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Physical Assay and Growth Cycle Studies of a Defective Adeno-Satellite Virus

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Electron microscopic particle counting of the defective adeno-satellite virus (ASV), by use of pseudoreplication and negative staining with phosphotungstic acid, was shown to be a reproducible quantitative assay procedure. Particles of satellite type 4 that were counted in fluids from infected cultures had the same morphology as particles that banded at a buoyant density of 1.43 g/cc in cesium chloride. Other satellite virus serotypes examined in the same manner had a buoyant density of 1.37 to 1.38 g/cc. A comparison of satellite titers obtained by complement fixation and by particle counting demonstrated that an increase in satellite particles resulted in a corresponding increase in CF titers; however, electron microscopy was at least 10 times more sensitive than complement fixation for detecting satellite virus. Growth cycle studies of satellite virus in cells co-infected with adenovirus, as assayed by particle counting, indicated that the kinetics of satellite virus production closely followed the kinetics of its helper adenovirus production, with an eclipse period of 12 to 16 hr. The eclipse period of the satellite remained the same when cultures were preinfected with satellite 24 hr prior to adenovirus inoculation. However, when cultures were infected with adenovirus 12 hr before satellite virus, the eclipse period of the satellite was shortened to between 4 and 6 hr. Thus, satellite virus replication seems dependent upon a relatively late event in the adenovirus replication cycle. When cells were co-infected with adenovirus and its defective satellite, the yield of adenovirus was markedly reduced from that obtained in cells singly infected with adenovirus.

The defective 20-mμ particles present in various preparations of adenovirus have recently been studied by a number of investigators (1, 7, 11, 14). Initially, these small particles were detected by electron microscopic examination, and early reports (11; C. A. Baechler and F. A. Brandon, Abstr. 23rd Annual Meeting Electron Microscopy Soc. Am., p. 17, 1965; M. D. Hoggan, Federation Proc. **24**:248, 1965) raised the question as to whether these particles were adenovirus breakdown products or separate viruses. Subsequent work by Atchison et al. (1) established that the small particles are antigenically distinct from adenovirus and that the particles are defective and require an adenovirus "helper" in order to replicate. Because of the similarity between these apparently defective particles and the defective tobacco necrosis satellite virus or TNSV (9), the term adeno-satellite virus was subsequently suggested for these particles (12).

To characterize further the adeno-satellite viruses as one of a growing group of nonconditionally defective animal viruses which require "helper" viruses to replicate, reliable quantitative assays for these defective viruses were needed. We have studied different methods of assaying satellite virus, including complement fixation and electron microscopic particle counting. The high particle concentration of satellite virus in infected cell fluids has allowed us to study certain aspects of the virus-cell relationship with particle counting, a technique which has proven valid and precise for other animal viruses (19, 21). In the present communication, we report the application of recent modifications of the electron microscopic particle counting technique (10) to the study of the satellite virus growth cycle. The kinetics of satellite virus production in cells doubly infected with satellite virus and adenovirus was studied, and data are presented on the interference with adenovirus replication by its satellite.

MATERIALS AND METHODS

Cells and media. The procedures used in this laboratory for preparing tissue cultures of primary African green monkey kidney cells (GMK) have been described (13). Cells were grown in M-H medium and maintained in Eagle's medium containing lactalbumin hydrolysate.

Virus strains. SV15, a simian adenovirus, upon serial passage in our laboratory was found to contain a satellite virus which has been antigenically characterized as a distinct serotype different from those recently described by Hoggan et al. (7). It will be referred to as satellite virus type 4, and the contaminated adenovirus stock, as SV15(4). This strain of SV15, presently associated with satellite type 4 and free from other satellite serotypes, was originally received from W. McD. Hammon of the University of Pittsburgh. SV15 stocks at that laboratory contained satellite type 1 (1, 7). In our laboratory, the SV15 adenovirus seeds were freed from satellite virus by triple plaque purification; this adenovirus stock will be referred to as SV15(0).

The other satellite serotypes used were also isolated in our laboratory. Type 1 satellite was recovered from a strain of human adenovirus type 7 (14), and type 2 satellite was recovered from another strain of adenovirus type 7 which had been passaged only in human kidney cells (3). Other adenoserotypes in our laboratory also had satellite particles (14). In the presence of helper adenovirus, all satellite strains grow to particle concentrations greater that 10^9 per milliliter in cells which support the replication of helper adenovirus. No significance can currently be attached to the association of adenovirus type 7 with the other satellite serotypes used in our laboratory, since this adenoserotype has been a relatively common isolate in clinical specimens tested here. A preparation of SV15 which contains satellite particles of a particular serotype will be referred to as SV15 (1, 2, . . .5, etc.) depending on the satellite serotype, as suggested by Hoggan et al (7).

Plaque assay. Plaque assays of adenovirus were performed with cells in 1-oz flat bottles. Virus dilutions were made in maintenance medium, and 0.1-ml inocula were adsorbed for 90 min at 37 C on drained monolayers, with frequent rotation of the bottles. The monolayers were overlaid with 1.5% agar in Eagle's basal medium with 2% fetal bovine serum and neutral red. Plaques were counted from day 5 to day 11 postinoculation.

