The Structural and Functional Diversity of Adenovirus Capsid Components

By E. NORRBY

Department of Virology, Karolinska Institutet,
School of Medicine Stockholm, Sweden

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Adenoviruses offer a number of advantages for integrated morphological and functional studies of structural virus components. During the course of multiplication of these viruses structural components are produced in considerable excess. These components are soluble, both in the traditional and in the true sense of the word, and have dimensions which allow their examination by electron microscopy. In addition most of these soluble components can be easily identified by a number of different biological tests.

During the last four years there has been a considerable accumulation of information on structural and biological characteristics of adenovirus capsid components (cf. Norrby, 1968 a). This new phase of intensive studies of structural adenovirus components was initiated by the demonstration that vertex capsomeres differed immunologically from non-vertex capsomeres and that they carried projections (Valentine & Pereira, 1965; Norrby, 1966a). In the present article an attempt is made to give a brief summing up of our present state of knowledge in this field. In addition some recent findings will be presented. Components with subcapsomere position in virus particles (internal components) will be dealt with only en passant, partly due to the dearth of information on the nature of these structures. It is hoped that this review will be of value to non-adenovirus virologists and that it may facilitate a following up of future developments. Emphasis will be placed on discussions of the correlation between the biological subgrouping of different human adenoviruses (Rosen, 1960, Table 1) and structural-functional characteristics of their soluble components. In conclusion, criteria for establishment of serotypes will be proposed.

Table I. Separation of human adenoviruses into subgroups on the basis of their haemagglutinin (HA) activity

<table>
<thead>
<tr>
<th>Rosen’s subgroup number; HA activity</th>
<th>Serotype numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Complete agglutination of monkey erythrocytes</td>
<td>3, 7, 11, 14, 16, 20, 21, 25, 28</td>
</tr>
<tr>
<td>II. Complete agglutination of rat erythrocytes</td>
<td>8–10, 13, 15, 17, 19, 22–24, 26, 27, 29, 30</td>
</tr>
<tr>
<td>III. Partial agglutination of rat erythrocytes</td>
<td>1, 2, 4, 5, 6, 12, 18, 31*</td>
</tr>
</tbody>
</table>

* Types 12, 18 and 31 were originally described as non-haemagglutinating.

Terminology

The terminology for structural components currently used (Fig. 1) was presented by Ginsberg et al. (1966). They proposed the term hexon for nonvertex capsomeres,
since these appear in a position surrounded by six other capsomeres. In one virion 240 out of the total of 252 capsomeres are hexons. Vertex capsomeres plus projections are called pentons, for analogous reasons. The two parts of a penton, the vertex capsomere and the projection, are referred to as penton base and fibre, respectively. In addition the term dodecon (Gelderblom et al. 1967) is used to denote aggregates of 12 pentons (see p. 226).

In the following the terms complete and incomplete haemagglutinins (HAs) will be used to denote components giving a direct agglutination of red cells and components which require the presence of antibodies against a heterologous serotype in order to give agglutination, respectively (Fig. 3). The test by which incomplete HAs are demonstrated is called a haemagglutination-enhancement (HE) test.

\[
\text{Hexon-non-vertex capsomere} \quad \bigcirc
\]

\[
\text{Penton base-vertex capsomere} \quad \bullet
\]

\[
\text{Fibre-vertex projection} \quad \longrightarrow
\]

\[
\text{Penton-vertex capsomere plus projection} \quad \bullet
\]

\[
\text{Virion}
\]

\[
\text{Dodecon}
\]

**Fig. 1.** Schematic description of monomeric and some experimentally identified polymeric forms of adenovirus capsid components.

**Some aspects of biological characteristics of monomeric and polymeric forms of capsid components.**

Figure 1 gives a schematic description of some different monomeric and polymeric forms of structural components, which have been identified experimentally.

Hexons (non-vertex capsomeres) represent the dominating soluble component in harvests from tissue cultures. Most hexons occur in a dispersed form, but there is some tendency to aggregate into dimers, trimers, tetramers, etc. Disruption of virus particles by sodium dodecyl sulphate (Smith, Gehle & Trousdale, 1965a), heating (Russell, Valentine & Pereira, 1967) or by acetone at room temperature (Laver et al. 1968) yields a specific polymer comprising nine hexons (Fig. 1). This type of polymer might represent a functional aggregate. Heat-treatment of virus particles under certain conditions can lead to a release of vertex capsomeres plus their neighbouring five hexons
Diversity of adenovirus capsid components

(Russell et al. 1967). The partially degraded capsid which remains is composed of 180 hexons. This kind of structure can easily be constructed from 20 groups of nine hexons with the configuration depicted in Fig. 1. More than 90% of the groups of nine hexons seen in the electron microscope display a counter-clockwise appearance suggesting a tendency to a specific orientation (E. C. Follett, personal communication). This might be due to a specific affinity between one side of the groups of nine hexons and the carbon layer on grids used for electron microscopy.

