
<th>Purified virus</th>
<th>Crude virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexon</td>
<td>160</td>
<td>&lt; 5</td>
<td>10</td>
<td>20</td>
<td>160</td>
</tr>
<tr>
<td>Fibre</td>
<td>&lt; 5</td>
<td>80</td>
<td>&lt; 5</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Dodecon</td>
<td>&lt; 5</td>
<td>10</td>
<td>10</td>
<td>80</td>
<td>160</td>
</tr>
<tr>
<td>Purified virus</td>
<td>20</td>
<td>80</td>
<td>80</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Crude virus</td>
<td>160</td>
<td>160</td>
<td>20</td>
<td>80</td>
<td>80</td>
</tr>
</tbody>
</table>

Table 5. HA-inhibition and neutralization: cross-reaction of adenovirus 19 component antisera

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Hexon</th>
<th>Fibre</th>
<th>Dodecon</th>
<th>Purified virus</th>
<th>Crude virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibre*</td>
<td>&lt; 5</td>
<td>10</td>
<td>10</td>
<td>640</td>
<td>640</td>
</tr>
<tr>
<td>Dodecon</td>
<td>20</td>
<td>40</td>
<td>80</td>
<td>2560</td>
<td>2560</td>
</tr>
<tr>
<td>Purified virus</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td>160</td>
<td>640</td>
<td>1280</td>
</tr>
<tr>
<td>Crude virus</td>
<td>20</td>
<td>40</td>
<td>160</td>
<td>2560</td>
<td>5120</td>
</tr>
<tr>
<td>Neutralization</td>
<td>160</td>
<td>&lt; 2</td>
<td>40</td>
<td>320</td>
<td>640</td>
</tr>
</tbody>
</table>

* Indirect haemagglutinin.

Splitting of dodecon by heat

Since the dodecon antigen contains the morphological elements of the fibre antigen (Gelderblom et al. 1967), attempts were made to convert dodecon to fibre antigen. Heating of dodecon for 1 hr at 55° or 60° yielded a material which reacted as an indirect haemagglutinin (Table 3), while the direct haemagglutinating activity was destroyed. Fibre antigen was stable at these temperatures.
Cross-reactivity of component antisera

Results of cross-reactions in complement fixation and HA-inhibition and results of neutralization tests are shown in Tables 4 and 5. The results obtained with the second rabbit immune serum against each antigen are not shown. Qualitatively they corresponded in all instances with the data listed in the tables.

Judged by neutralization or HA-inhibition (Table 5), most sera had a reasonably high antibody content. The homologous complement fixation titres were low for dodecon and virus particle antisera and fairly high for hexon and fibre antisera. In complement fixation tests (Table 4), hexon and fibre antigens did not show any cross-reaction with one another. Dodecon antiserum reacted weakly with hexon antigen, but there was no reciprocal complement fixation. Crude virus preparations, as expected, exhibited both group- and type-specificity. The same was true for purified virus.

The HA-inhibition tests showed several interesting features (Table 5). HA-inhibition titres were approximately eightfold lower with fibre antigen than with dodecon. Hexon antiserum showed a weak HA-inhibition against dodecon. Dodecon and purified virus antisera reacted equally with virus-associated haemagglutinin and dodecon but fibre antisera did not react with virus-associated haemagglutinin. It is noteworthy that the same sera were also devoid of neutralizing capacity, while all other sera had appreciable neutralizing potency.

In two instances (hexon and fibre antiserum v. purified virus antigen) a positive complement-fixation reaction, but no HA-inhibition, was found. The reason for this will be discussed below.

