Biological Characterization of Structural Components of Adenovirus type 12

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SUMMARY

Two products with a large mass and five different soluble components of adenovirus type 12 have been identified. The former were virus particles and empty capsids, which both carried group-specific complement-fixing (CF) antigen and were active as complete haemagglutinins (HAs). Their buoyant densities in CsCl were 1.330 to 1.340 and 1.295 to 1.305, respectively. The soluble components were identified as hexons, pentons, isolated fibres, dimers of fibres and isolated vertex capsomeres. Hexons were trypsin-resistant, thermostable and carried group-specific CF antigen; ultrastructurally they resembled hexons of other human adenoviruses. Pentons were active as an incomplete HA, causing agglutination in the presence of heterologous antisera against representative members of all three subgroups of adenoviruses. Furthermore, they were trypsin-sensitive and thermolabile. Isolated fibres represented another incomplete HA, exhibiting agglutinating activity only in the presence of antisera against members of subgroup III, e.g. type 2. This component was also identified as the dominating type-specific CF antigen. The length of fibres was estimated as 28 to 32 nm. by electron microscopy. The dimers of fibres represented a complete HA, the activity of which became apparent only after other material was removed. Both isolated fibres and their dimers were trypsin-resistant and thermostable. The assumed pentons and fibre dimers occurred in too low concentration to allow ultrastructural identification. Isolated vertex capsomeres were demonstrated by a haemagglutination enhancement antibody consumption test. They were completely destroyed by trypsin treatment and exhibited a moderate thermostability. Electron microscopy confirmed the presence of capsomere-like structures. These structures occasionally displayed a five-sided contour, thereby differing from hexons.

The sequence of elution of different components from an anion exchanger was: isolated vertex capsomeres, hexons + pentons, fibres and dimers of fibres. In zonal centrifugation experiments the components sedimented at decreasing rates in the following order: hexons, pentons + isolated vertex capsomeres, dimers of fibres and isolated fibres. Probably on account of the morphological characteristics of the various components they presented a different pattern of elution in exclusion chromatography. Dimers of fibres, pentons and even isolated fibres were eluted before hexons. The position of the peak activity of type 12 fibres in the elution diagram was similar to that of types 1, 2 and 5 fibres.
INTRODUCTION

The information concerning physico-chemical and biological characteristics of structural components of adenovirus type 12 is relatively meagre. Two capsid components have hitherto been identified (Huebner et al. 1964). These are the typespecific and group-specific complement-fixing (CF) antigens, which have been assumed to represent fibres (isolated vertex projections) and hexons (nonvertex capsomeres), respectively. The nature of an additional antigen which was identified in immunodiffusion tests (Berman & Rowe, 1965) has not as yet been revealed. Adenovirus type 12 has been demonstrated to cause a partial agglutination of rat erythrocytes in the presence of heterotypic antisera (Bauer & Wigand, 1963; Schmidt, King & Lennette, 1965). With regard to this activity type 12 behaves like members of Rosen’s (1960) subgroup III.

The need for more knowledge about characters of different structural components of adenovirus type 12 is obvious. Such knowledge is a necessary prerequisite for an analysis of the relationship between these components and tumour-specific antigens appearing in connection with the process of oncogenesis (Huebner et al. 1963). Advantage was taken in this study of experience gained from studies in this laboratory of soluble components of other adenovirus serotypes in particular types 3 (Norrby, 1966a, b; Norrby & Skaaret, 1967) and types 1, 2, 5 and 6 (Wadell & Norrby, 1969a; Wadell, Norrby & Skaaret, 1969).

METHODS

Virus and cells. The prototype HUm strain of adenovirus type 12, kindly provided by Dr L. Kjellén, was used. In some experiments the prototype strain of type 6 was also used. Virus stock material was prepared in a human bone marrow cell line, MAS-A cells (Kjellén, 1961). Eagle’s medium + 5 % calf serum was used for maintenance of cells. The materials were harvested when a clearcut cytopathic degeneration of the whole cell sheet had developed. The medium was discarded and the cells, which remained attached to the glass, were washed twice in physiological saline + 0.01 M-tris buffer, pH 7.3. They were then scraped off in one-fifteenth of the original volume of medium and subjected to five cycles of freezing and thawing. Cell debris was removed by low-speed centrifugation.