Rosen's subgroup number

Serotypes analysed

Length of fibres (nm.)

Fig. 2. Diagrammatic presentation of the relative position in the elution diagram after exclusion chromatography on Sephadex G200 of fibres of various serotypes representing different adenovirus subgroups. The curved line represents extinction values at 280 nm. of components of normal calf serum, added as a reference in the fractionations. The length (nm.) of different fibres as determined by electron microscopy of virus particles is also indicated.

Table 2. Some characteristics of fibres of different adenovirus serotypes

<table>
<thead>
<tr>
<th>Subgroup number</th>
<th>Serotypes studied</th>
<th>Length of fibre (nm.)</th>
<th>Incomplete HA</th>
<th>Immunological specificities</th>
<th>References:</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3, 11, 16</td>
<td>9 to 11</td>
<td>No</td>
<td>Type-specific HI antigen</td>
<td>Norrby, 1966a, Norrby, 1968b</td>
</tr>
<tr>
<td>II</td>
<td>9, 15</td>
<td>12 to 13</td>
<td>Yes</td>
<td>Type-specific HI antigen, intersubgroup-specific (II+III) and subgroup-specific HE antigen</td>
<td>Norrby et al. 1967; Norrby, 1968c.</td>
</tr>
<tr>
<td>III</td>
<td>4, 6</td>
<td>17 to 18</td>
<td>Yes</td>
<td>Type-specific HI antigen, intersubgroup-specific (II+III) and subgroup-specific HE antigens</td>
<td>Wadell et al. 1967; Norrby et al. 1969a; Valentine &amp; Pereira 1965; Pettersson et al. 1968; Wadell &amp; Norrby, 1969b.</td>
</tr>
</tbody>
</table>

Fibres (vertex projections). There is a considerable variation in length of fibres of different serotype origin. The most sensitive technique for separating isolated, elongated structures of the kind represented by fibres appears to be exclusion chromatography. Figure 2 illustrates the relative position of different isolated fibres in the elution diagram of normal calf serum components (included as a reference) after fractionation on Sephadex G200 (Pharmacia Fine Chemicals, Uppsala, Sweden) and
Table 2 gives some additional data on characteristics of various fibres. Fibres of all subgroup I members studied (types 3, 11, 16) elute in a position intermediate between the IgG and albumin-peaks. The length of fibres extending from dodecons or virions has been estimated as 9 to 11 nm. (Pl. 1, 2; Norrby, 1966a, 1968b). Fibres of members of subgroup II (types 9 and 15 have been studied) are somewhat larger, 12 to 13 nm. (Pl. 1, 2; Norrby et al. 1967; Norrby, 1968c) and elute just after the peak of IgG after fractionation on Sephadex G200. The length of fibres of different members of Rosen's subgroup III varies somewhat. Type 4, the aberrant member of the group, which with regard to most biological activities other than haemagglutination belongs to subgroup I

(cf. Norrby, 1968a), carries fibres with a length of 17 to 18 nm. (Pl. 1, 2; Wadell, Norrby & Schöning, 1967). Fibres of most members of subgroup III (types 1, 2, 5) plus type 12 have a length of 28 to 31 nm. (Pl. 1, 3; Valentine & Pereira, 1965; Pettersson, Philipsson & Höglund, 1968; Norrby & Ankerst, 1969; Norrby, Wadell & Marusyk, 1969a). They elute from a Sephadex G200 column in a position intermediate between the void volume and the peak of IgG. However, fibres of type 6 elute somewhat later than these fibres and ultrastructural examination of virus particles has shown that the former are 3 to 4 nm. shorter (Pl. 1) (Norrby et al. 1969a).

Fibres of members of subgroups II and III can be most readily demonstrated as incomplete HAs in HE tests. The presumed background to this test is schematically illustrated in Fig. 3. It is assumed that antibodies present in antisera against a heterologous serotype can aggregate fibres via their proximal parts, antigen δ (Fig. 3)
Ultrastructure of virus particles of different serotype representatives of Rosen's subgroups I, type 3 (a); II, type 15 (b); and III, types 4 (c), 6 (d) and 2 (e). Negative contrasting with sodium tungstosilicate.

E. NORRBY

(Facing p. 224)
Ultrastructure of dodecons of adenovirus types 3(a), 4(b), 9(c) and 11(d). Negative contrasting with sodium tungstosilicate. Reproduced with permission of *Current Topics Microbiol. Immunol.*

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Ultrastructure of 15 to 16S (a and b) and 8 to 9 S (c and d) complete HAs of adenovirus type 6. Negative contrasting with sodium tungstosilicate.

