

Replication of Mammalian Parvoviruses

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Cover: Particles of minute virus of mice, approximately 250 Å in diameter
(electron micrograph courtesy of Peter Tattersall).



Antigenic Differences Among AAV Species of Different Densities

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When purified virions of adeno-associated virus (AAV) are centrifuged to equilibrium in CsCl gradients four bands are usually observed (Hoggan et al. 1966; Hoggan 1971; Johnson et al. 1971; Johnson and Hoggan 1973). The lightest band, with a density between 1.32 and 1.34 g/cm³, contains noninfectious particles which are devoid of DNA. Additional noninfectious virions which contain DNA molecules of less than genome length are found in the density range of 1.34–1.38 g/cm³. The major band of infectious AAV exhibits a density of 1.39–1.42 g/cm³. However, particles which contain a complete viral genome are found also at a density of 1.46–1.51 g/cm³. The resolution of infectious virions into two classes of particles in CsCl has been shown to occur with other parvoviruses, for example, Haden virus (Johnson and Hoggan 1973), minute virus of mice (MVM) (Clinton and Hayashi 1975, 1976), LuIII (Gautschi and Siegl 1973), and H-1 (Kongsvik et al., this volume). The polypeptide composition and the biological properties of these two species of virions are different in the cases of MVM (Clinton and Hayashi 1976) and H-1 (Kongsvik et al., this volume). In this report we have employed immune electron microscopy and infectivity assays to detect physical and biological differences between the "heavy" ($\rho=1.46\text{--}1.51\text{ g/cm}^3$) and "light" ($\rho=1.39\text{--}1.42\text{ g/cm}^3$) virions of AAV.

MATERIALS AND METHODS

GROWTH AND PURIFICATION OF VIRUS. AAV1 was grown in KB spinner culture with adenovirus type 2 (Ad2) (Johnson et al. 1971), and the virions of different densities were separated by banding three times in isopycnic CsCl gradients (Hoggan et al. 1966).

INFECTIVITY TITRATION. AAV1 was titrated in human-embryo-kidney tube cultures preinfected with Ad2 helper. Infectious titer is reported as the highest dilution of virus capable of inducing AAV1 complement-fixing antigen in 50% of the tubes (TCID₅₀) (Hoggan et al. 1966).

PREPARATION OF ANTISERA. After separation by isopycnic centrifugation, the two bands containing the complete AAV1 genome were used for the production of hyperimmune sera in guinea pigs as described previously (Hoggan et al. 1966). Hereafter, the major infectious band (1.39–1.42 g/cm³) will be designated as light (L) AAV, and serum produced against it as anti-L serum. Similarly, the minor band with higher density (1.46 g/cm³) is designated as heavy (H) AAV and its corresponding antiserum as anti-H serum.

SEROLOGY. Complement-fixation tests were carried out in microtiter plates as described previously (Hoggan et al. 1966). Immune electron microscopy (IEM) was done according to the method of Thomas and Hoggan (1974).

RESULTS

Immune Electron Microscopy of Heavy and Light AAV1 Particles

The results of experiments in which H and L AAV1 particles were reacted with anti-H and anti-L sera and examined by immune electron microscopy are demonstrated in Figure 1. When H particles of AAV were reacted with anti-H serum, medium-size to large antibody-virus aggregates were formed (Fig. 1A). When the same H antiserum was reacted with L particles, a few small aggregates could be found by searching a number of fields; however, the majority of the particles were single and naked (Fig. 1B). Conversely, when anti-L serum was reacted with H virus as shown in Figure 1C, the majority of particles were found to be free of antibody. Nonetheless, a few particles heavily coated with antibody were observed. We feel that these represent contaminating L-band particles in the H preparation. When L serum was used in a homologous reaction (anti-L serum vs. anti-L particles), large aggregates containing several thousand particles associated with 4+ antibody could be seen (Fig. 1D).