Preparation of hyperimmune sera. Rabbits were inoculated intramuscularly with 4 ml. of the material to be used for immunization mixed with Freund’s complete adjuvant. An intravenous booster was given 5 weeks later and the animals were exsanguinated after another week. In various experiments antisera against whole virus material of different prototype strains, against particles of adenovirus types 9 or 12 purified by isopycnic centrifugation, and against different purified soluble components of adenovirus type 3 (Norrby, 1969a) were employed.

Serological tests. The techniques for assay of complete and incomplete haemagglutinins (HAs) were described by Norrby (1966b). Type 12 material could agglutinate not only rat (Bauer & Wigand, 1963; Schmidt et al. 1965), but also human group O erythrocytes. A similar observation was recently made in studies of some members of subgroup III, types 1, 2, 4, 5 and 6 (Wadell, 1969). Since the bottom patterns obtained with rat cells could regularly be discerned more readily than these with human group O cells, rat erythrocytes were used in most tests. The cells were collected and washed on the day they were used. Since different rats have red cells of different agglutinability,
Structural components of adenovirus type 12 185
cells from individual rats were pretested with a reference preparation of HA. The
observation by Schmidt et al. (1965) that the agglutinability of rat erythrocytes for
adenovirus type 12 HA was not correlated with that for other members of Rosen’s
subgroup III, serotypes 1, 2, 4, 5 and 6, was confirmed. The agglutination patterns of
rat cells with type 12 components at 37° in many tests were not as distinct as those
normally seen in tests with the other serotypes mentioned.

The haemagglutination-enhancement antibody consumption (HEC) test (Norrby &
Skaaret, 1967) which was designed for the identification of vertex capsomeres, isolated
or combined with other components, e.g. fibres, was performed as follows. Hyperim-
mune sera against adenovirus type 12 particles were tested in a chessboard titration
against incomplete adenovirus type 3 HA (pentons), used in the form of erythrocyte-
absorbed virus material (Norrby, 1966b). This test allowed a determination of the
haemagglutination-enhancing (HE) titre of serum and the titre of incomplete adeno-
virus type 3 HA. In the final test 0.1 ml. of the hyperimmune serum against adeno-
virus type 12 particles, containing two HE units (referring to incomplete adenovirus
type 3 HA) was added to 0.2 ml. of serial twofold dilutions of materials to be studied.
After incubation for one hr at room temperature two HA units (HAU) of incomplete
adenovirus type 3 HA were added and the mixture incubated for another hr at room
temperature. Finally, 0.2 ml. of 0.5% green monkey (Cercopithecus aethiops) erythro-
cytes was added. After careful mixing the red cells were allowed to settle at 37° in an
incubator. The last tube exhibiting a clearcut negative pattern was considered to
contain one HEC unit (HECU). In tests for adenovirus type 6 HEC-test-positive
material the antiserum against type 12 particles was exchanged for a corresponding
antiserum against adenovirus type 6 material.

Complement-fixing (CF) tests were performed by use of the drop technique as
modified by Svedmyr, Enders & Holloway (1952). Two units of an antiserum against
adenovirus type 9 particles, two units of complement and six amboceptor units were
employed per antigen dilution.

