Infectivity Titrations and Particle Counts of Adenovirus Type 5

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SUMMARY: Infectivity titrations and electron microscopic spray counts were performed on purified preparations of adenovirus type 5. The number of virus particles/50 % infective units varied from 10 to 100 with different preparations.

Infectivity titrations of adenoviruses are usually based on their cytopathic effect in cultures of HeLa cells and end-points, calculated from all-or-none responses, are given as the dilution of the preparation under test which gives a 50 % response. It has been shown (Pereira & Kelly, 1957) that the dose-response curve of the infective action of adenovirus type 5 in HeLa cells is consistent with the hypothesis of independent action of infective units, a single unit being sufficient to initiate infection. The present investigation was designed to determine the relation between these infective units and electron microscope counts of the numbers of virus particles.

METHODS

Virus strain. The prototype strain of adenovirus type 5 was received from Dr R. J. Huebner (National Institutes of Health, Bethesda, Maryland, U.S.A.) and propagated in HeLa cell cultures. The materials used in the present study consisted of pooled cells from cultures showing advanced cytopathic effect. Each pool contained cells either from ten bottles (each with about 10^7 cells at the time of inoculation) or from 200 to 300 tubes used in routine titrations of the strain under study.

Virus purification. The method used was based on the technique described by Gessler, Bender & Parkinson (1956). Pooled cells were washed with 25 ml. distilled water and centrifuged at 600 *g* for 10 min. The supernatant fluid was discarded and the packed cells resuspended in 2 ml. of M/15 phosphate buffer (pH 7.3). This suspension was added to 4 ml. of Arcton 63 (Imperial Chemical Industries, London, S.W. 1) and homogenized in an M.S.E. universal container blender for 2 min. at maximum speed. The homogenate was centrifuged for 10 min. at 600 *g* and the aqueous fraction decanted and saved. Another 2 ml. volume of phosphate buffer was added to the cell + Arcton fraction and followed by homogenization and centrifugation as above. This process was repeated a third time and the three aqueous fractions were pooled. The material obtained was opalescent, and when examined in the electron microscope was seen to contain a considerable amount of amorphous debris in addition to the virus particles. Further purification was achieved by submitting this material to several cycles of freezing at -10° and thawing at 37° .

At each cycle a precipitate formed and this was separated by centrifugation at 1000 g for 10 min., without measurable loss of virus activity. Three or four cycles of freezing and thawing were usually sufficient to yield almost water-clear preparations suitable for spray counts in the electron microscope (see Pl. 1).

Virus titrations. These were performed in cultures of HeLa cells propagated and maintained by methods previously described (Pereira & Kelly, 1957). Virus dilutions were made in broth saline (10 % tryptic digest broth in normal saline) at 0.5 log steps, using a fresh pipette for each dilution. Volumes of 0.1 ml. of appropriate dilutions were inoculated into each of 4 cultures of HeLa cells which were incubated without rotation at 37° for 27 days, with 3 fluid changes at intervals of 6 to 7 days. The 50 % end-point was obtained by the method of Reed & Muench (1938), and recorded as the number of 50 % tissue culture doses (TCD₅₀)/ml. The standard error of each titration was calculated according to Pizzi's (1950) formula.

Table 1. *Particle counts and infectivity titres of adenoviruses type 5*

Material	Particles/ml. ($\times 10^{10}$)	Infective doses/ ml. ($\times 10^{10}$)	Particles/ infective dose
H554-c	6.5	0.10	65
H554	6.8	0.68	10
H565	37.4	0.46*	82
H566	20.7	0.20*	103

* From geometric mean of two titrations.

Particle counts. The purified virus preparations were mixed with equal volumes of an aqueous suspension of polystyrene latex spheres of diameter 0.16 μ (Dow Chemical Company: Lot no. LS 055 A). The latex suspension had a count of 1.0×10^{11} particles/ml. as determined from its dry weight, the known density (1.05 g./cm.³) of the spheres and their diameter which was measured in terms of the spacing of an aluminium replica of a standard ruling. Bovine albumin was added to the mixture (0.05 %, w/v) which was then sprayed in the usual way (Williams & Backus, 1949; Backus & Williams, 1950) on to carbon films carried on electron microscope specimen supports. These were lightly shadowed with palladium and photographs were taken of the microdroplets at a magnification of $\times 7,000$. The virus particles could be clearly identified as electron-dense spheres of c. 70 m μ . diameter and the ratio of the count of these particles to the count of the latex particles was determined. The counts were checked for randomness and statistical significance by the method given by Luria, Williams & Backus (1951).

RESULTS

Infectivity titres and particle counts obtained from four virus preparations are recorded in Table 1. The standard errors of the particle counts varied between 10 and 20 % and those of the infectivity titrations between 0.18 and 0.30 log₁₀ units.

The mean number of virus particles/infective dose ranged from 10 to 103. This variation may be due to differences in sensitivity of separate batches of HeLa cell cultures. It is also possible that the different preparations used contained varying proportions of inactive virus particles, as no special precautions were taken to insure maximum infectivity of the starting materials. Preliminary experiments had shown that infectivity titres of adenovirus type 5 were not significantly decreased by the Arcton 63 treatment described above.

COMMENTS

The numbers of virus particles/infective dose obtained in the present study are very similar to the corresponding values found for other animal viruses (Isaacs, 1957; Schwerdt & Fogh, 1957; Dumbell, Downie & Valentine, 1957). They differ, however, from the ratio of 10^4 particles/TCD₅₀ of adenovirus type 4, reported by Tousimis & Hilleman (quoted by Tousimis & Hilleman, 1957). This discrepancy is rather too large to be due to differences in the properties of the two virus types and is probably accounted for by the short period of incubation (3 days) of the test cultures in the titration technique used by Tousimis & Hilleman. The sensitivity of adenovirus titrations in HeLa cells varies greatly with the length of this period of incubation and maximum titres are reached only after incubation for 3-4 weeks (Pereira, unpublished).

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H. G. PEREIRA & R. C. VALENTINE—ADENOVIRUS PARTICLE COUNTS. PLATE 1
(Facing p. 181)

WILLIAMS, R. C. & BACKUS, R. C. (1949). Macromolecular weights determined by direct particle counting. I. The weight of the bushy-stunt virus particle. *J. Amer. chem. Soc.* **71**, 4052.

EXPLANATION OF PLATE

Electron micrograph of a typical spray droplet used in making the virus particle count. The larger spheres are the latex indicator particles ($0.16\ \mu$ diameter) and the smaller dense objects (*c.* $70\ \text{m}\mu$ diameter) the adenovirus particles. The droplets were metal shadowed with palladium. $\times 16,000$.

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