Identification of Soluble Components of Adenovirus Type 11

By E. NORRBY

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

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SUMMARY

Five or possibly six different soluble adenovirus type 11 components have been identified. Zonal centrifugation separated, listed in order of decreasing sedimentation rates: (1) a complete haemagglutinin (HA), (2) an incomplete HA plus group-specific complement-fixation (CF) antigen, and (3) components absorbing haemagglutination inhibition (HI) and haemagglutination enhancement (HE) antibody. The complete HA carried toxin activity. The incomplete HA, which also exhibited toxin activity, could be separated from group-specific CF antigen by anion-exchange chromatography. The incomplete HA exhibited haemagglutination activity only in the presence of antibody which presumably interacts with vertex capsomere antigen. Antisera against members of all of Rosen's subgroups contained HE antibody. The major portion of antigen capable of absorbing HE antibody was associated with incomplete HA, but a part of it appeared in a separate fraction. The incomplete HA also exhibited some capacity to absorb HI antibody.

Treatment with trypsin completely destroyed HE antibody-absorbing antigen and eliminated all toxin activity. Repeated erythrocyte absorptions removed all activities except group-specific CF antigen and some HE antibody-absorbing antigen.

By comparison with data obtained in parallel studies of adenovirus types 3, 4 and 5 it is suggested that the different type 11 components are of the following nature:

The incomplete HA might represent isolated penton components—vertex capsomeres plus projections—and the complete HA symmetrical aggregates of 12 such components. The group-specific CF antigen most likely is carried by non-vertex capsomeres (hexon components). HE antibody-absorbing structures not related to incomplete HA appeared heterogeneous and probably included both intact and fragments of free vertex capsomeres. Slowly sedimenting HI antibody-absorbing components presumably represent fibre components, i.e. isolated vertex projections.

INTRODUCTION

Adenovirus type 11 agglutinates monkey erythrocytes and is therefore included in Rosen's subgroup I of adenoviruses (Rosen, 1960). However, the members of this subgroup show certain differences in haemagglutinating characteristics (cf. Norrby, 1967). Rosen (1960) noticed that some of them, e.g. serotypes 11 and 16, agglutinated monkey erythrocytes equally well at 4°, 20° and 37°. In contrast, other serotypes, e.g. types 3 and 7, agglutinated the cells only at temperatures above 20°. It was later found (Simon, 1962) that the haemagglutinin of these latter serotypes was eluted from red cells at 4°. Other differences between members of Rosen's subgroup I concern their
capacity to agglutinate cells derived from different monkey species (Rosen, 1960; Simon, 1962; Stöhr & Wigand, 1965). Recent studies of adenovirus type 3 have demonstrated the occurrence of five different soluble components (Norrby, 1966a, b; Norrby & Skaaret, 1967). The present paper describes results from attempts to identify the corresponding components in adenovirus type 11 material.

When applicable, the terminology for adenovirus components proposed by Ginsberg et al. (1966) will be used. However, for reasons given in a recent review (Norrby, 1967) the terms will be used to specify soluble components characterized by their morphological and physico-chemical rather than their immunological properties.

**METHODS**

**Virus and cell culture**

The prototype strain SLOBITSKI of adenovirus type 11 was used. Before arrival in this laboratory it had been passed about 10 times in HeLa cells. Stock materials were prepared by growing the virus either in a human bone-marrow cell line, denoted ‘Masa cells’ (Kjellén, 1961), or in a human embryonic lung cell line, Lu 106 cells. Parker’s medium 199 plus 2 % calf serum was used for maintenance of Masa cells and Earle’s medium containing 0·5 % lactalbumin hydrolysate and 3 % calf serum for Lu 106 cells. All media contained 100 IU penicillin, 50 μg. streptomycin and 50 μg. Kanamycin per ml.

Virus material was harvested at an advanced stage of cell degeneration. Medium and cells were concentrated 10 to 20 times by forced dialysis using either polyethylene glycol (Carbowax 6000, Union Carbide Chemicals Co., U.S.A.) or simply negative pressure. The concentrate was frozen and thawed 3 to 5 times, after which cell debris was eliminated by low-speed centrifugation. Insoluble virus components, i.e. complete particles and empty capsids, were eliminated by subjecting the concentrates to three consecutive cycles of high-speed centrifugation at 20,000 rev./min., (rotor 40, Spinco Division, Beckman Instruments Inc., California, U.S.A.). This treatment reduced infectivity titres by a factor of 10^5 or more.