Fractionation techniques. Rapidly sedimenting and soluble virus components were
separated by use of a discontinuous CsCl gradient. Into a 30 ml. tube, fitting the
buckets of the SW25 rotor (Spinco, Beckman Instruments, California, U.S.A.), was
introduced a bottom layer of 7 ml. CsCl (Hopkins and Williams, Chadwell Heath,
Essex, England) solution in 0.05 M-tris+HCl with a density of 1.40 g./ml. and an
intermediate layer of 13 ml. CsCl solution, density 1.20. After addition of 10 ml.
top layer of virus material the tubes were centrifuged at 25,000 rev./min. (SW 25,
Spinco) for 90 min. Fifteen fractions of equal volume were then collected via the
bottom of the tube. The techniques for further fractionation of rapidly sedimenting
components by equilibrium centrifugation in CsCl gradients and of soluble compo-
nents by zonal centrifugation, anion exchange chromatography on DEAE-Sephadex
A 25 and exclusion chromatography on Sephadex G200 were described by Norrby
(1966a, b), Norrby & Skaaret (1967) and Norrby, Grönberg & Magnusson (1964).

Electron microscopy. Purified virus products were dialysed on a membrane filter
(VM 50 nm., Millipore Filter Corporation, Bedford, Mass., U.S.A.) against a 1%
ammonium acetate solution. One drop of the dialysed material and one drop of a 4%
solution of sodium tungstosilicate were mixed on carbon-coated grids. Excess fluid
was removed by a filter paper and the preparations were left to dry in the air before
examination in a Philips EM 200 electron microscope at primary magnifications of
RESULTS

Characterization of rapidly sedimenting components

Soluble and rapidly sedimenting components were separated by centrifugation of concentrated virus material in a discontinuous CsCl gradient as described under Methods. The fractions collected were tested for presence of incomplete HA in the presence of an antiadenovirus type 2 serum and for complete HA. All complete HA was recovered in the interphase between the CsCl layers with densities of 1.40 g./ml. and 1.20 g./ml., in which region two sharp bands were also clearly visible. Soluble components remaining on top of the low density layer exhibited only incomplete HA activity.

![Fig. 1. Distribution of HA (□—□), and group-specific CF antigen activity (●—●), of non-soluble type 12 components after equilibrium centrifugation in a CsCl gradient at 30,000 rev./min. (SW 39, Spinco) for 20 hr.](image)

Fractions containing the non-soluble complete HA were pooled and the density adjusted to 1.32 g./ml. by addition of a saturated CsCl solution. This mixture was then subjected to an equilibrium centrifugation at 30,000 rev./min. (SW 39, Spinco) for 20 hr. The content of complete and incomplete HA and group-specific CF antigen of individual fractions collected was determined (Fig. 1). Two sharp peaks of complete HA were distinguishable. These occurred at densities of 1.33 to 1.34 g./ml. and 1.29 to 1.30 g./ml. Group-specific CF antigen was also predominantly recovered at these densities although relatively more CF antigen than complete HA was recovered in fractions with densities of 1.31 to 1.32 g./ml. No incomplete HA was detected by tests in the presence of an antiadenovirus type 2 serum. Infectivity titrations (not included in Fig. 1) demonstrated that only the high-density particles, 1.33 to 1.34 g./ml., were infectious, as could be expected (Smith, 1965; Piña & Green, 1965).

Capacity of antisera against heterologous adenovirus serotypes to demonstrate soluble incomplete HA

Soluble components collected from centrifugation of material in a discontinuous CsCl gradient, as described above, were tested in chessboard titrations with hyperim-
mune sera against types 2, 3, 5, 9, 11 and 15. Much incomplete HA was demonstrable with the aid of antisera against the members of subgroup III, i.e. types 2 and 5, in agreement with previous findings (Schmidt et al. 1965). However, the antisera against members of subgroups I (types 3, 11) and II (types 9, 15) were also capable of indicating some incomplete HA. The latter phenomenon was further investigated by testing the HE activity of hyperimmune sera directed against hexons, pentons and fibres of type 3 (Norrby, 1969a). Only sera against pentons were active in demonstrating adenovirus type 12 incomplete HA, suggesting a participation of vertex capsomere antigen in this reaction.