Electron microscopy. Samples of tissue culture fluids for electron microscopy were extracted with an equal volume of fluorocarbon (Freon 113) for 1 min with vigorous mixing on a Vortex mixer (5, 8). Particle counts of adenovirus or satellite virus either in clarified tissue culture fluids or in purified preparations were not significantly altered by treatment with fluorocarbon. However, the amount of cellular debris was markedly reduced, and particle clumping was minimized by the fluorocarbon treatment; therefore, this treatment led to more reproducible counts. A drop (0.05 ml) of treated specimen was placed on each of several squares of 2% agar, allowed to dialyze dry at 25 C, covered with 0.25% parlodion in amyl acetate, and allowed to drain. After the collodion film had dried, the agar was introduced into a solution of 0.75% phosphotungstic acid (PTA) with a *p*H range from 4.7 to 5.8, in a 60-mm plastic petri dish. The collodion film containing the virus particles was stripped from the agar and floated on the surface of the stain. The floating film was picked up with a 500-mesh steel grid and dried (21). All grids were examined within 72 hr. A Hitachi HU-11B was used at an instrumental magnification of 5,000 ×, and six random fields per specimen were photographed. Particles were counted at 15 × magnification and converted to particles per milliliter based on the calculated area of the photographed field and the dilution factor, as described (10).

Density gradient centrifugation. Equilibrium cesium chloride density gradient centrifugation procedures used in the present study for purifying adenovirus and its satellite virus were similar to those previously described from this laboratory (10, 11, 20). Adenovirus inocula were purified by layering 2 ml of virus stock over a preformed CsCl gradient with a density range from 1.30 to 1.37 g/cc. After 3 hr at 175,296 × *g* in a Spinco SW39 rotor, the gradient was collected by bottom puncture by use of a Szybalski fractionation device. Ten-drop fractions were collected, and fractions with densities ranging from 1.32 to 1.36 g.cc were pooled. Alternatively, the virus band at a density of 1.34 g/cc, representing 99% of the original adenovirus infectivity, was collected by side puncture with a tuberculin syringe. Densities were calculated from refractive indices.

Satellite virus stocks were concentrated by centrifugation for at least 5 hr at 78,000 × *g*. Virus pellets were then soaked overnight in tris(hydroxymethyl)-aminomethane (Tris) buffer (*p*H 7.3) at 4 C before being resuspended, layered over a preformed CsCl gradient with a density range from 1.30 to 1.50, and centrifuged to equilibrium. At least 16 hr at 175,300 × *g* in a Spinco SW39 rotor was required for satellite particles to reach equilibrium; alternatively, 5 hr of centrifugation at 358,200 × *g* in a Spinco SW65 rotor was sufficient to reach equilibrium. The procedure for fractionation of gradients and collection of satellite virus bands was the same as for adenovirus. Satellite type 4 banded at a density of 1.43 g/cc; pelleting followed by double banding in CsCl to equilibrium was employed for preparation of purified satellite virus inocula. All inocula were dialyzed against 0.15 M NaCl before use in tissue culture.

Single-cycle growth curves. Replicate monolayers containing 2×10^5 to 5×10^5 cells in tubes were infected with a multiplicity of 100 to 1,000 satellite particles per cell and 10 to 100 plaque-forming units (PFU) of adenovirus per cell. As described above, adenovirus inocula were first purified by CsCl density gradient centrifugation. All inocula were treated for 30 sec in a Raytheon sonic oscillator (10 kc, 250 w) immediately prior to use. Experiments which involved satellite and adenovirus co-infection were carried out with twofold concentrates of satellite and of adenovirus in order that the final fluid volume and virus concentration were the same as in the singly infected cultures. After 90 min of adsorption, all cultures were rinsed three times with prewarmed Tris buffer (*p*H 7.3), and maintenance medium was added. After various periods, as indicated below, the fluid and cell phases were frozen at −20 C.

Complement fixation (CF). Two full units of complement were employed in a microdroplet method (Melnick, *in preparation*). Antisera were prepared in rabbits against satellite virus type 4 and SV15(0), each of which had been purified by double banding in

CsCl gradients. Animals were inoculated intramuscularly with 1.0 ml of virus in complete Freund's adjuvant and exsanguinated on day 28 postinoculation.

RESULTS

Validity and precision of counting satellite virus particles. To determine the validity of satellite particle counting, serial twofold dilutions of an unpurified preparation of SV15(4), containing approximately 200 satellite particles per field at 5,000 × (or 10^{10} particles per ml), were made and counts were performed on each dilution. The relationship between particle counts and relative virus concentration is shown in Fig. 1. A line best fitting the mean counts of 24 separate determinations for each point passes through the origin, indicating the validity of the technique. The vertical bars show the range of the satellite particle counts for each point. The standard error of the mean of the counts for each dilution was 20% ± 5, well within the range reported by others using this technique (19). Negative staining with PTA was found to be superior to positive staining with uranyl acetate because PTA facilitated particle identification. A limitation of the electron microscopic assay was the requirement of particle concentrations of at least 10^7 per ml. Nevertheless, this assay proved more sensitive than complement-fixation titration (see below).