E. NORRBY
Diversity of adenovirus capsid components

Table 5; Pereira & de Figueiredo, 1962) into complexes which are polyvalent with regard to their capacity to interact with receptors on red cells. Antibodies against antigen δ cannot interact with this part of a fibre when it occurs combined with vertex capsomeres into pentons. Fibres of members of subgroup I cannot function as incomplete HA. They therefore have to be identified by complement fixation tests or by haemagglutination-inhibition antibody consumption (HIC) tests (Norrby & Skaaret, 1967).

Pentons (vertex capsomeres plus projections) of all serotypes studied can be identified as an incomplete HA (Norrby, 1966b, 1968c, d; Norrby & Skaaret, 1968; Norrby & Ankerst, 1969; Norrby et al. 1967; Waddell & Norrby, 1969a, Wadell, Norrby & Skaaret, 1969). In earlier studies (Pereira & de Figueiredo, 1962) it was suggested that pentons of some members of subgroup III represented a complete HA. This conclusion was based on the presence of complete HA activity in preparations of pentons isolated by anion exchange chromatography. In more recent studies (Wadell et al. 1969) preparations of this kind could be further separated by zonal centrifugation into monomeric and oligomeric forms of pentons. Only the latter were found to carry complete HA activity (see p. 227).

The penton incomplete HA can be demonstrated in HE tests after aggregation by antibodies, present in heterotypic antiserum, which can react with vertex capsomeres, e.g. their antigen specificity β (Fig. 3, Table 5). Pentons of some serotypes carry a toxin (cell-detachment) activity which is destroyed by treatment with trypsin (Table 3; Pereira, 1960; Norrby & Skaaret, 1967). This treatment causes a breakdown of the vertex capsomere part of the penton, which leads to the appearance of isolated fibres. The effect of trypsin is concentration-dependent. At very low concentrations of the enzyme only toxin activity is destroyed whereas the capacity of pentons to function as an incomplete haemagglutinin remains unchanged (Wadell & Norrby, 1969b; U. Pettersson, personal communication).

Table 3. Some characteristics of different kinds of complete and incomplete HAs

<table>
<thead>
<tr>
<th>Characteristic features</th>
<th>Type of complete HA</th>
<th>Type of incomplete HA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dodecons</td>
<td>Dimer of pentons</td>
</tr>
<tr>
<td>Rate of sedimentation (S)</td>
<td>50 to 100</td>
<td>15 to 16</td>
</tr>
<tr>
<td>Toxin activity*</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Degradation by trypsin</td>
<td>Variable</td>
<td>Yes</td>
</tr>
<tr>
<td>Presence of vertex capsomeres†</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Effect of heterotypic anti-sera on agglutinating activity</td>
<td>None</td>
<td>Increase</td>
</tr>
</tbody>
</table>

* Refers to serotypes, which exhibit this activity.
† Determined by the HEC test.

Penton bases (vertex capsomeres) as isolated structures are generally present only in relatively low concentrations among spontaneously occurring soluble components. However, among the different serotypes studied, type 12 materials form an exception to this rule. In preparations of this serotype the relative concentration of free vertex capsomeres is markedly higher than that of vertex capsomeres combined with fibres
into pentons (Norrby & Ankerst, 1969). Treatment of type 3 materials with 2 M-guanidine + HCl caused a dissociation of pentons into vertex capsomeres and projections (Norrby & Skaaret, 1967). Other substances such as formamide (Neurath, Rubin & Stasny, 1968) and pyridine (U. Pettersson, personal communication) can also bring about such a dissociation.

Since isolated vertex capsomeres cannot function as an incomplete HA, a special technique, the haemagglutination-enhancement antibody consumption (HEC) test, was designed for their identification (Norrby & Skaaret, 1967). A schematic description of this test is given in Fig. 4. The test measures the consumption of HE antibody reacting with vertex capsomeres, some antigen specificities of which are common to all human adenoviruses (Table 5). The test sample, which either should not interact with the red cells finally added or should be deprived of all complete HA, is incubated with homologous antiserum. To demonstrate the remaining penton HE antibody in this mixture a preparation of heterologous pentons is added. In a situation where the test sample contains pentons or free vertex capsomeres these will absorb the penton HE antibody of serum and no agglutination by indicator pentons will occur.

As was mentioned above, the penton base is susceptible to trypsin digestion, which treatment destroys the toxin activity of penton. There are some findings indicating that not only pentons, but also isolated vertex capsomeres may carry toxin activity (Wadell & Norrby, 1969; U. Pettersson, personal communication).

**Soluble HAs** of three different kinds have been identified (Fig. 1, 3). These are (a) dodecahedral aggregates of 12 pentons plus some extra structure, the type of complex called dodecons (Pl. 2), (b) dimers of pentons (Pl. 3a, b) and (c) dimers of fibres (Pl. 3c, d). Some characteristics of these different HAs as well as their occurrence in preparations of different serotypes of human adenoviruses have been summarized in Tables 3 and 4, respectively.