We have noticed repeatedly that antibody-virus aggregates formed with H and L particles and their homologous antisera each have their own

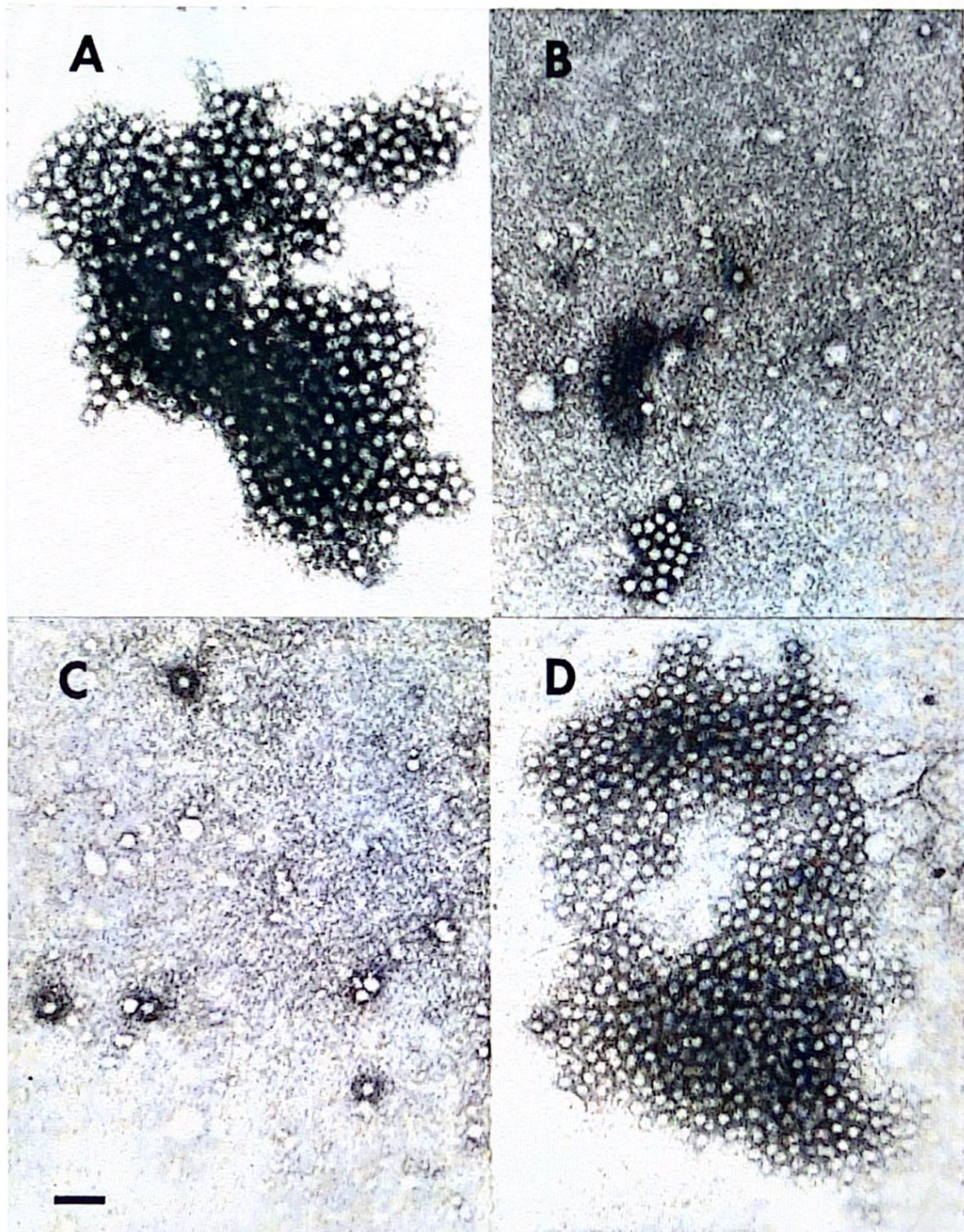


FIGURE 1

Demonstration of antigenic differences between heavy (H) and light (L) AAV1 particles using immune electron microscopy. (A) AAV H vs. anti-AAV-H serum. (B) AAV L vs. anti-AAV-H serum. (C) AAV H vs. anti-AAV-L serum. (D) AAV L vs. anti-AAV-L serum. (Bar represents 100 nm.)