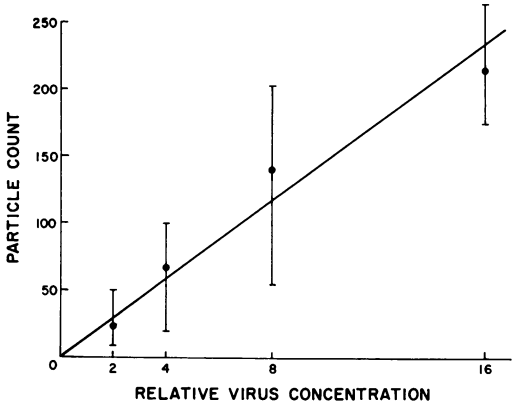

FIG. 1. *Particle counts on serial dilutions of a fluorocarbon-extracted preparation of satellite virus type 4. Each point represents the mean of 24 separate determinations. The vertical bar represents the range of particle counts.*

Density gradient equilibrium centrifugation. The question of whether satellite particles counted in unpurified virus preparations were of the same buoyant density as purified satellite virus was studied by use of equilibrium density gradient centrifugation in CsCl. SV15(4) stocks were centrifuged to equilibrium, and particle counts were performed on the fractions obtained. As shown in Fig. 2, the densest peak of satellite virus appeared at 1.43 g/cc in CsCl, and a second, larger peak appeared at a density of 1.31 g/cc. Adenovirus counts were maximal at a density of 1.33 to

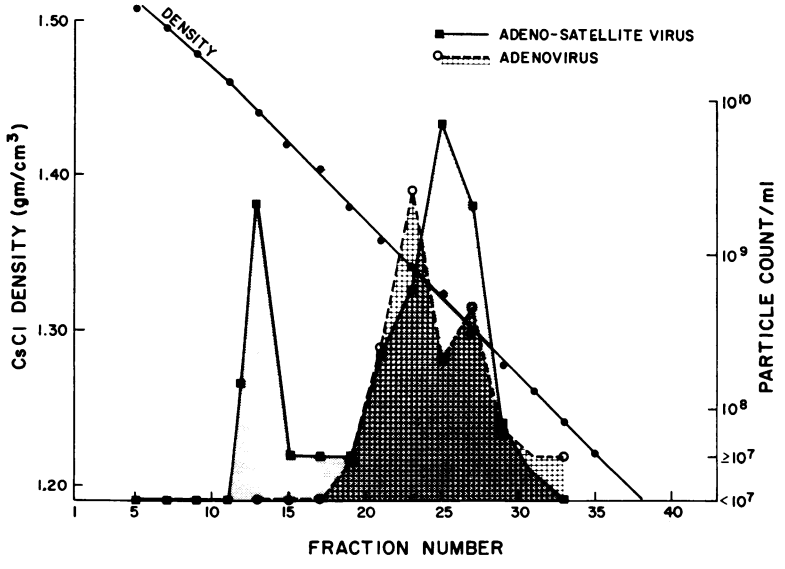

FIG. 2. *Equilibrium buoyant density centrifugation of satellite virus type 4 and simian adenovirus SV15 in CsCl. A 2-ml amount of virus stock was layered over a preformed CsCl gradient with a density range of 1.30 to 1.50 g/cc. Centrifugation was carried out in a Spinco SW39 rotor for 18 hr at 39,000 rev/min. Ten-drop fractions were collected by bottom puncture, and refractive index measurements were used for density determinations. Particle counts were made of alternate fractions.*

1.34 g/cc. With this technique of buoyant density determination, strains of satellite type 1 and 2 gave a density in CsCl of 1.37 to 1.38 g/cc. The reasons for the difference in density of type 4 remain unknown at this writing. They cannot be ascribed to differences in particle diameter, host cell, or adenovirus "helper."

A field of purified adeno-satellite virus is shown in Fig. 3 (at a magnification of 250,000 × from a plate magnification of 25,000 ×). This type of preparation was obtained by concentration of the virus followed by double banding to equilibrium at a density of 1.43 g/cc in CsCl. The distinctive morphology of satellite can be noted. The particles have a clearly hexagonal outline, and one can see capsomeres on the surface of the particles. Measurements of such particles gave a size range from 18 to 20 mμ. Noteworthy is the lack of similarity between these particles and the hemagglutinin particles of similar size obtained from human adenovirus type 3 (15). Generally, neither groups of adenovirus capsomeres nor star-shaped particles with projections (hemagglutinins) were noted either in CsCl density gradient purified preparations or in fresh adenovirus preparations. Photographs of fields from a fluorocarbon-extracted SV15(4) stock (Fig. 4) demonstrate the minimal degree of clumping present in preparations. The final magnification of the figures is 75,000 ×, the same as used for particle counting (a plate magnification of 5,000 viewed optically at 15 ×); the satellite particles are easily recognized. The capsomeres on the adenovirions are not visible because of the difficulty in obtaining precise focus at the low magnification used for particle counting. A higher magnification photograph of an SV15(4) stock is shown in Fig. 5. The smaller satellite particles surround the larger helper adenovirus, and the adenovirus capsomeres are clearly evident.