Fig. 2. Separation of soluble type 12 components by anion exchange chromatography on DEAE-Sephadex A25. Different components were eluted by introduction of a linear NaCl gradient, 0-0.35 M, in 0.04 M-tris HCl buffer pH 8.4. Complete HA, \( \square - \square \); incomplete HA demonstrated in the presence of an antiadenovirus type 2, (■ - - - ■) and antiadenovirus type 3, (▲ . . ▲) serum; HEC test positive material ▲ — ▲; group-specific CF antigen (● — — ●), determined in the presence of an antiserum against particles of type 9 virus.

Separation of soluble type 12 components by anion exchange chromatography

Soluble adenovirus type 12 components were fractionated on DEAE-Sephadex A25, in 0.04 M-tris + HCl buffer, pH 8.4. All components with demonstrable biological activities were retained by the column under these conditions. They were eluted by introduction of a linear 0 to 0.35 M-NaCl gradient (Fig. 2). The group-specific CF antigen, hexons (fractions 38 to 41), was eluted before a type-specific CF antigen as described by Huebner et al. (1964). In our experiments the latter component was identified either as a CF antigen reacting with homologous antiserum against virus particles, or more conveniently in the form of an incomplete HA (fractions 53, 54) (Fig. 2). This incomplete HA was active in the presence of an antiadenovirus type 2 serum, i.e. against a member of subgroup III, but not sera against types 3 (included in Fig. 2) or 9. The latter sera, however, revealed the presence of small quantities of another incomplete HA. This component, which eluted together with hexons, could also be demonstrated with antisera against members of subgroup III, although this generally required these sera to be used at a lower dilution than that required to indicate the late eluting incomplete HA. In most experiments the activity of the early eluting incomplete HA was difficult to demonstrate. This was partly explained by small
quantities of this component present, but also because it gave a very weak agglutination
and positive fractions generally exhibited marked prozones of negative bottom patterns.
HEC tests, which indicate the presence of vertex capsomeres—isolated or combined
with fibres to pentons—indicated a relatively broad distribution of active components.
The major part of these eluted before all other components, but considerable activity
was recovered in a position in the elution diagram corresponding to that of hexons and
early eluting incomplete HA. HA tests, finally, demonstrated the occurrence of a
soluble complete HA, which eluted immediately after the late eluting incomplete HA.

![Graph](image1)

Fig. 3. Separation of soluble components of untreated and trypsin-treated type 12 materials
and of type 6 material, included as a reference. Symbols as for Fig. 2.

**Fractionation of soluble type 12 components by zonal centrifugation**

Adenovirus type 12 soluble components with different biological activities were
further characterized by fractionation by rate zonal centrifugations in linear 5 to
20% sucrose gradients at 23,000 rev./min. (SW 25, Spinco) for 42 hr. A preparation of
soluble components of type 6 was included as a reference (Fig. 3). The group-specific
Structural components of adenovirus type 12

CF antigen of type 12 was recovered close to the bottom of the tube in a position identical with that of the corresponding component of type 6. In contrast, the incomplete HAs of both serotypes, which were demonstrable in the presence of an antiserum against type 2, had barely reached the middle of the gradient. The activity of this component of type 12 was not distributed in the form of a symmetrical peak; significant amounts of components carrying this activity were also recovered in gradient fractions of somewhat higher densities. Incomplete HA demonstrable in the presence of an antiadenovirus type 3 serum and HEC-test-positive material was similarly distributed. With type 12, peaks of these two activities were found intermediate between group-specific CF antigen and slowly sedimenting incomplete HA. Small amounts of HEC-test-positive material were detectable in the first fractions collected, i.e. those nearest to the bottom of the tube. The distribution of components carrying the corresponding two activities in the type 6 reference material was somewhat different. One peak of these two activities appeared in the first two fractions collected, i.e. closer to the bottom of the tube than group-specific CF antigen, and another in a somewhat lower density region of the gradient than the latter component. The position of the more slowly sedimenting peak of these two activities of type 6 was similar to that of the peak of the corresponding type 12 activities. However, most experiments indicated that components carrying activities of the latter type sedimented slightly more slowly. In the experiment illustrated in Fig. 3 no soluble complete type 12 HA was found. In some experiments traces of this activity were recovered in gradient fractions of somewhat higher densities than those containing the peak of slowly sedimenting, subgroup-specific incomplete HA activity. Zonal centrifugations of soluble complete HA isolated by anion exchange chromatography (Fig. 2) confirmed these sedimentation characteristics. The position of this type 12 component in the gradients was similar to that of the more slowly sedimenting population of complete type 6 HA. However, no type 12 components were found, corresponding to the rapidly sedimenting complete HA of type 6. In addition, as already mentioned, the peak of HEC and incomplete HA activities associated with this peak of type 6 complete HA was absent in the type 12 material.