characteristics and can be recognized under the electron microscope. Aggregates formed by H virus and its antiserum appear loosely clumped, with the center-to-center spacing between particles greater than the center-to-center spacing seen with L virus and its homologous serum. The higher-magnification electron micrographs shown in Figure 2 A and B demonstrate the results of mixing both H and L virus with both

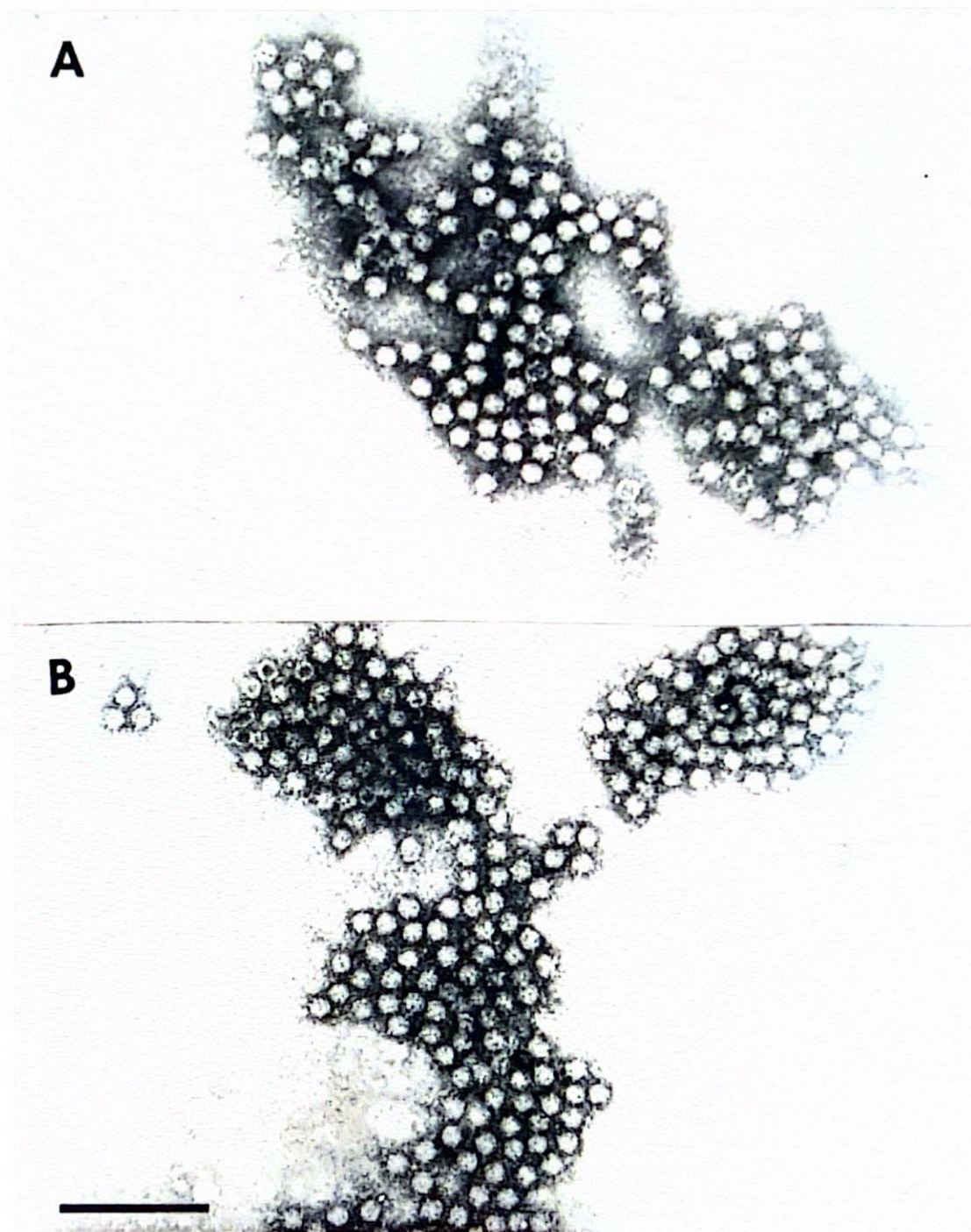


FIGURE 2

Demonstration of homogeneous heavy-particle aggregates and homogeneous light-particle aggregates in a mixed reaction. (A) Characteristic AAV-H-particle aggregate. (B) Characteristic AAV-L-particle aggregate. (Bar represents 100 nm.)

antisera. In such a combination, aggregate configurations are formed. An aggregation of loosely packed particles with the characteristics of H particles is illustrated in Figure 2A. Figure 2B shows a representative L-particle aggregate. The two aggregates shown were found on the same grid. In such a mixture, individual aggregates were always homogeneous

with respect to particle type. In addition, we rarely (if ever) saw unreacted particles, although they were fairly common in reactions involving one virus type and its homologous antiserum.

Infectivity of H and L Particles

We reported that the H band contained fewer particles than the L band and that these particles were always smaller (Hoggan 1971). In general, no grossly visible band can be seen in the H density region, although particles can be detected using the electron microscope. Occasionally, however, we find preparations in which the widths of H and L bands appear almost equal. With one such preparation used in our immune-electron-microscopy studies we have also been able to compare directly the H and L particles in terms of physical particle counts and infectivity. The results are shown in Table 1. It should be noted that even though the L band contained only 18% more particles than the H band it had over 300 times the infectivity.

DISCUSSION

Our present observations clearly suggest that there is a difference in the antigenicity of H and L particles of AAV, even though we are unable to completely separate the two populations. Whereas our earlier studies indicated that the same three polypeptides are present in both H and L virus bands, the relative amounts of the two minor polypeptides (92,000 and 80,000 