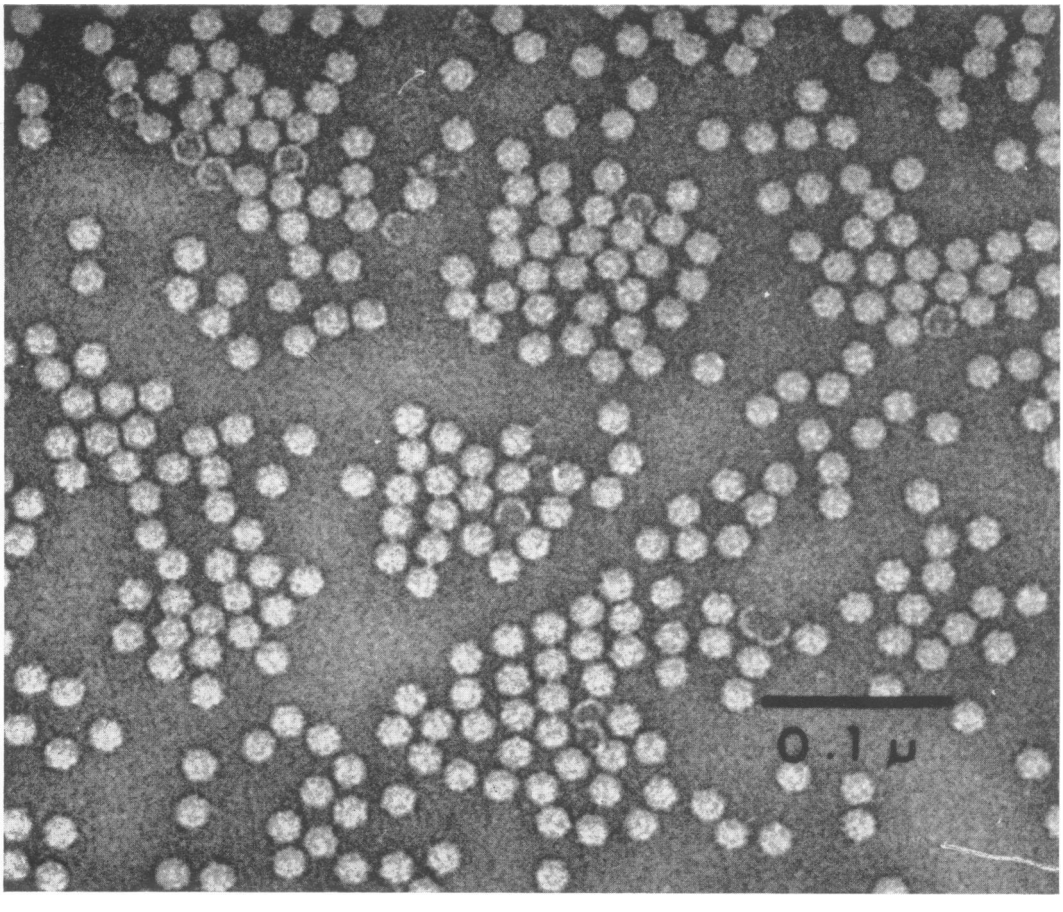

FIG. 3. *Field of satellite virus type 4 purified by double banding in CsCl. The scale line represents 100 mμ.*

Correlation of satellite virus particle counts with CF titers. Another approach to demonstrate that the particles enumerated in the electron microscope were serologically reactive satellite viruses was to compare satellite virus CF antigen titers with particle counts. In the CF tests, antigen titers were determined by reacting each twofold dilution of satellite virus with 4 units of rabbit antiserum prepared against doubly banded satellite type 4. No reactivity between this serum and adenovirus antigens was detectable. The results of comparative titrations are shown in Table 1. The ratio of particle counts to CF antigen titers, expressed as counts or CF antigenic units per ml, ranged from 6.2×10^6 to 1.4×10^7. Approximately 10^9 satellite particles per ml are required for detection by the complement fixation procedure; electron microscopy could detect satellite virus at a concentration of 10^7 particles per ml, and therefore was about 100 times more sensitive than CF assay. The precise lower limit of satellite virus detection by electron microscopy was somewhat variable, and was dependent on recognition of 20-mμ particles with hexagonal outlines.

The ratios of particle counts to CF antigen unit titers were similar whether unpurified mixed adenovirus and satellite stocks or purified satellite preparations were used as antigens. This suggests that satellite virus, unlike its adenovirus "helper," does not produce any large excess of soluble CF antigen.