Dodecons vary in their rate of sedimentation, from 50 to 100 S (Norrby, 1966a; Norrby, 1968b; Norrby et al. 1967). This is a true variation reflecting structural differences which occur between dodecons of different serotypes (cf. Pl. 2). These differences concern both the size of the central part of the dodecon, i.e. the aggregate of vertex capsomeres and some extra structure, and the length of fibres. Dodecons and
isolated pentons share a number of biological characteristics. However, the sensitivity of dodecons of different serotypes to trypsin digestion varies somewhat. This may be due to the fact that vertex capsomeres forming a part of a dodecon complex cannot be as readily attacked by trypsin as free structural components.

Dimers of pentons carry all the properties of isolated pentons (Norrby & Skaaret, 1968, Wadell et al. 1969), but sediment with a rate that is about 1.3 times greater, 15 to 16 S. Dimers of pentons represent the only complete HA which displays an increase in agglutinating activity in the presence of heterologous antiserum (a true enhancement). This is caused by antibodies reacting with vertex capsomere antigen. The effect has been interpreted as being due to a steric reorientation of a considerable fraction of non-agglutinating penton dimers into agglutinating polymers.

The third kind of soluble HA, dimers of fibres, shares a number of properties with isolated fibres (Norrby, 1968 c; Norrby & Skaaret, 1968; Norrby et al. 1969 a; Wadell & Norrby, 1969). However, it is a larger complex sedimenting at a rate of 8 to 9 S as compared with about 6 S for free fibres. A comparison of sedimentation characteristics and exclusion chromatography behaviour of this kind of complete HA clearly demonstrates that it is a markedly elongated structure.

The polyvalent and univalent character of complete and incomplete HAs, respectively, with regard to their capacity to interact with receptors on cells should be emphasized (cf. the schematic illustration in Fig. 3). The question why some pentons or fibres occur in monomeric and others in oligomeric forms of specific configurations cannot be answered at present. Aggregation of pentons into dodecons seems to occur around some extra component (Norrby, 1966 a; Wadell et al. 1967). Possibly the dimerization of pentons and fibres is also dependent upon some extra component.

In preparations of various human adenovirus serotypes either one or two of the different kinds of HAs have been identified. As can be seen from Table 4, all theoretically possible combinations, except a simultaneous occurrence of dodecons and dimers of pentons, have been found. Possibly the presence of either one of these two penton polymers may mutually exclude the occurrence of the other.

In the absence of heterotypic antiserum, preparations of members of subgroup III

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**Table 4. Occurrence of different types of soluble HAs among various human adenovirus serotypes**

<table>
<thead>
<tr>
<th>Rosen's subgroup</th>
<th>Serotype</th>
<th>Dimer of pentons</th>
<th>Dimer of fibres</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>+</td>
<td>-</td>
<td>Norrby, 1966a</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>+</td>
<td>-</td>
<td>Neurath &amp; Rubin, 1968</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>+</td>
<td>-</td>
<td>Norrby, 1968b</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>-</td>
<td>+</td>
<td>Norrby &amp; Skaaret, 1968</td>
</tr>
<tr>
<td></td>
<td>3–16</td>
<td>-</td>
<td>+</td>
<td>Norrby &amp; Skaaret, 1968</td>
</tr>
<tr>
<td>II</td>
<td>9, 15</td>
<td>+</td>
<td>-</td>
<td>Norrby, 1968c; Norrby et al. 1967</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>+</td>
<td>-</td>
<td>Norrby &amp; Wadell, 1967; Wadell et al. 1967</td>
</tr>
<tr>
<td></td>
<td>1, 2, 5, 6</td>
<td>-</td>
<td>+</td>
<td>Wadell &amp; Norrby, 1969a; Wadell et al. 1969</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>Norrby &amp; Ankerst, 1969</td>
</tr>
</tbody>
</table>

give only a partial agglutination of rat erythrocytes. This has recently been shown (Wadell, 1969) to be due to a competition for receptors on red cells between complete and incomplete HAs, the latter of which occur in excess (Wadell & Norrby, 1969a; Wadell et al. 1969). In contrast, preparations of members of subgroup II contain markedly more complete than incomplete HAs (Norrby et al. 1967; Norrby, 1968c) and therefore give a pattern of complete agglutination of rat erythrocytes. An additional factor of importance for the haemagglutination pattern obtained is the number of specific receptors occurring on cells of different kinds. It was recently found (Wadell, 1969) that members of subgroups II and III can agglutinate not only rat erythrocytes but also human O red cells. However, presumably owing to the fact that the latter cells carry relatively few receptors, they display a markedly partial agglutination which readily escapes detection. Certain members of subgroup II, which give complete agglutination, are exceptions to this rule.