daltons) compared to the major polypeptide (66,000 daltons) were less in H particles than in L particles (Johnson et al. 1971). We further reported that the average diameter of H particles was 12% less than the diameter of the L particles (Hoggan 1971) and suggested that these findings might reflect a lack of, or configurational alteration of, a protein at the capsid surface. Recent support for this theory comes from an

TABLE 1
Relative Infectivity of Heavy and Light AAV Particles

	Physical particles per ml	TCID ₅₀ per ml
Heavy band ($> 1.46 \text{ g/cm}^3$)	3.2×10^{12}	3×10^6
Light band (1.39 g/cm^3)	3.9×10^{12}	1×10^9

experiment in which we measured the ratio of nucleic acid to protein in the two bands by double-labeling the virions. We found that H particles have a higher DNA/protein ratio than L particles. In one experiment the ratio of ^3H -labeled DNA to ^{14}C -labeled protein was 0.575 for H particles and 0.424 for L particles.

How our data on AAV relate to the findings of Clinton and Hayashi (1975) on minute virus of mice is not clear. They found a proteolytic activity associated with infected cell culture fluid which converted one of the MVM polypeptides into a smaller one and at the same time converted H MVM particles into L MVM particles. Of course, if this change represented a simple loss of protein, the particles would become denser, not lighter. Clinton and Hayashi have postulated that the capsid protein may undergo a conformational change which might alter the L particles' ability to be penetrated or solvated by CsCl. Clinton and Hayashi (1975, 1976) have also reported that H MVM particles were as infectious as L MVM particles although they adsorbed very poorly to the host cells. If the cell monolayers were washed after adsorption, most of the H-particle infectivity was lost.

We have found that the H particles of AAV are much less stable in CsCl than the L particles. After two to three bandings they tend to lose all structural integrity, and the proteins migrate to the top of the gradient. In contrast, L particles have been rebanded as many as five times without appreciable loss. Whether this relative instability or the fact that they adsorb very poorly to KB cells (K. I. Berns, personal communication) accounts for the apparent lower infectivity of H particles is currently under study.

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