Table 6. Absorption of hexon antiserum with hexon antigen

<table>
<thead>
<tr>
<th>Serum</th>
<th>CF reaction against Hexon</th>
<th>HA-inhibition against Dodecon</th>
<th>HA-inhibition against Crude virus</th>
<th>Neutralization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unabsorbed</td>
<td>160</td>
<td>160</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Absorbed</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
<td>&lt; 10</td>
</tr>
</tbody>
</table>

Table 7. Absorption of fibre antiserum with fibre antigen

<table>
<thead>
<tr>
<th>Serum</th>
<th>HA-inhibition against Fibre</th>
<th>HA-inhibition against Dodecon</th>
<th>HA-inhibition against Crude virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unabsorbed</td>
<td>10*</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>Absorbed</td>
<td>N.D.*</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
</tr>
</tbody>
</table>

* Because of the presence of excess fibre antigen, not determined.

Serum absorption studies

Component antisera were absorbed with their homologous antigens in order to gain more insight into the relationship of the antigens.

Absorption experiments with dodecon antiserum failed, i.e. HA-inhibiting serum titres did not diminish after absorption, but were successful with hexon and fibre antisera. In experiments with hexon antiserum (Table 6), not only complement-fixation antibodies but also HA-inhibiting and neutralizing antibodies were removed...
by absorption with hexon antigen. From fibre antiserum (Table 7), HA-inhibiting antibodies against dodecon were absorbed. This gives evidence that the ‘cross-reacting antibodies’ in these sera were elicited by the respective antigens, and not by impurities in the material used for immunization.

DISCUSSION

The neutralizing capacity of hexon antisera against type 19 (Table 5) appears to be contradictory to the group-specific character of the hexon antigen. Similar observations, however, have been made by Wilcox & Ginsberg (1963a) with type 5. Even very highly purified hexon antigen of type 5 invariably elicited type-specific neutralizing antibodies (Ginsberg, personal communication). This has led to the conclusion that hexon antigen must be endowed with both group- and type-specific antigenic determinants. The results of absorption experiments (Table 6) are compatible with this interpretation. Hexon and fibre antigens did not exhibit cross-reactivity, which is in accordance with precipitation studies made with type 5 (Wilcox & Ginsberg, 1963b; Valentine & Pereira, 1965) and type 2 (Köhler, 1965). Type 19 fibre antigen did not induce neutralizing antibodies, which were elicited by type 5 fibre antigen (Wilcox & Ginsberg, 1963a). There is no obvious reason for this discrepancy.

In the complement-fixation reaction, the dodecon reacted mainly as a type-specific antigen. There was, however, a weak complement fixation by dodecon antiserum against hexon antigen and a weak HA-inhibition by hexon antiserum against dodecon haemagglutinin. This may indicate a subtle serological relationship between both antigens, indicated by the observation that HA-inhibiting antibodies v. dodecon were absorbed from hexon antiserum by hexon antigen (Table 6).

Not only hexon but also dodecon elicited the production of neutralizing antibodies. We conclude therefore that for the initiation of cell infection the vertex capsomeres as well as the hexon capsomeres of the virus particle are important, while the projections (fibres) are not, since fibre antisera did not neutralize the infectivity.

The close serological relationship of fibre and dodecon antigen is apparent from cross-reactions in HA-inhibition (Table 5), from heating experiments (Table 3), and from the absorption experiment with fibre antiserum (Table 7). Norrby (1966b) also found common antigenic determinants in type 3 (of group I) between direct and indirect haemagglutinins, shown by heating experiments as well as by serum absorption studies. Similar conclusions may be reached when the degree of cross-reactivity of dodecon and fibre antigen with other types is compared (Table 2). Type 10 sera and one type 25 serum reacted with type 19 antigens in all three tests listed. From the study of other types of group II (unpublished results) it appears that, in most cases, fibre antigen and dodecon have a similar degree of cross-reactivity with other types. While all these findings were to be expected on the basis of the structural relationship between dodecon and fibre antigens (Gelderblom et al. 1967), some differences between them also need to be noted.