**Preparation of hyperimmune sera**

Tenfold concentrates of whole virus material of prototype strains of adenovirus types 2, 3, 4, 5, 9 and 11 were prepared. All serotypes were grown in the cell lines mentioned above except some adenovirus type 5 materials, which were prepared in primary rabbit-kidney cell cultures. A primary immunization of the rabbits was given with 4 ml. of the concentrated virus material mixed with Freund’s complete adjuvant. Four to five weeks later the animals were given an intravenous booster of 1 to 2 ml. and were then exsanguinated after another week.

**Tests for biological activities**

* Determination of complete haemagglutinin (HA). Serial twofold dilutions of material in 0·4 ml. were set up. To each dilution was added 0·2 ml. of a 0·5 % suspension of green monkey (Cercopithecus aethiops) erythrocytes. The cells were allowed to settle at room temperature. The last tube exhibiting a bottom pattern of complete or clearcut partial agglutination was considered to contain one haemagglutinating unit (HAU).

* Determination of incomplete HA. The method was the same as for complete HA with
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the addition of heterotypic adenovirus antiserum to the diluent, and incubation for 1 hr at room temperature before addition of erythrocytes. In most experiments an anti-adenovirus type 3 serum was used. In order to determine the dilution to be used in the tests, the serum was tested in chess-board titrations against preparations of incomplete HA (erythrocyte absorbed material; see below under Results). Serial twofold dilutions were used and the volume of each reagent was 0.2 ml. Erythrocytes were added after incubation for 1 hr at room temperature. The highest dilution of serum causing maximum haemagglutinin enhancement (HE) was considered to contain one HE unit (HEU). Two to four HEU of serum were incorporated per dilution for quantitative estimation of incomplete HA.

Haemagglutination inhibition antibody consumption (HIC) tests. The haemagglutination inhibition (HI) antibody activity of sera to be used in this type of test was first determined. Serial twofold dilutions of serum in 0.2 ml. were mixed with 0.2 ml. HA to give 4 HAU per serum dilution. Erythrocytes were added after incubation for 1 hr at room temperature. The highest dilution of serum causing a complete inhibition of the HA activity was considered to contain one HI unit.

In the HIC test three different reagents besides the red cells were included. Serial twofold dilutions of test material in a volume of 0.2 ml. were carefully mixed with 0.1 ml. containing two HI units of an anti-adenovirus type 11 serum. After incubation for 1 hr at room temperature an additional volume of 0.1 ml. 'whole' virus material containing two units of complete HA was added per dilution. Erythrocytes were admixed after incubation for another hour at room temperature. The last tube exhibiting a bottom pattern of complete or clear-cut partial agglutination, i.e. the one in which the major fraction of HI antibodies were still absorbed by the diluted test material, was considered to contain one HIC test unit (HICU).

Haemagglutination enhancement antibody consumption (HEC) test. The general principle and the method were the same for the HEC as for the HIC test (cf. Norrby & Skaaret, 1967). However, the participating reagents were different. The pre-testing of reagents comprised a chess-board titration, performed as described above, of incomplete adenovirus type 3 HA (erythrocyte absorbed material; Norrby, 1966b) against an anti-adenovirus type 11 serum. This test permitted a determination of both concentration of incomplete HA and HE antibody titre. In the subsequent HEC test each serial twofold dilution of material to be tested was first allowed to interact for 1 hr at room temperature with an anti-adenovirus type 11 serum containing 2 HE serum units (referring to incomplete adenovirus type 3 HA). Thereafter two units of incomplete adenovirus type 3 HA were added per dilution and the mixtures incubated for another hour at room temperature. Finally erythrocytes were added and the tests incubated at 37°. The last tube exhibiting a completely negative bottom pattern, i.e. the one in which the major fraction of HE antibodies could still be absorbed by the material tested, was considered to contain one HEC test unit (HECU).