Distribution of soluble type 12 components after exclusion chromatography

Preparations of soluble type 12 components mixed with normal calf serum were fractionated on Sephadex G200 (Pharmacia Fine Chemicals, Uppsala, Sweden) (Fig. 4). All soluble complete HA was recovered in the void volume whereas incomplete HA demonstrable in the presence of an antiserum against type 3 exhibited a peak of activity a few fractions later. The predominating incomplete HA, i.e. the one demonstrable by an antiadenovirus type 2 serum, appeared in a position intermediate between the void volume and the peak of 7S γ-globulin. The peak of this incomplete HA was somewhat skewed towards the void volume, a pattern of distribution similar to that encountered in the zonal centrifugation experiments described above. Separate experiments were made to compare the exclusion chromatography behaviour of this component with that of the corresponding component of members of subgroup III, types 1, 2, 5 and 6. All components were prepared by rate zonal centrifugations (Wadell et al. 1969). Mixtures of components from two or three serotypes, plus normal calf serum added as a reference, were fractionated. The behaviour of the slowly sedimenting incomplete HAs of types 1, 2, 5 and 12 was similar. The peak
activities of all components were recovered in a position intermediate between the void volume and the 7 S γ-globulin peak. Experiments including slowly sedimenting type 6 incomplete HA demonstrated, as was described separately (Norrby, Wadell & Marusyk, 1969), that this component eluted somewhat later than the corresponding components of the other serotypes studied.

The group-specific CF antigen of type 12 eluted somewhat earlier than 7 S γ-globulin (Fig. 4). HEC-test-positive components, finally, showed a broad distribution ranging from the void volume to the 7 S γ-globulin peak.

The effect of trypsin and heat treatments on the activity of different soluble type 12 components

A preparation of soluble components of type 12 was dialysed against tris+HCl buffered, 0.04 M pH 7.2, physiological saline containing 10⁻³ M-CaCl₂. Trypsin (2 × crystalline, Fluka AG, Buchs SG, Switzerland) dissolved in the same buffered saline was added to a final concentration of 0.4%, and the material was incubated for 3 hr at 37°. After this incubation, soy-bean trypsin inhibitor (5 × crystalline, Nutritional Biochemicals Corporation, Cleveland, Ohio) was added in an amount equivalent to that of trypsin. Components carrying biological activities remaining after this treatment were separated by zonal centrifugation in a linear 5 to 20% sucrose gradient at 23,000 rev./min. SW 25, Spinco for 42 hr (Fig. 3). Compared with
untreated material, trypsin destroyed all HEC-test-positive material as well as the rapidly sedimenting incomplete HA demonstrable for example with an antiadenovirus type 3 serum. There were no significant differences in the position of the peaks of remaining activities, i.e. group-specific CF antigen and slowly sedimenting incomplete HA, as compared to those present in untreated material. An elimination of some of the more rapidly sedimenting components carrying the latter activity was suggested, however. Since the effect of trypsin on the soluble complete HA could not be deduced from this experiment, preparations of this component, obtained by anion exchange chromatography, were treated separately under conditions identical with those described above. The treatment did not destroy the activity of the soluble complete HA; but a slight increase in activity was encountered in some experiments.