Kinetic studies of satellite virus production in monkey kidney cells co-infected with adenovirus. To study the kinetics of satellite production in cells co-infected with adenovirus, it was first necessary to determine the temporal kinetics of adenovirus production in cells infected only with adenovirus. A representative growth curve of

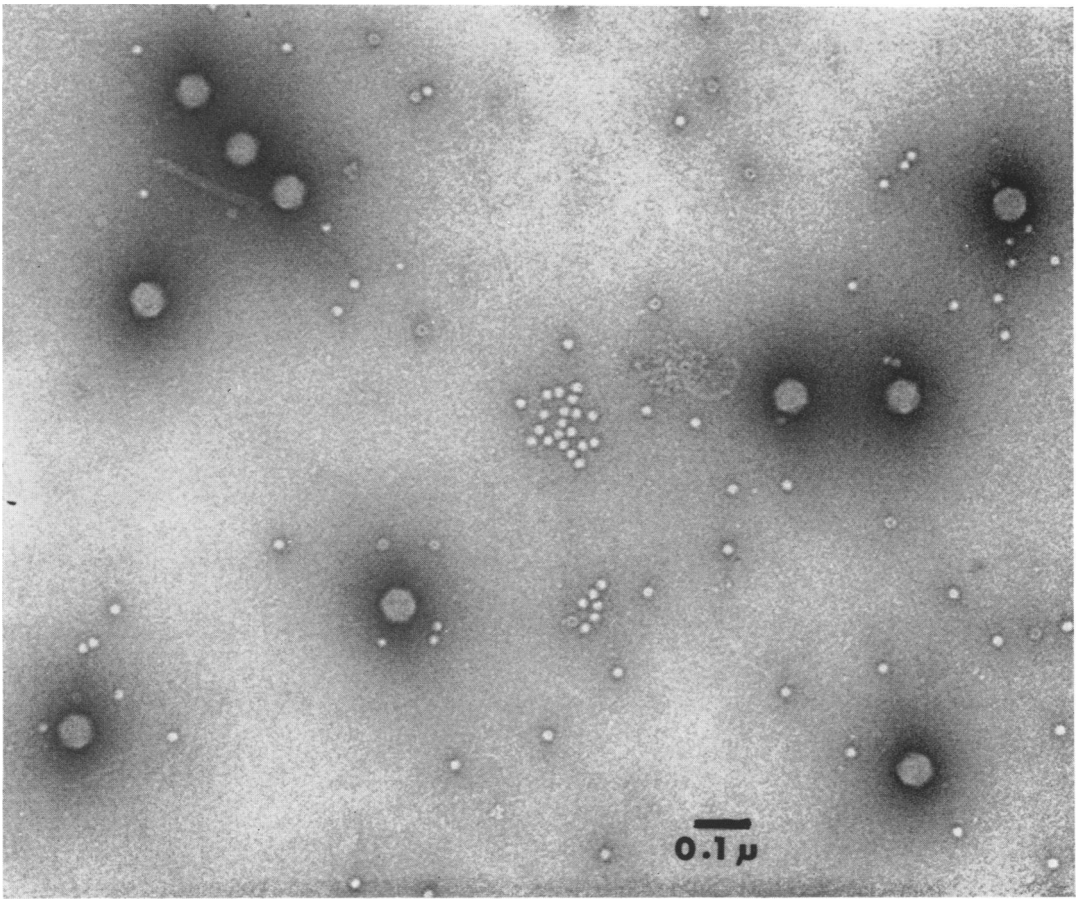

FIG. 4. *Typical field of satellite and adenovirus used for particle counting. This is a 15 × print of a plate photographed at an instrumental magnification of 5,000 × to demonstrate the differentiation between satellite particles and cellular debris during routine particle counts. The scale represents 100 mμ.*

SV15(0) is shown in Fig. 6, in which both the PFU and particle counts are expressed per cell. SV15(0) had an eclipse period of 12 to 14 hr postinfection; both the infectivity and particle titer then increased over the next 24 hr, reaching a maximal production of 100,000 adenovirus particles per cell and about 7,000 PFU per cell, and yielding virus with a particle to infectivity ratio of 14. When the fluid and cell phases were separated at different times during the growth cycle, 90 to 99% of the adenovirus was cell-associated throughout the time period studied. In other experiments, the eclipse period noted for the appearance of adenovirus particles varied from 12 to 16 hr postinfection.

In cultures co-infected with both adenovirus and satellite virus at a satellite multiplicity of greater than 100 particles per cell, satellite particles were first detectable by electron microscopy at 12 to 14 hr postinfection. A growth curve of SV15(4) in simultaneously infected cultures is shown in Fig. 7. At the end of the adenovirus eclipse period, there was a burst of satellite virus particle production where titers reached concentrations greater than 10^{10} particles per ml, or greater than 250,000 satellite particles per cell. Satellite virus production reached a plateau by 24 hr and did not significantly increase after 24 to 36 hr postinoculation. In cultures infected with satellite alone, no increase in particles was noted; however, production of satellite virus could be elicited by superinfection with adenovirus up to at least 7 days after infection.