Determination of group-specific complement-fixing (CF) antigen activity. The drop technique of Fulton & Dumbell (1949) as modified by Svedmyr, Enders & Holloway (1952) was used. Two units of an anti-adenovirus type 5 serum and two units of complement were applied per antigen dilution. In order to avoid cross-reactions with non-viral material the rabbit hyperimmune serum used was prepared against material obtained from primary rabbit kidney cultures maintained on serum from the animal to be immunized.
Determination of toxin (cell-detachment) activity. The technique previously described (Norrby, 1966b) for determination of toxin activity of adenovirus type 3 material was used. Serial twofold dilutions were made in complete tissue culture medium of material previously dialysed against the same kind of medium. One ml. of each dilution was added to two Lu 106 tissue-culture tubes from which the medium had been decanted. The tubes were cautiously rocked once every hour during the first 4 hr of incubation and then once again 1 hr before the reading taken 18 to 20 hr later. Tubes in which more than 50% of the cell sheet had been detached from the glass were counted as positive. End-point titres were calculated according to Reed & Muench (1935) and expressed in arbitrary units.

Technique of zonal centrifugation

The techniques of preparation, centrifugation and fractionation of linear 5 to 20% sucrose gradients were described by Norrby (1966a, b).

Fractionation by anion exchange chromatography

DEAE-Sephadex A 25 (Pharmacia Fine Chemicals, Uppsala, Sweden) was used. The gel was allowed to swell in an 0-04 M-tris + HCl buffer at pH 8-4 and then washed carefully in the same buffer. Material (4 to 8 ml.) to be fractionated was dialysed against the same buffer and then layered on to the gel, which was packed in columns measuring 1.5 x 40 cm. The temperature of fractionation was maintained at approximately 17°. By use of an electrical pump the flow rate was maintained at approximately 5 to 10 ml./cm.²/hr. Elution was obtained by introduction of a linear 0 to 0.5 M-NaCl gradient in the above-mentioned tris buffer. Equal fractions of 3.0 to 3.5 ml. were collected automatically by use of a drop counter.

RESULTS

Isolation of complete HA by zonal centrifugation

Zonal centrifugation was found to be a useful technique for purification of complete adenovirus type 3 HA (Norrby, 1966a) and was therefore tried also in attempts to purify the complete adenovirus type 11 HA. However under conditions of centrifugation identical with those used for type 3, all complete adenovirus type 11 HA was recovered in the sediment. In order to identify clearly the relatively rapid sedimenting component carrying the complete HA activity of type 11 preparations a centrifugation time of 7 hr at 25,000 rev./min., in a Spinco SW 25 rotor, was chosen (Fig. 1).

Complete HA was recovered in the high-density region of the gradient, whereas incomplete HA and group-specific CF (hexon) antigen remained close to the meniscus. Incomplete HA was demonstrable in the presence not only of an anti-adenovirus type 3 serum, as shown in the figure, but also of other hyperimmune sera tested i.e. against types 2, 4, 5 and 9. The major part of toxin activity was recovered in the low-density region of the gradient, but in addition a considerable part of this activity was present in fractions containing complete HA.
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Separation of hexon components and incomplete HA from more slowly sedimenting components by zonal centrifugation

Virus components remaining in the upper half of a gradient after a centrifugation of the kind shown in Fig. 1 were dialysed against phosphate-buffered physiological saline (0·066 M, pH 7·2) concentrated by forced dialysis and subjected to one more zonal centrifugation. In order to achieve an additional separation of soluble components the conditions of centrifugation were changed to 42 hr at rotor 24,000 rev./min., in a Spinco SW 25 rotor. Hexon components and incomplete HA were recovered in the high-density region of the gradient (Fig. 2). The major fraction of all HEC test positive and a smaller part of all HIC test positive material were also present in the corresponding fractions. Remaining HIC and HEC test positive components were recovered in the low-density part of the gradient as a second peak of activities.

Separation of soluble components by anion exchange chromatography

Anion exchange chromatography on DEAE-Sephadex A 25 was successfully applied for separation of hexon antigen, incomplete and complete HA of adenovirus type 3 (Norrby, 1966b). The same technique was found to be an effective tool for separation
of the corresponding three types of soluble components in adenovirus type II preparations (Fig. 3). However, the elution characteristics of soluble components of the two serotypes were markedly different. The incomplete HA of type II appeared as a separate peak of activity just before the albumin peak. At much higher concentrations of NaCl, about 0.4 M, the complete HA and finally hexon antigen were eluted as two partly separated peaks of activity. Two peaks of toxin activity were demonstrable. Their position corresponded to that of the peaks of incomplete HA and complete HA, respectively. The distribution of HIC and HEC test positive material was also determined in fractions not containing complete HA. Both these activities were eluted

Fig. 2. Distribution of different biological activities after zonal centrifugation of components recovered from the upper half of a gradient of the kind illustrated in Fig. 1. The centrifugation was performed at 24,000 rev./min., in a Spinco SW 25 rotor for 42 hr. The following activities were recorded: □—□, incomplete HA determined in the presence of an anti-adenovirus type 3 serum; ●—●, group-specific CF antigen determined with an antiserum against adenovirus type 5; ○—○, HEC, and ▲—▲ HIC, test activities.

mainly together with incomplete HA, but in addition low-titre HIC test positive material was eluted just before and some HEC test positive material immediately after this peak of activity. In all experiments there was a complete recovery of hexon components, but in some losses of incomplete and complete HA were encountered.

