

## The Growth of Vaccine Strains of Rubella Virus in Cultured Human Synovial Cells<sup>1</sup> (35296)

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(Introduced by T. H. Spaet)

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Arthritis is a common manifestation of naturally occurring rubella and also the most troublesome complication of rubella vaccination in adults. The incidence of arthritis has been reported to be as high as 40% in women given the HPV-77 strain of rubella vaccine and 25% in those receiving the Cendehill strain (1). The actual nature of rubella synovitis is not clear. Recently, however, we have shown that rubella virus will grow in monolayer cultures of cells derived from explants of nonrheumatoid synovial membranes (2), suggesting that rubella arthritis may be due to virus infection of the synovial membrane. This study reports our observations on the growth of two vaccine strains of rubella, HPV-77 and Cendehill, in monolayer cultures of synovial cells, and suggests a correlation between the growth curves of these strains in tissue culture and their potential to cause synovitis postvaccination.

**Methods.** We have previously described the methods used to grow monolayer cultures from explants of human synovial membrane obtained from patients undergoing orthopedic surgery (2, 3). The HPV-77 virus was the Merck Sharp and Dohme vaccine obtained at random from the hospital pharmacy. The Cendehill strain was the Smith Kline and French vaccine. For measuring growth curves, 30-mm petri dishes containing  $10^4$  nonrheumatoid synovial cells were infected with  $10^2$  TCID<sub>50</sub> (tissue culture infective dose) of virus. The virus was allowed to adsorb for 1 hr at 4°, and then, at zero time,

the temperature was raised to 37°. At varying time intervals thereafter, the infected cells were washed 8 times with phosphate buffered saline, scraped off the petri dishes into 0.5 ml of medium and lysed by freeze-thawing. Debris was removed by centrifugation at 10,000 rpm for 10 min. Virus titers in 0.2 ml of the lysate were assayed by the 50% end point dilution method of Reed and Muench (4) with Vero cells as the indicator culture. In all other experiments,  $10^4$  nonrheumatoid synovial cells were incubated with  $5 \times 10^2$  TCID<sub>50</sub> of virus, and were observed for CPE which was noted between 14 and 21 days later.

**Results.** Seven nonrheumatoid synovial cell strains were infected with HPV-77 virus. All 7 cell strains began to show cytopathic effects (CPE) consisting of increased granularity and decreased size beginning 12–14 days after infection, which progressed to rounding, increased refractility and detachment of the cells (Fig. 1). Four strains were infected with the Cendehill virus. These cells showed only minimal CPE.

Comparative growth curves of wild type rubella virus, strain F-8 (5), HPV-77 virus and the Cendehill strain in the same strain of nonrheumatoid synovial cells are shown in Fig. 2. The growth of Cendehill virus was considerably less than the F-8 strain at 24 hr. This is in keeping with the minimal CPE observed in Cendehill-infected cells and the lower frequency of arthritis in recipients immunized with the Cendehill vaccine.

**Conclusion.** Two attenuated vaccine strains of rubella virus still retain their ability to grow in monolayer cultures of nonrheumatoid synovial cells, and by extension probably