Kinetics of satellite particle production in monkey kidney cultures preinfected with adenovirus. The growth rate of satellite virus was studied in cultures preinfected with adenovirus in an attempt to determine whether the temporal kinetics of satellite virus production might be altered as in the Rous sarcoma system (6). Cultures containing 3.4×10^5 cells were infected with a multiplicity of 10 PFU per cell of SV15(0). After 12 hr, replicate cultures were drained and superinfected with satellite virus type 4 at a multiplicity of 100 particles per cell. After an adsorption period of 2 hr at 37 C, fresh maintenance

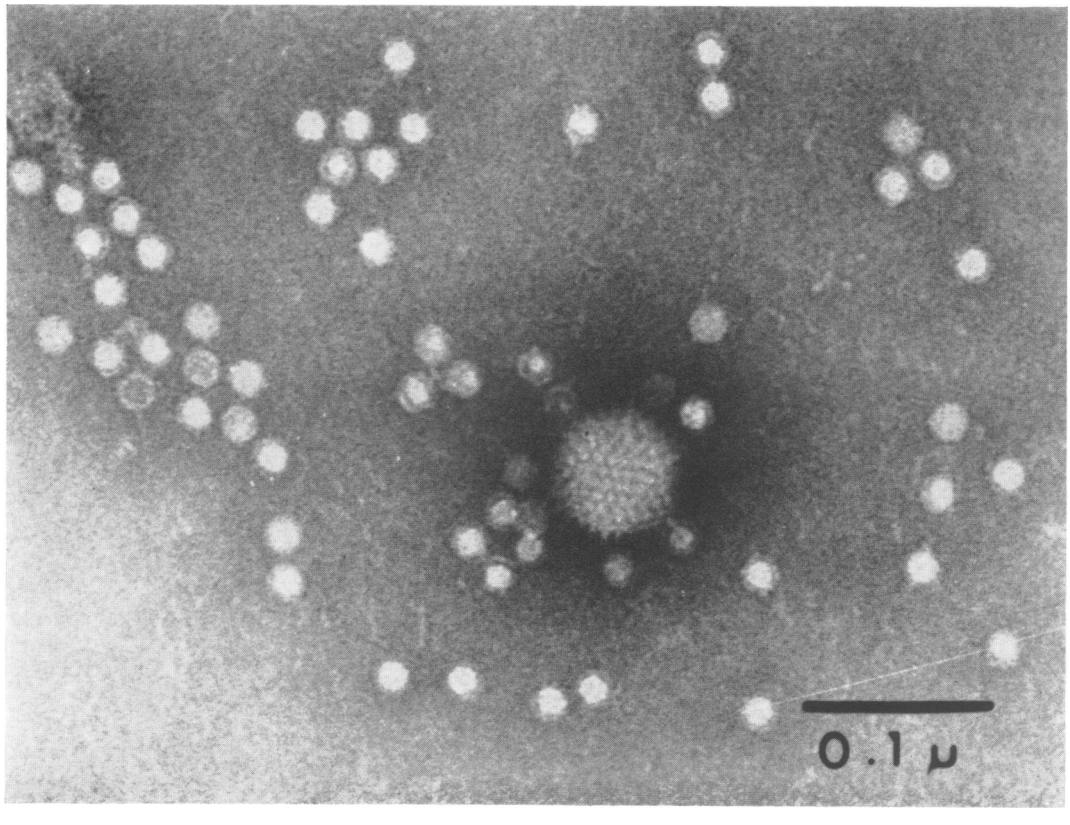

FIG. 5. *Single SV15 adenovirus particle surrounded by adeno-satellite virus type 4. The scale line represents 100 mμ.*

TABLE 1. *Comparison of adeno-satellite virus particle counts with complement-fixing antigen titers*

Prepn no.	Satellite virus particle count/ml	CF units/ml	Ratio of particle count to CF titer
1	1.5×10^{9}	240	6.2×10^{6}
2	2.6×10^{10}	1,920	1.4×10^{7}
3[a]	1.0×10^{11}	15,360	6.5×10^{6}

[a] Purified by double banding in CsCl. Particle count was made of a 1:10 dilution.

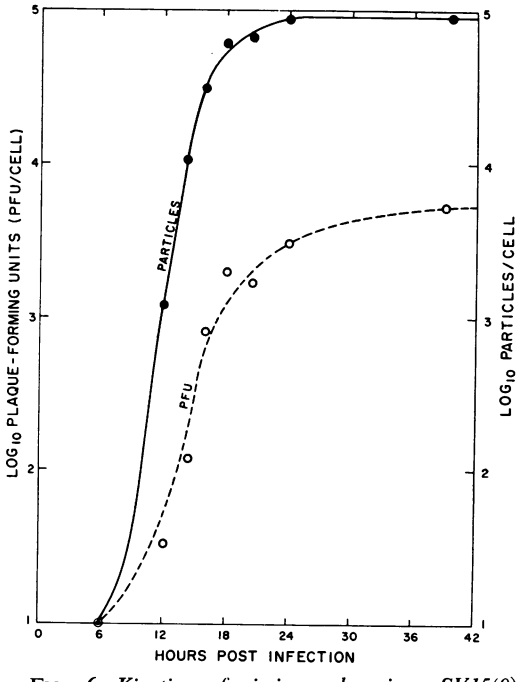

FIG. 6. *Kinetics of simian adenovirus SV15(0) particle and plaque-forming unit (PFU) production of GMK cells. Cultures containing 3×10^{5} cells were infected with 100 PFU per cell of purified adenovirus, adsorbed 2 hr at 37 C, and washed three times with Tris buffer (pH 7.3); then 1 ml of maintenance medium was added. At various times after infection, cultures were frozen and assayed as described in the text.*

medium was added and cultures were incubated at 37 C. Samples were removed and frozen at various times after satellite virus inoculation. The growth curves of satellite virus and of its "helper" adenovirus under these conditions are shown in Fig. 8.