The effect was studied of incubation at 56°C for 1 and 5 hr of different soluble components isolated by anion exchange chromatography. All early eluting incomplete HA, demonstrable in the presence of a type 3 serum, was destroyed within 1 hr HE tests in the presence of an antiadenovirus type 2 serum suggested the release of subgroup-specific, incomplete HA. The titre of HEC-test-positive material was slightly reduced after incubation for 5 hr. The activities of group-specific CF antigen, late eluting incomplete and complete HA remained unchanged even after incubation for 5 hr.

Ultrastructural characteristics of purified components

Group-specific CF antigen was prepared by anion exchange chromatography followed by exclusion chromatography. Electron microscopic examination of these purified components revealed the presence of capsomeres (Pl. 1a) similar to those previously demonstrated for other serotypes (Valentine & Pereira 1965; Norrby 1966a; Pettersson, Philipson & Högland, 1967). The appearance of type 12 capsomeres of this kind suggested a polygonal (occasionally six-sided) tubular structure. The inner and outer diameters measured 20 to 30 Å and 75 to 90 Å respectively.

HEC-test-positive material was prepared by anion exchange chromatography. The absence of demonstrable amounts of group-specific antigen was carefully controlled. Ultrastructural examination showed the presence of capsomere-like structures (Pl. 1b) although, predictably, in much lower quantities than in preparations of group-specific CF antigen. The appearance of the capsomeres in these two kinds of preparations was slightly different. The HEC-test-positive components displayed a more compact tubular appearance and in addition a five-sided contour could frequently be distinguished (see inserts Pl. 1b). Inner and outer diameters measured 25 to 35 Å and 75 to 95 Å, respectively.

Slowly sedimenting incomplete HA, prepared by zonal centrifugation, contained fibre-like structures carrying a knob (Pl. 1c) similar to those previously demonstrated for type 5 (Valentine & Pereira, 1965). The length of what was assumed to represent intact structures averaged 280 to 300 Å. Components with a similar ultrastructural appearance were found radiating from vertices of virus particles (Pl. 1d, e) purified by the centrifugation procedures presented above.

The ultrastructure of rapidly sedimenting incomplete HA and of soluble complete HA could not be clarified owing to difficulties in obtaining preparations containing purified components at sufficiently high concentration.
(a) Group-specific CF antigen; (b) HEC test positive material; (c) slowly sedimenting incomplete HA; and (d, e) virus particles.

E. NORRBY AND J. ANKERST

(Facing p. 191)
DISCUSSION

Previous studies of adenovirus type 12 (Huebner et al. 1964) showed the presence of a group-specific and type-specific soluble structural component. In our study purified preparations of these two types of components, identified as group-specific CF antigen and incomplete HA (slowly sedimenting) were found to contain capsomere-like and fibre-like structures. Comparison with data presented on types 5 (Valentine & Pereira, 1965) and 3 (Norrby, 1966a) indicate that these products represent non-vertex capsomers and vertex projections. The length of fibres, isolated or extending from virus particles, was found to be 280 to 320 Å, a value which tallies with that presented for some members of subgroup III, types 1, 2 and 5 (Valentine & Pereira, 1965; Norrby et al. 1969).

In addition to the above-mentioned two components, three more types of soluble virus products were demonstrated in the present study. These newly identified soluble components were a complete HA, a rapidly sedimenting incomplete HA and HEC-test-positive components lacking HA activity. The soluble complete HA could be demonstrated only in fractionated material. This is probably due to a competition for receptors on red cells by the relatively large amounts of incomplete HA present in crude virus material. Soluble complete HAs with general characteristics identical with those of the type 12 HA were recently described for types 1, 2, 5 and 6 (Wadell et al. 1969), types 9 and 15 (Norrby, 1968b) and 16 (Norrby & Skaaret, 1968). This type of component, present in preparations of type 6, was shown by Norrby et al. (1969) to represent dimers of fibres. A morphological confirmation of the presumed fibre dimer nature of type 12 soluble complete HA has to await the availability of purified and highly concentrated preparations of this component. The second type of soluble HA, dimers of pentons, which was identified in preparations of some members (types 1, 2, 5 and 6) of Rosen's subgroup III, could not be demonstrated in the present study (cf. Fig. 3).