Table 5. Summary of biological characteristics of structural adenovirus components*

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Structure</th>
<th>Designation</th>
<th>Specificity</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-vertex capsomers; hexons</td>
<td>α</td>
<td>Group</td>
<td>Group</td>
<td>Localized at the inside of the capsid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>?</td>
<td>Intersubgroup and intra-subgroup</td>
<td>Common to a restricted number of serotypes in various combinations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ε</td>
<td>Type</td>
<td>Available at the surface of the capsid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reacts with neutralizing and virus particle-HI antibody</td>
</tr>
<tr>
<td>Vertex capsomeres; penton bases</td>
<td>β</td>
<td>Group</td>
<td>Group</td>
<td>The complex of base and fibre (= penton) carries (a) toxin activity, (b) incomplete HA activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>?</td>
<td>Intersubgroup and intra-subgroup</td>
<td>Common to a restricted number of serotypes in various combinations</td>
</tr>
<tr>
<td>Vertex projections; fibres †</td>
<td>δ</td>
<td>Intrasubgroup</td>
<td>Intrasubgroup</td>
<td>Proximal part of projections. Present only in members of the same subgroup (not present in members of subgroup I)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>?</td>
<td>Intersubgroup</td>
<td>Shared between members of subgroups II and III</td>
</tr>
<tr>
<td></td>
<td></td>
<td>γ</td>
<td>Type</td>
<td>Distal part of projection. Reacts with HI and neutralizing antibody</td>
</tr>
<tr>
<td>Pentagonal; dodecon-associated</td>
<td>?</td>
<td>Unknown</td>
<td></td>
<td>Forms a core of dodecons. Possibly subcapsomere component of vertices of virus particles (?)</td>
</tr>
<tr>
<td>'Meshwork'-like</td>
<td>?</td>
<td>Unknown</td>
<td></td>
<td>Arginine-rich internal components.</td>
</tr>
</tbody>
</table>

† Incomplete HA of members of subgroups II and III.

Additional structural components, as yet only partly characterized, have been described. A spontaneous disruption of dodecons of type 3 (Norrby, 1966a) and type 4 (Wadell et al. 1967) caused a release of ring-like or occasionally pentagonal particles. This type of component has been assumed to form the core of dodecons. It has
Diversity of adenovirus capsid components

Furthermore been speculated that it may occur in a subcapsomere position at the vertices of virions. It has not as yet been identified by any biological test.

Recently, methods have been described for the isolation of internal components of virions (Laver et al. 1968; Prage, Petterson & Philipson, 1968; Russell, Laver & Sanderson, 1968). Biochemical and biological characterization of these components is currently being undertaken.

![Diagram](image)

**Vertices of virions**

**Dodecon**

**Pentons**

**Hexons**

**IgG antibodies against hexons**

![Diagram](image)

**Fig. 5. Schematic description of the background to the selective HI of virions by anti-hexon antibodies. The two mechanisms include (a) aggregation of virions and (b) steric blocking of fibres by antibody attached to para-vertex hexons.**

**Immunological specificities carried by different structural components**

The present state of knowledge of immunological specificities and other biological characteristics of structural components has been summarized in Table 5.

*Hexons.* These structural components carry an antigenic specificity (α) which is common to all human and non-human adenoviruses, except the GAL virus of birds (Pereira et al. 1963). The possible presence in hexons of a type-specific antigen inducing neutralizing antibody is a matter of controversy. In some studies a strong neutralizing activity of anti-hexon sera was found (Wilcox & Ginsberg, 1963; Kjellén & Pereira, 1968; Gelderblom et al. 1968; Norrby, 1969), whereas in others, highly purified hexons could not induce the production of neutralizing antibody (Pettersson, Philipson & Höglund, 1967). This discrepancy between different results can be explained in two different ways. Either the antigen mainly responsible for the induction of neutralizing antibody is a separate component which occurs intimately associated with hexons and which was removed only by the purification procedure employed by Pettersson et al. (1967), or this procedure removed or, more likely, altered the
specificity of a component which represents an integrated part of the hexons. Available evidence does not allow a choice between these two possible explanations.