The effect of repeated erythrocyte absorptions on the concentrations of different soluble components

Concentrations of soluble components were subjected to repeated absorption with packed erythrocytes for 1 hr at room temperature. When seven consecutive absorptions were made with cells in a final concentration of 10% (Table 1) complete HA was rapidly eliminated, whereas a more gradual reduction in titres of incomplete HA, toxin activity and HIC test positive material occurred. The titres of HEC test
Soluble components of adenovirus type 11 positive material were markedly reduced by the first five absorptions, but the low titre remaining thereafter was not affected by further treatments. The absorptions did not cause any change in titres of group-specific CF antigen.

Fig. 3. Distribution of biological activities after anion exchange chromatography on DEAE-Sephadex A 25 of soluble adenovirus type 11 components. The components were eluted by a linear 0 to 0.5 M-NaCl gradient in 0.04 M tris+HCl buffer, pH 8.4. The following activities were recorded: ■ — ■, complete HA; ▲ — ▲, incomplete HA demonstrated in the presence of an anti-adenovirus type 3 serum; △ — △, toxin activity; ○ — ○, group-specific CF activity determined in the presence of an anti-adenovirus type 5 serum; ○ — ○, HEC, and ▲ — ▲ HIC test activities (tested only in fractions not containing complete HA).

The effect of trypsin treatment on the activity of some different soluble components

Purified preparations containing a mixture of incomplete HA plus hexon antigen and slowly sedimenting HEC plus HIC test positive material were prepared by zonal centrifugations performed under the conditions shown in Fig. 2. These preparations were treated with trypsin (2 × crystallized; Koch–Light Laboratories, Colnbrook, Buckinghamshire, England) in a final concentration of 0.4% for 3 hr at 37° in a water bath (Table 2). The enzyme activity was interrupted by addition of equivalent amounts of soy-bean trypsin inhibitor (5 × crystallized; Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.). After treatment of a mixture of incomplete HA and hexon antigen, the activity of the former was markedly reduced, whereas the CF titre of the latter remained unchanged. The treatment also eliminated all HEC test activity, but no reduction in HIC test activity was recorded. Similarly slowly sedimenting HIC test positive material remained unaffected by the treatment, whereas slowly sedimenting
components carrying HEC test positive activity were destroyed. The effect of trypsin treatment was also analysed by the zonal centrifugation technique. A preparation of soluble components from which the complete HA had been removed was subjected to

Table 1. *The effect of repeated erythrocyte absorptions on different biological activities of soluble components of adenovirus type II*

<table>
<thead>
<tr>
<th>No. of absorptions*</th>
<th>Complete HA (units/0.4 ml.)</th>
<th>Incomplete HA (units/0.4 ml.)†</th>
<th>Toxin (arbitrary units)</th>
<th>HICU/0.4 ml.</th>
<th>HECU/0.4 ml.</th>
<th>Group-specific CFU/0.02 ml.‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10240</td>
<td>N.M.</td>
<td>64</td>
<td>N.M.</td>
<td>N.M.</td>
<td>256</td>
</tr>
<tr>
<td>1</td>
<td>160</td>
<td>1280</td>
<td>64</td>
<td>N.M.</td>
<td>N.M.</td>
<td>256</td>
</tr>
<tr>
<td>2</td>
<td>&lt;10</td>
<td>640</td>
<td>45</td>
<td>8</td>
<td>64</td>
<td>256</td>
</tr>
<tr>
<td>3</td>
<td>N.T.</td>
<td>160</td>
<td>11</td>
<td>4</td>
<td>32</td>
<td>256</td>
</tr>
<tr>
<td>4</td>
<td>N.T.</td>
<td>20</td>
<td>&lt;6</td>
<td>&lt;2</td>
<td>16</td>
<td>256</td>
</tr>
<tr>
<td>5</td>
<td>N.T.</td>
<td>&lt;10</td>
<td>N.T.</td>
<td>N.T.</td>
<td>8</td>
<td>256</td>
</tr>
<tr>
<td>7</td>
<td>N.T.</td>
<td>N.T.</td>
<td>N.T.</td>
<td>N.T.</td>
<td>8</td>
<td>256</td>
</tr>
</tbody>
</table>