Purified virus particles reacted in complement-fixation tests with hexon and fibre antisera, but with the latter only at high antigen concentrations. Since there are 240 hexon capsomeres but only 12 fibre elements on the virus surface, this difference is easily understood. Purified type 5 virus particles were also found to react more strongly with hexon than with fibre antisera (Wilcox & Ginsberg, 1963b). According to our
morphological study (Gelderblom et al. 1967), the substrate involved in haemagglutinating activity appears to be fibre + knob for fibre antigen; 'penton base', fibre + knob (clustered into a regular array of 12 units) for dodecon; and penton base + fibre + knob or vertex capsomere + projection for virus particle. A number of serological and biological differences between the three haemagglutinating particles of adenovirus 19 have been observed (empty capsids appear to be similar to the virion in all respects except infectivity):

(1) The host range of agglutinable blood cells is different. Virus particles agglutinate only rat erythrocytes while dodecon agglutinates both rat and human erythrocytes. In some other adenovirus types of group II (types 9, 13, 15) virion and incomplete virion agglutinate monkey erythrocytes, while dodecon does not (Wigand & Stöhr, 1965). Erythrocytes which are agglutinated by dodecon but not by virion (human blood cells for adenovirus 19) may require close proximity of several projections—present in the dodecon only—for HA to occur, while for rat erythrocytes no such necessity may exist. The slower adsorption of adenovirus 19 haemagglutinins to human than to rat blood cells (Inteewaun & Wigand, 1967) would be compatible with this interpretation. With types 9, 13 and 15, however, monkey blood cells are agglutinated by virion but not by dodecon. It may be therefore that in this HA the vertex capsomeres are active, these are covered by projections in the dodecon but are relatively accessible on the surface of the virus particle.

(2) Fibre antigen has a different HA mechanism from the two 'direct' haemagglutinins. This is shown by the need for heterologous immune serum for HA with fibre antigen and by the apparent failure of the fibre antigen to be absorbed to rat erythrocytes. The fibre antigen appears to have a monovalent type-specific antigenic determinant reacting with the erythrocyte surface, and another, of broader specificity, reacting with bivalent antibodies of the heterologous immune serum. 'Direct' haemagglutinins, however, react as bivalent antigens with more than one erythrocyte, which leads directly to HA.

(3) Differences in the serological specificity of virus-associated haemagglutinin and dodecon have been shown earlier for some other group II viruses (Wigand & Wunn, 1966). Adenovirus 19 dodecon elicits HA-inhibiting antibodies against virus-associated haemagglutinin, while fibre antigen does not (Table 5). Since the only constituent which is present in dodecon but absent from fibre antigen is presumably the penton base, it is reasonable to assume that the vertex capsomere (= penton base) of the virion plays some role in viral HA with rat erythrocytes. The same structural element in the dodecon may be inactive in HA because it is covered by the projections but active as an antigenic determinant in the rabbit.

It was mentioned above that hexon and fibre antisera reacted with virion antigen in complement-fixation reaction, but not in HA-inhibition (Tables, 4, 5). If it is true that the vertex capsomeres of the virion are active in HA but hexon capsomeres and projections are active in complement-fixation reactions this finding is readily explained.

Indirect haemagglutinins have been demonstrated in all four adenovirus groups. For groups II and III they have been shown to be fibre antigens. However, Norrby (1966b) on the basis of centrifugation experiments considers the incomplete haemagglutinin of type 3 (group I) to be penton antigen. In addition, Norrby & Wadell (1967) recently found in type 4, an atypical member of group III, two fractions of different sedimentation rates with incomplete haemagglutinin activity which might be identical
with penton and fibre antigen. Further studies are needed concerning the specificity of
the immune serum inducing HA with incomplete haemagglutinins. As to group III,
most sera inside the group (Rosen, 1960; Pereira & de Figueiredo, 1962), and even
some adenovirus immune sera of types outside group III (Bauer & Wigand, 1963) are
active. For type 4 of group III, Norrby & Wadell (1967) found differences in the serum
types evoking HA with the two incomplete haemagglutinins. As we have shown, for
type II viruses only a few immune sera of this group are active. The nature of anti-
body involved remains to be determined.

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