The eclipse period of the satellite virus was shortened to 4 to 6 hr; satellite progeny appeared and increased with the increase in adenovirions. Plaque titration of adenovirus showed that infectivity had begun to increase at 12 hr postinoculation at the time the satellite was added. Particle production reached levels of 100,000 adenovirions per cell, similar to titers in cultures co-infected with adenovirus and satellite virus at the same time.

The kinetics of satellite virus production in GMK cultures preinfected with satellite virus and then superinfected with adenovirus 12 and 24 hr later were essentially those of cultures simultaneously infected with adenovirus and satellite virus. Both the satellite and the adenovirus had an eclipse period of 12 to 16 hr after the infection with adenovirus. These results suggested that satellite virus replication was dependent on completion of a relatively late event in the adenovirus replicative cycle.

Interference between satellite virus and adenovirus. In the course of growth cycle studies in cells co-infected with adenovirus and satellite virus type 4, it was noted that, in instances of satellite virus multiplicities of infection greater than 10 particles per cell, there was a reduction in adenovirus yields. In Table 2, the reduction of adenovirus yields in cultures co-infected with

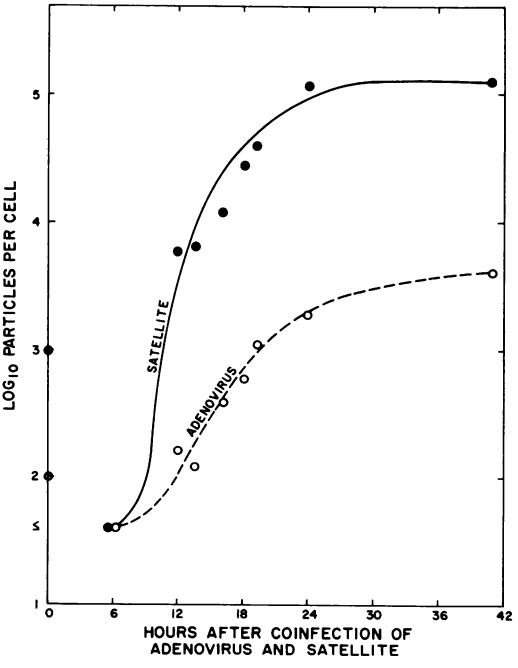

FIG. 7. *Kinetics of satellite virus type 4 in cultures co-infected with the satellite and adenovirus. Cultures containing 2.5×10^{5} cells were infected with 1,000 particles of satellite virus (purified by double banding in CsCl) per cell. At the same time, adenovirus was added at a multiplicity of approximately 100 PFU per cell. Subsequent steps were as described in the legend to Fig. 6.*

SV15(0) and satellite is compared with the time of satellite inoculation. Yields of adenovirus were reduced by 90% in the presence of its satellite when satellite virus was added either 12 hr before or at the same time as the adenovirus. However, satellite virus interference with adenovirus was not absolute, as indicated by the failure of satellite to markedly interfere with the adenovirus when added 12 hr after adenovirus inoculation. Adenovirus particle to PFU ratios were similar in harvests with and without satellite virus, and the eclipse period of the adenovirus in cultures infected with SV15(4) was the same as in cultures infected with SV15(0). However, in cultures preinfected with SV15(0) and superinfected 12 hr later with satellite type 4, adenovirus yields were the same as in cultures infected only with the adenovirus. This indicates that satellite virus does not interfere with the replication of its "helper" adenovirus under all conditions.

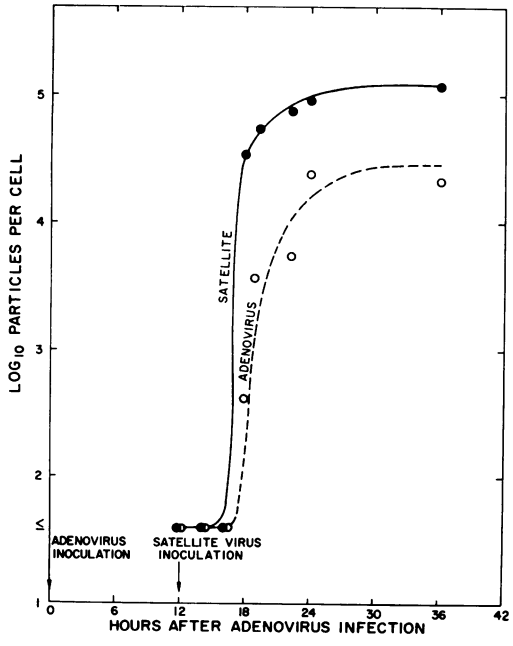

FIG. 8. *Kinetics of satellite virus production in cells infected 12 hr prior with adenovirus, SV15(0). Replicate cultures containing 3.5 × 10⁵ cells were infected with 50 PFU of purified adenovirus per cell, adsorbed for 2 hr at 37 C, and washed; maintenance medium was then added. At 12 hr after the inoculation of adenovirus (indicated in the figure with an arrow), the medium was removed and the cultures were superinfected with 100 particles of purified satellite virus type 4 per cell, adsorbed, and washed as with adenovirus. Cultures were frozen at various times postinfection and were assayed for satellite and adenovirus as described in the text.*