* For each individual absorption 1/10 volume packed erythrocytes was added. After incubation for 1 hr at room temperature the cells were removed by low-speed centrifugation.
† Demonstrated in the presence of an anti-adenovirus type 3 serum.
‡ Quantified in tests including an anti-adenovirus type 5 serum.
N.M. Not measurable due to the presence of excess complete HA.
N.T. Not tested.

Table 2. *The effect of trypsin treatment on preparations of incomplete HA plus group-specific CF antigen and slowly sedimenting HIC plus HEC test positive material*

<table>
<thead>
<tr>
<th>Biological activities</th>
<th>Incomplete HA (units/0.4 ml.)†</th>
<th>HECU/0.4 ml.</th>
<th>HICU/0.4 ml.</th>
<th>Group-specific CFU/0.02 ml.§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of preparation*</td>
<td>Trypsin treatment †</td>
<td>640</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Incomplete HA plus</td>
<td>0</td>
<td>&lt;2</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>group-specific CF antigen</td>
<td>+</td>
<td>20</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Slowly sedimenting</td>
<td>0</td>
<td>&lt;8</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
<tr>
<td>HIC plus HEC test</td>
<td>0</td>
<td>&lt;2</td>
<td>8</td>
<td>N.T.</td>
</tr>
<tr>
<td>positive material</td>
<td>0</td>
<td>N.T.</td>
<td>N.T.</td>
<td>N.T.</td>
</tr>
</tbody>
</table>

* Components isolated by zonal centrifugation were used.
† 1/10 volume of 4 % trypsin dissolved in physiological saline containing 0.066 M-phosphate buffer (pH 7.2) was added to samples denoted +, whereas controls received the buffered saline only. After incubation for 3 hr in a 37° water bath another 1/10 volume containing equivalent amounts soy-bean trypsin inhibitor was added.
‡ Demonstrated in the presence of an anti-adenovirus type 3 serum.
§ Quantified in tests including an anti-adenovirus type 5 serum.
N.T. Not tested.

trypsin treatment under the conditions described above. The product was then centrifuged in a sucrose gradient under conditions identical with those recorded in Fig. 2. Peaks of hexon components and of slowly sedimenting HIC test positive material were recovered. Their position was similar to those of the corresponding components present in untreated material, which was centrifuged in parallel.
DISCUSSION

Five, or possibly six, different soluble components of adenovirus type 11 were distinguished in the present study. Further ultrastructural and immunological analyses are needed to clarify the relationship between these soluble products and structural components participating in the formation of virions. However, results obtained in parallel studies of adenovirus types 3 (Norrby, 1966a, b; Norrby & Skaaret, 1967) and 4 (Norrby & Wadell, 1967; Wadell, Norrby & Schönning, 1967) provide a basis for speculation on their possible nature.

The complete HA of type 11 sedimented relatively rapidly like the complete HAs of type 3 (Norrby, 1966a) and type 4 (Norrby & Wadell, 1967), but differently from the complete HAs of types 5 (Norrby & Wadell, 1967), 1, 2 and 6 (Wadell et al. to be published). This suggests that in principle the composition of the former three complete HAs might be the same, i.e. dodecahedral aggregations of 12 penton components, which was demonstrated for types 3 and 4 (Norrby, 1966a; Wadell et al. 1967). Electron microscopic examinations of purified complete HA of adenotype 11 have verified the correctness of this interpretation (Norrby, 1968). As shown by the results of zonal centrifugation experiments the complete HA of adenovirus type 11, like the corresponding component of type 3 (Norrby, 1966b), carries toxin activity. This is to be expected, since both HAs are thought to be symmetrical aggregates of 12 penton components, which are the basic units carrying toxin activity (Valentine & Pereira, 1965, Norrby, 1966b).

