VIROLOGY

Mixed Infection of African Green Monkey Kidney Cells by Adenovirus 7 and *SV* 40

It has been shown that mixed infection with adenovirus 12 and *SV* 40 can occur in African green monkey kidney cells (AGMK)[1]; moreover, the double infection results in an enhancement of adenovirus growth[2]. In both the foregoing investigations it was demonstrated with electron microscopy that less than 1 per cent of the cells contained adenovirus 12 particles 72 h after infection with adenovirus alone, yet with adenovirus plus *SV* 40, 75 per cent of the cells contained adenovirus. Determinations of adenovirus infectivity in human embryonic kidney cell cultures using limiting dilution titrations also consistently demonstrated enhancement of adenovirus growth by *SV* 40. Similar results were obtained with adenovirus 5 by infectivity titrations and electron microscopy.

To investigate further this phenomenon, we measured haemagglutinin production as another parameter of adenovirus growth. Adenovirus 7 was chosen because of its capacity to agglutinate rhesus monkey erythrocytes. The prototype strain of adenovirus 7 was obtained from Dr. J. Kasel (National Institute of Allergy and Infectious Diseases, National Institutes of Health). It was serially passed in *HEp*-2 cells and titrated in primary human embryo kidney cells[2]. Primary AGMK tube cultures were inoculated with adenovirus 7 only and with adenovirus 7 plus *SV* 40 simultaneously using methods previously described[1,2]. The tubes were incubated at 37° C on a roller drum for 72 h, frozen and thawed three times, and titrated. Haemagglutinin titres for rhesus monkey erythrocytes were determined using a micro-titre method[3]. To two-fold serial dilutions of 0·025 ml. tissue culture fluid, were added 0·025 ml. saline and 0·025 ml. 1 per cent erythrocytes in each micro-titre well. The titre plates were incubated for 1 h at 37° C and the end-point was read as the last dilution (of the original two-fold) in which a definite agglutination pattern was obtained. All experiments were performed in duplicate, and haemagglutination observations of each tube also were performed in duplicate. The quadruplicate haemagglutination results were the same for all titrations in this investigation. Infectivity titrations were carried out in roller tube cultures of primary human embryo kidney cells. Identical cultures with single and double infection were fixed, sectioned and examined with the electron microscope[1]. The results are summarized in Table 1.

Table 1. Infection of African Green Monkey Kidney Cell Cultures with Adenovirus 7 only and Adenovirus 7 plus *SV* 40

Inoculum	Haemagglutinin titre*	Cells with adenovirus particles by electron microscopy*	Infectivity titre*
Adenovirus 7 ($10^{5.2}$ $TCID_{50}$)	0	0	$10^{6.2}$ $TCID_{50}$/ml.
Adenovirus 7 ($10^{5.2}$ $TCID_{50}$) plus *SV* 40 ($10^{6.4}$ $TCID_{50}$)	1 : 32	20 per cent	$10^{7.9}$ $TCID_{50}$/ml.

* 72 h after inoculation.

Although approximately 20 per cent of the cells examined electron-microscopically had intranuclear adenovirus particles, in a few cells there was morphological evidence of mixed infection with *SV* 40 particles and adenovirus particles within the same nucleus. The close association of the two viruses provides a morphological basis for the serological evidence of 'hybridization' of adenovirus 7 and *SV* 40 presented by Huebner *et al.*[4].

Our findings show that the development of adenovirus haemagglutinin as well as mature infectious virus particles is enhanced by mixed infection with *SV* 40. This is consistent with the work of Brandon and his associates[5], who report good evidence that haemagglutinin is an intrinsic part of the infective particle. In their experiments adenovirus 3 was adsorbed and eluted on monkey erythrocytes through three cycles, and more than 90 per cent of the infective particles accompanied the haemagglutinin. Thus, our investigation has provided additional affirmative evidence that adenovirus growth is enhanced by *SV* 40. Moreover, the quantitative haemagglutination technique is much less cumbersome for determining this phenomenon than infectivity titrations or electron microscopy.

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[1] O'Conor, G. F., Rabson, A. S., Berezesky, I. K., and Paul, F. J., *J. Nat. Cancer Inst.*, **31**, 903 (1963).
[2] Rabson, A. S., O'Conor, G. F., Berezesky, I. K., and Paul, F. J., *Proc. Soc. Exp. Biol. and Med.*, **116**, 187 (1964).
[3] Sever, J. L., *J. Immunol.*, **88**, 320 (1962).
[4] Huebner, R. J., Chanock, R. M., Rubin, E. A., and Casey, M. J., *Proc. U.S. Nat. Acad. Sci.* (in the press).
[5] Brandon, F. B., and McLean, I. W., jun., *Adv. Virus Res.*, **9**, 157 (1962).

CYTOLOGY

Isolation of Macronuclei from the Ciliate *Tetrahymena pyriformis* GL

Several different non-ionic detergents have recently been used for the isolation of nuclei from mammalian cells[1-5]. One of these detergents, 'Triton *X*-100', was found to solubilize readily cytoplasmic components, without affecting the structural integrity (at the light-microscope level) of nuclei from rat liver cells and mouse plasma cell tumours[5]. 'Triton'-isolated nuclei contained more than 90 per cent of the DNA in the original cell homogenate, retained rapidly labelled RNA and NAD-pyrophosphorylase activity[5]. The detergent digitonin has been used with some success for the isolation of macronuclei from stationary phase *Tetrahymena pyriformis*, mating type II, var. 1 (ref. 6).

This communication describes a simple method for the isolation of macronuclei from the ciliate *Tetrahymena* after cell lysis in 'Triton *X*-100'.

Growth conditions for *T. pyriformis* GL and the method for collecting cells were the same as reported earlier[7]. Macronuclei were isolated from organisms in the following growth stages: (1) exponential multiplication at the optimum temperature for growth (29° C); (2) exponential cultures incubated for 2 h at 34° C; and (3) maximum stationary phase. Brief periods of incubation at 34° C were also used to induce synchronized cell division[8,9].

The gathered organisms were washed once at room temperature in a diluted Ringer's sodium phosphate (RSP) buffer (final concentration: 0·047 M sodium chloride, 0·002 M potassium chloride, 0·001 M magnesium sulphate, 0·0125 M sodium phosphate buffer, *p*H 7·3) and resuspended in the RSP-buffer to give a final density not exceeding 4×10^6 cells/ml. The suspension was gently shaken to avoid cell aggregation. From this suspension, 100 ml. was removed with a plastic pipette in 10-ml. portions and slowly added to 100 ml. of medium *A* (0·5 per cent v/v 'Triton *X*-100' and 0·5 M sucrose in RSP) in a 500-ml. Erlenmeyer flask kept in crushed ice. An L-shaped glass rod (2 × 27 cm, area of blade approximately 15 mm²) rotating at 200 r.p.m. in medium *A* was used for mixing. Five min after addition of the last aliquot of the cell suspension to medium *A*, almost all cells were completely lysed. From this time on, 200 ml. of medium *B* (2 per cent w/v proteose peptone, Difco, and 0·1 per cent w/v liver fraction *L*, Wilson Laboratories, in distilled water) were slowly added to the mixture while stirring continued.

Frequent examination of samples under a phase-contrast microscope showed that lysis in medium *A* started at the anterior part of the cell and swept along the long