The presence of a type-specific hexon antigen was suggested already from early studies on CF antigen activity of human adenovirus preparations (Pereira, 1956). These results were recently confirmed in tests including purified hexons (Döhner, 1968). Additional data clearly demonstrating that hexons carry a type-specific antigen have been accumulated (Norrby, 1969; Norrby, Marusyk & Hammarskjöld, 1969b). One reason for this conclusion was the finding that although antihexon sera do not contain any HE antibody or HI antibody reacting with soluble HAs, they do contain a type-specific antibody which can inhibit effectively the haemagglutinating activity of virions. This effect can be due to two different mechanisms which have been schematically outlined in Fig. 5. These mechanisms are (a) an aggregation of virus particles (alternative (a), Fig. 5) or (b) a steric interference between IgG antibodies, attached to hexons with a paravertex position, and projections extending from vertex capsomeres (alternative (b), Fig. 5). The latter interference might prevent projections from attaching to receptors on cells. As was described above, fibres of different serotypes vary in length between 10 and 30 nm. Since the length of a maximally extended IgG antibody is 15 to 20 nm. (Valentine & Green, 1967), one would anticipate a variation in the capacity of an antibody of this kind to block sterically the fibres of different serotypes. In order to analyse this problem, antisera against hexons of different serotypes containing the corresponding amount of homologous CF antibody and lacking HI activity in tests with soluble components were tested in HI tests with different virions (Table 6) (Norrby & Wadell, 1969). Sera against hexons derived from serotypes carrying fibres with a length similar to or shorter than that of serotype 4 (17 to 18 nm.) inhibited effectively the activity of homologous virions, whereas anti-type 6 and anti-type 2 hexon sera only displayed low activities. This might be interpreted as suggesting that in the case of members of subgroups I and II and in addition type 4, steric interference of the kind described plays a major role, whereas the low virion-HI activity of the antihexon sera of subgroup III members other than type 4 is due to an aggregation of the particles. However, recent data obtained in immuno-electron-microscopy studies suggest that the true explanation may be a more complex one (Norrby, unpublished).

The fact that the virion-HI activity of antihexon sera is demonstrable only with

Table 6. Virus particle HI tests with anti-hexon sera against different representative members of Rosen's subgroups

<table>
<thead>
<tr>
<th>Anti-hexon serum type*</th>
<th>Type 3 virus particles</th>
<th>Type 15 virus particles</th>
<th>Type 4 virus particles</th>
<th>Type 6 virus particles</th>
<th>Type 2 virus particles</th>
<th>Homologous soluble HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (I)†</td>
<td>1600</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
</tr>
<tr>
<td>15 (II)†</td>
<td>&lt; 8</td>
<td>1600</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
</tr>
<tr>
<td>4 (III)</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
<td>400</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
</tr>
<tr>
<td>6 (III)†</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
<td>16‡</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
</tr>
<tr>
<td>2 (III)</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
<td>&lt; 8</td>
<td>8‡</td>
<td>&lt; 8</td>
</tr>
</tbody>
</table>

* Sera were diluted to contain corresponding quantities of homologous hexon CF activity (640 units per 0.02 ml).
† Roman figures within parenthesis denote subgroup belonging.
‡ Traces of agglutination were detectable in tubes counted as negative.
Diversity of adenovirus capsid components

Homologous virions implies not only that hexons carry a type-specific component, but also that this component is available at the surface of virions, whereas the group-specific part of hexons is turned towards the interior of the particle. The occurrence of such a specific orientation of hexons in the capsid has been verified by additional experimental studies, including immuno-electron microscopy (Norrby et al. 1969b). These findings can explain previous observations that only homotypic antibodies attach to the surface of virions (Smith et al. 1965b).

Absorption of antihexon sera with purified heterologous hexons has demonstrated not only the presence of type and group antigenic specificities but also subgroup specificities (Norrby, 1969; Norrby & Wadell, 1969). It is of some interest that by use of this technique it was shown that hexons of the aberrant subgroup III member, type 4, are more closely related to hexons of members of subgroup I than to those of subgroup III. In this connexion it might be mentioned that heterologous neutralization of virions has been demonstrated by incubation of antigen–antibody complexes at low pH (Kjellén, 1966; Kjellén & Pereira, 1968). This effect was seen only between virions of one serotype and antisera against other serotypes belonging to the same subgroup. Since this effect presumably was due to antibodies reacting with hexons, it suggests that antigen specificities of a subgroup nature occur in these components.

Penton bases. The antigenic specificities of penton bases are responsible for the interaction with HE antibody converting the incomplete penton HA into an agglutinating complex (Fig. 4). Since a penton HE activity can be demonstrated by any heterologous antiserum the penton base must contain one antigenic specificity (β) which is common to all human adenoviruses. In addition it contains some intersubgroup and intrasubgroup specificities as demonstrated by determination of the immunological characteristics of HE antibodies in absorption experiments (Norrby, 1969; Wadell & Norrby, 1969b). No type-specific component has been found by use of this technique. This, however, does not exclude the possible presence of a component of this kind. Immunological specificities of vertex capsomeres of the aberrant subgroup III member, type 4, like those of non-vertex capsomeres (see above), are more similar to those of the corresponding components of subgroup I than of subgroup III members.