The occurrence of an incomplete adenovirus type 11 HA as a separate component was clearly demonstrated in anion chromatography experiments. It sedimented together with hexon components, but differed from them by being sensitive to trypsin. The incomplete HA could be converted into a haemagglutinating aggregate by antibody present in sera against members of Rosen's subgroups I, II and III. This suggests that the antigen responsible for interaction with HE antibody, which Norrby has suggested is the β-antigen of vertex capsomeres (Norrby, 1966b; Norrby & Wadell, 1967; Norrby, 1967), is group-specific. The major fraction of the total HEC and a small part of the total HIC test activities were found in zonal centrifugation, anion exchange chromatography and erythrocyte absorption experiments to form a part of incomplete HA. The same experiments also demonstrated that the major part of the total toxin activity was carried by incomplete HA. All these characteristics are similar to those exhibited by the incomplete adenovirus type 3 HA (Norrby, 1966b; Norrby & Skaaret, 1967). Norrby (1966b) has suggested that the latter component represents penton components, which is supported by recent findings in electron microscopy studies (Norrby, to be published). A corresponding identification between incomplete adenovirus type 11 HA and penton components therefore appears likely.

There are also some differences between incomplete HAs of adenovirus types 3 and 11. They exhibit somewhat different patterns of elution from anion exchangers. The incomplete HA of type 3 could be obtained by thermal degradation of the complete HA of this type (Norrby, 1966b). Attempts to bring about the same degradation of complete adenovirus type 11 HA failed due to the relatively greater thermostability of this component. Another difference concerns the possibility of removing the incomplete HAs by erythrocyte absorptions. Both incomplete HAs were removed more slowly than their corresponding complete HA by this treatment. However, whereas
the incomplete HA of type 3 could be completely eliminated only in the presence of heterotypic antibody (Norrby, 1966b), no similar addition of antiserum was needed for a complete removal of the incomplete HA of type II.

The characteristics of components carrying the group-specific CF antigen of adenovirus types 3 and II in most regards appeared similar. Thus they both sedimented together with the incomplete HA, were not removed by erythrocyte absorptions and were resistant to treatment with trypsin. However, it should be noted that they behaved markedly differently in anion exchange chromatography experiments. The group-specific CF antigen of type II was associated with components which eluted at much higher NaCl concentrations than those of type 3. This emphasizes the individuality of the presumed non-vertex capsomeres—hexon components—of the two serotypes (Norrby, 1966a; Norrby, 1967).

The major part of all HEC test positive components formed a part of the incomplete HA. However, there was also evidence for the occurrence of HEC test positive material of a different nature. A small fraction of HEC test positive material remained, even after repeated erythrocyte absorptions in excess of those sufficient to remove all incomplete HA and toxin activity. Furthermore some HEC test positive material eluted somewhat after the peak of incomplete HA and toxin activity in anion exchange chromatography experiments. Additional characterization of this isolated HEC test positive material was difficult due to the fact that it was present only in small quantities. Norrby & Skaaret (1967) suggested that the corresponding components occurring in adenovirus type 3 preparations represented isolated vertex capsomeres. These were found to sediment slightly more slowly than hexon components. Presumably some of the isolated HEC test positive adenovirus type II material also represents free vertex capsomeres. However, a considerable fraction of this activity was recovered in fractions containing slowly sedimenting HIC test positive material. A similar kind of small particle HEC test material has hitherto not been encountered in studies in this laboratory of any other adenovirus serotype. It is difficult to ascertain whether the slowly sedimenting HEC and HIC test positive activities are carried by one and the same component. Against such a relationship is the finding that trypsin treatment removed slowly as well as rapidly sedimenting HEC test positive activity, although no effect was recorded on the behaviour of slowly sedimenting HIC test positive components in sucrose gradients. Possibly, therefore, the slowly sedimenting HEC test positive material represents fragments of vertex capsomeres. Further studies are needed to elucidate the nature of components of this type.

Finally, HIC test positive activity resides to a minor extent in incomplete HA. The rest of this activity was carried by more slowly sedimenting components. After treatment with trypsin all HIC test positive activity remained, but was present exclusively in the latter form. It appears likely that this adenovirus type II component, like the corresponding type 3 component (Norrby & Skaaret, 1967), represents fibre components, i.e. isolated vertex projections. The difference in absorbability of adenovirus type 3 and II incomplete HAs also concerned their postulated fibre components. Thus repeated erythrocyte absorptions removed the HIC test positive activity of adenovirus type II material, whereas no reduction of the corresponding activity of type 3 material was noticed.
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