The rapidly sedimenting incomplete HA of type 12, the biological and physico-chemical characteristics of which are similar to that of pentons of other serotypes (cf. Norrby, 1969b) occurred only in small quantities. For this reason it was not possible to identify this component in the electron microscope. It seemed to carry only a minor fraction of all HEC-test-positive activity, i.e. vertex capsomere antigen. In contrast hereto, studies of naturally occurring soluble components of other serotypes—e.g. 3 (Norrby & Skaaret, 1967), 9 (Norrby et al. 1967) and 11 (Norrby, 1968a)—demonstrated that almost all HEC-test-positive material appeared to be carried by pentons. Treatment of type 3 material with guanidine-HCl in adjusted concentrations caused a release of HEC-test-positive material assumed to represent isolated vertex capsomeres. These components sedimented somewhat more slowly than hexons, eluted before pentons from an anion exchanger (Norrby & Skaaret, 1967) and gave a broad band of activity in exclusion chromatography experiments (aggregation of components?) (E. Norrby, unpublished). Similar characteristics were displayed by the major part of spontaneously occurring HEC-test-positive components present in type 12 material. For these reasons it seems likely that the capsomere-like structures ultrastucturally identified in preparations of this kind of component represent isolated vertex capsomeres. It is of interest that many of these capsomeres appeared five-sided, since they would be expected to be composed of 5 (or a multiple of this figure) structure units.
The reasons for the differences in relative frequencies of occurrence of soluble components of type I2 and the corresponding components of other human adenoviruses of similar haemagglutinating characteristics, i.e. members of Rosen's subgroup III (Wadell & Norrby, 1969a; Wadell et al. 1969) is unknown. One possible explanation could be that the pentons of type I2 are relatively fragile and that they easily fall apart into vertex capsomeres and fibres. This could explain on one hand the absence of any detectable penton dimers carrying complete HA activity and the relatively small amounts of penton incomplete HA which were found, and on the other hand the relatively high concentration of free vertex capsomeres present in the materials.

The rapidly sedimenting components were found to be of two different kinds. These components banded at densities of about 1.335 and 1.300 in CsCl gradients which, as described by Smith (1965) and Piňa & Green (1965), represent the buoyant densities of virus particles and empty capsids. The ultrastructure of the former was confirmed. Predictably (Norrby et al. 1964), both these components carried complete HA and group-specific CF activity. It should be underlined that the HA activity carried by these structures is very labile. Storage at 4°C appears to lead to a rapid release of fibres (E. Norrby, unpublished).

Human adenoviruses types I2 and 18 were classified by Rosen (1960) as non-haemagglutinating and therefore were not included in any of the three subgroups proposed by him. More recent studies (Bauer & Wigand, 1963; Schmidt et al. 1965) showed that type I2 materials carry a haemagglutinating activity similar to that of subgroup III members. As described above this also concerns the capacity to agglutinate human group O erythrocytes (Wadell, 1969). Thus from the point of view of haemagglutination, i.e. fibre characteristics, type I2 should be associated with subgroup III. However most of the general biological characteristics of type I2 structural components described above and the immunological properties of type I2 hexons (Norrby & Wadell, 1969) and vertex capsomeres (Wadell & Norrby, 1969b) clearly differ from those of subgroup III members. For these reasons it would seem justified to assign type I2 (and probably therefore also types 18 and 31) to a separate subgroup. This has already been proposed on grounds of the high guanidine-cytosine content of the DNA of these serotypes (Piňa & Green, 1965) and their oncogenic characteristics (Huebner, 1967).

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