Fibres. The immunological complexity of fibres increases in parallel with the increase in their length (Tables 2, 5). The shortest fibres (9 to 11 nm.), which are carried by members of subgroup I, seem to contain only one antigen. This is the antigen γ, which is responsible for the induction of type-specific HI antibodies. Antibodies against the γ antigen in addition carry some neutralizing activity. This is most effectively demonstrated by the rapid technique of fluorescent foci inhibition (U. Pettersson, personal communication), but less effectively so, or not at all, with other techniques (Norrby, 1969; Kjellén & Pereira, 1968; Pettersson et al. 1968). The most plausible explanation for this behaviour seems to be that antibodies against fibres do not completely block but rather cause a delay of the process of virus replication. Fibres of members of subgroup II in addition to the type-specific antigen γ carry antigenic specificities of a subgroup and intersubgroup-specific (II and III) nature (Norrby, 1968; Wadell & Norrby, 1969). The longest fibres, i.e. those belonging to members of subgroup III, contain at least one more antigenic specificity, which is subgroup-specific (Wadell & Norrby, 1969). This corresponds to the antigen described by Pereira & de Figueiredo (1962). Antigenic specificities which reside in the proximal parts of isolated fibres can interact with HE antibodies (Fig. 4).
Other components. The immunological specificity of the internal component of dodecons and additional components recently isolated from the cores of virions has not as yet been clearly elucidated.

The occurrence of serotypes with a capsid composed of a mosaic of structural components related to one or two heterologous serotypes

Typing of adenoviruses is performed by use of neutralization and HI tests. The results of these two tests do not always agree and some cross-reactions occur between prototype strains in either one or both of the tests. In addition, so-called intermediate strains have been described which behave like one prototype in the neutralization test but a completely different one in the HI test (cf. Wigand & Fliedner, 1968). The recognition of two distinct type-specific antigens in virions allowed an analysis, on the level of structural components, of the background for these cross-reactions. As was mentioned, these two antigens are the distal parts of fibres (γ) which react with HI antibodies, specifically demonstrable in tests with soluble components, and the e antigen of hexons. The latter can be readily demonstrated in virion-HI tests with most serotypes (Table 6). Presumably this antigen is identical with or related to the antigen, which is of dominating importance for the induction of neutralizing antibody.

Table 7. Antibody activities of antisera from hyperimmunized animals against different type 4, 16 and 3-16 components in HI tests with types 4 and 16 virions and homologous soluble HA and in CF tests with homologous hexons

<table>
<thead>
<tr>
<th>Sera against adenovirus type</th>
<th>Type 4 virions</th>
<th>Type 16 virions</th>
<th>Homologous soluble HA</th>
<th>Homologous hexon CF activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 hexons</td>
<td>5120</td>
<td>80</td>
<td>&lt; 8</td>
<td>2560</td>
</tr>
<tr>
<td>16 hexons</td>
<td>640</td>
<td>2560</td>
<td>&lt; 8</td>
<td>2560</td>
</tr>
<tr>
<td>16 pentons</td>
<td>&lt; 8</td>
<td>320</td>
<td>1280</td>
<td>&lt; 8</td>
</tr>
<tr>
<td>3-16 virions</td>
<td>&lt; 8</td>
<td>160</td>
<td>1280</td>
<td>1280</td>
</tr>
</tbody>
</table>

Ratio of homologous/heterologous neutralization titres

Type 4 16/64 Type 16 2/4

An analysis of cross-reactions in neutralization tests between prototype strains (see Stevens et al. 1967) reveals some rather close cross-reactions between pairs of serotypes belonging to the same subgroup. However, an interesting reciprocal cross-reaction between types 4 (Rosen’s subgroup III) and 16 (Rosen’s subgroup I) has also been demonstrated. This cross-reaction was studied by use of the two kinds of HI tests mentioned above (Table 7). It was found that antiserum against type 4 hexons inhibited mainly homotypic virions. In contrast, antiserum to type 16 hexons inhibited both homotypic and to a considerable extent also type 4 virions. This one-way crossing parallels the findings in comparative neutralization tests. The ratio of homotypic to heterotypic neutralizing antibody titre is much lower for antisera to type 16 hexons (2 to 4) than for antisera to type 4 hexons (16 to 64). The fact that antiserum to type 16 pentons did not inhibit type 4 virions demonstrates that the structural similarity between type 4 and 16 must reside in their hexons. The reason for the one-way dominance in the reciprocal crossing must be that the antigenic specificity, which is shared between hexons of types 4 and 16, is available to a greater extent at the surface of
Diversity of adenovirus capsid components

Type 4 virions. The occurrence of additional antigenic components of a type-specific nature has been demonstrated in cross-absorption experiments (Norrby & Wadell, 1969).