TABLE 2. *Interference of satellite virus type 4 with simian adenovirus SV15 replication*

Inoculum[a]	Adenovirus yield, 48 hr	
	PFU/ml	Per cent of maximal yield
Adenovirus alone	4.3×10^8	100
Satellite plus adenovirus together at 0 time	4.8×10^7	11
Satellite plus adenovirus 12 hr later	3.7×10^7	9
Adeno plus satellite 12 hr later	3.3×10^8	77

[a] The inoculum of satellite virus was 100 particles per cell.

DISCUSSION

It has been shown for a number of viruses that electron microscopic particle counting provides accurate determination of particle concentrations (19). We have applied the modification described by Smith and Melnick (21) in which negative staining and pseudoreplication are utilized. Particle counts were made of the defective adeno-satellite virus under a variety of conditions in crude and purified preparations. The difficulty of recognizing what to count was greatly facilitated by extracting samples with fluorocarbon prior to electron microscopy (5). Electron microscopic particle counting of the satellite virus was shown to be a valid and relatively precise quantitative technique. The lower limit of concentration of virions for assay was 10^7 per ml; nevertheless, particle counting was about 100 times more sensitive than complement fixation, where 10^9 virions per ml were required for detectability.

By use of the particle-counting technique, it could be shown that particles with the morphological appearance of satellite virus had a buoyant density of 1.43 g/cc in CsCl. Other serotypes appeared to have a lower buoyant density of 1.37 to 1.39 g/cc. Using the calculations described by Weigle et al. and Breedis et al. (4, 22), one can calculate the differences in the nucleic acid content per particle, if one assumes that the proteins of the satellite capsids have the same buoyant density. This suggests that satellite virus type 4 has a nucleic acid content of 35% of the particle weight, whereas the other types would have a content of approximately 20%. It is probable that this difference in nucleic acid content would be detected with biochemical studies.

The kinetics of satellite virus particle produc-

tion in cells co-infected with simian adenovirus SV15(0) indicated an eclipse period of 12 to 16 hr, very close to that of the adenovirus "helper." The eclipse periods for both the satellite and adenovirus in cultures preinfected 12 to 24 hr before with satellite was the same as in co-infected cells. However, in cells preinfected 12 hr prior with adenovirus, the satellite eclipse was shortened to 4 to 6 hr. Using an immunofluorescence assay method, Ito et al. (J. Gen. Virol., *in press*) also obtained a similar shortening of the incubation period. One possible interpretation of these results is that a relatively late step in adenovirus replication was required to permit satellite virus replication to proceed.

The type of kinetics described here for satellite and adenovirus is similar to the growth cycles of the defective Rous sarcoma virus (RSV) and its avian leukosis "helper" (RAV) reported by Hanafusa and Hanafusa (6). However, since the satellite virus coat proteins are serologically distinct from those of their adenovirus helper (1), it is unlikely that production of coat protein (as in the case with the RSV-RAV system) is the mechanism by which adenovirus functions in production of its satellite. It is noteworthy that the ribonucleic acid-containing tobacco necrosis satellite virus (TNSV) is a defective virus with a coat protein also distinct from its "helper" virus. Reichmann (16) has suggested that its limited genomic information can apparently code for the capsid protein of TNSV and little more, although recent data indicate satellite virus deoxyribonucleic acid has a molecular weight approximating that of the papovaviruses (18).

Satellite virus has another characteristic similar to other defective viruses which require helper viruses in order to replicate. Satellite type 4 reduces the yield of adenovirus in cells co-infected with SV15. Similar systems in which the defective virus interferes with the replication of its helper have been reported for TNSV-TNV (9), and for herpes simplex, in which a defective mutant interferes with the multiplication of complete virus in dog kidney cells (17). The latter type of interference was thought to be the result of nonfunctional aggregation of nonfunctional and functional products specified by the defective and complete viruses, respectively. Unlike the relation between adenovirus and its satellite, the defective herpes mutant interfered with the "helper" herpes under all conditions tested.

The observation that the ratio of adenovirus particle to plaque-forming units was unchanged in cultures co-infected with satellite virus suggests that the reduction in adenovirus yields results from a decreased production of adenovirus, rather than from a change in the quality of the virus. Hoggan et al. (7) have also shown that the interference of satellite virus type 1 with adenovirus type 7 in human kidney was characterized by a reduction in adenovirus CF antigen production. A recent report by Atchison et al. (2) suggested that some type of interference was also operative in their system. A detailed study on the mechanism of this interference with adenovirus replication is currently in progress. The observation of interference with adenovirus replication by its satellite raises questions concerning the mechanisms of both enhancement and interference and their relationship to each other.

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