The character of intermediate strains has been studied in the author's laboratory with types 9-15, described by Cramblett et al. (1960), and type 3-16 (the San Carlos agent), serologically characterized by Hatch & Siem (1966). The working hypothesis that the intermediate strains should have a capsid representing a mosaic of hexons and fibres (or pentons) similar to those of the serotypes with which they can react in neutralization and HI tests, respectively, (cf. Norrby, 1968a), has been proved to be correct (Norrby, in preparation). In Table 7 an antiserum to type 3-16 virions has been included as a biological control. This serum shows a high HI titre with the two adenovirus type 16 HAs, but no reaction with type 4 virions. This implies that hexons of type 3-16 differ from those of type 4 and consequently also from those of type 16. This difference is further emphasized by the results of testing antisera to type 3, 3-16 and 16 hexons and fibres for HI antibodies against virions and soluble HAs (Table 8). Antisera against type 3 or 3-16 hexons both inhibited virion agglutination of types 3 and 3-16, but not 16. In contrast, antisera to fibres of types 3-16 and 16 reacted similarly, i.e. they inhibited all antigens of these two types, whereas antiserum to type 3 fibres only contained antibodies reacting with homotypic antigens. These results indicate the occurrence of close immunological similarities between type 3 and 3-16 hexons and between type 3-16 and 16 fibres.

Table 8. HI titres of antisera against hexons and fibres of types 3, 3-16 and 16 in tests with soluble HA and virions of all three types

<table>
<thead>
<tr>
<th>Sera against adenovirus type</th>
<th>HI titres in tests with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type 3</td>
</tr>
<tr>
<td></td>
<td>Soluble HA</td>
</tr>
<tr>
<td>3 hexons</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>3 fibres</td>
<td>640</td>
</tr>
<tr>
<td>3-16 hexons</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>3-16 fibres</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>16 hexons</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>16 fibres</td>
<td>&lt; 4</td>
</tr>
</tbody>
</table>

Some aspects of criteria to be applied for typing of adenoviruses

The importance of the problem concerning criteria to be adopted for typing of adenoviruses is emphasized by the above-mentioned demonstrations of the occurrence of a mosaic of multi-related structural components in some adenovirus capsids. Since there are two different type-specific components, it seems logical to let one of them form the basis for a separation into different serotypes and the other one for a further separation into different subtypes. The primary question, then, is which one of the type-specific components—hexons or fibres—should be given priority? In practice this means a choice between the use of the neutralization test (or when possible the virion-HI tests) or of the HI tests with soluble components. Although the latter test is advantageous from the point of view of convenience, the neutralization test should preferably be chosen. One reason for this is the fact that this test gives a more effective
separation into serotypes and that therefore fewer subtypes would be encountered. Other, less precise arguments for this choice are (a) that antihexon antibodies are presumably of relatively greater importance from the point of view of protection \textit{in vivo} and (b) that hexons are presumably phylogenetically ‘older’ than fibres. Taking this cue the following proposals are made.

(1) The primary separation of adenoviruses into serotypes should be based on an individualistic behaviour in the neutralization test. Since a number of low-grade crossings occur in this test ‘individualistic’ might arbitrarily be defined to mean an eightfold or greater reactivity between homologous antiserum and virus than between the same serum and heterologous serotypes. Special consideration has to be given to cross-reactions, which only one way is less than 8. Possibly one could indicate such a relationship by adding the relevant serotype within parenthesis. Thus serotype 16 could be denoted 16(4), whereas the designation of type 4 would not be affected. It should be pointed out that neutralization tests should be performed with relatively ‘slow’ systems, since these appear to measure exclusively anti-hexon antibody.

(2) A separation into subtypes (strains) should be made on the basis of an individualistic behaviour (more than eightfold homologous preference of antiserum) in the HI test employing \textit{soluble} HA as antigen. By this criterion serotype 29 (Rosen et al. 1962; Stevens et al. 1967) should be regarded as a subtype of type 15.

The situation in which the fibre specificity of one serotype is identical with or closely related to that of another serotype must be treated separately. Finally, the so-called intermediate strains represent a special case. These could be presented as before in a hyphenated way, but the serotype with which a cross-reaction occurs in the neutralization test should be preferably given first. Thus, type 9-15 should be called type 15-9, whereas type 3-16 would retain its designation.

It should be pointed out that the designation of adenovirus strains with different immunological specificities poses certain problems. Possibly some provisional system could be adopted. However, it is important that this system allows future modifications. One can envisage that one day it will be possible to designate different strains by use of an index system, which describes the combination of different antigenic specificities carried by various structural components, hexons, penton bases, fibres and possibly others. Future endeavours will presumably also make it possible to specify the basis for those variations, concerning for example hexons, by reference to their amino acid sequences. Possibly the molecular nature of these variations might resemble those found recently in studies of polypeptides of y-globulins.

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Diversity of adenovirus capsid components

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