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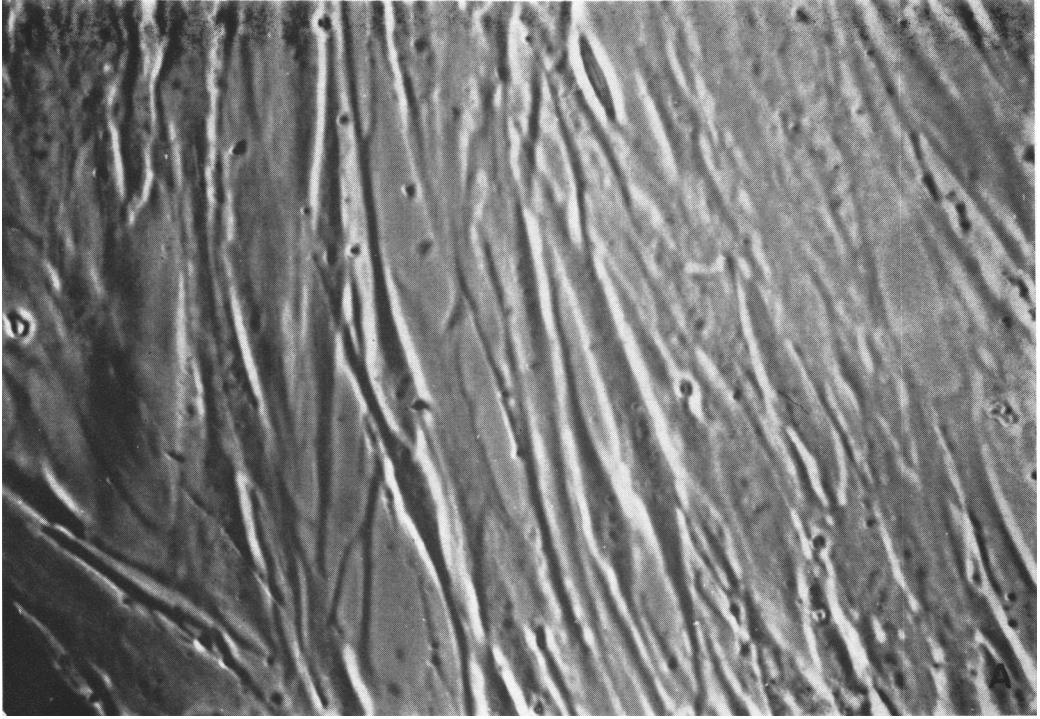
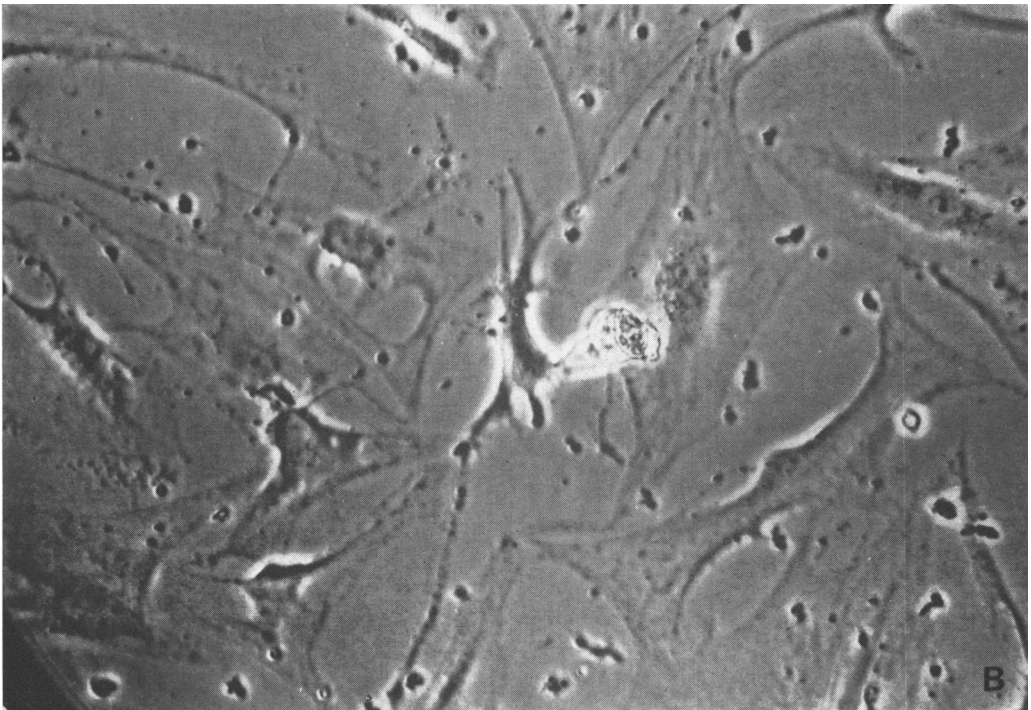


FIG. 1. A single strain of nonrheumatoid synovial cells 15 days after infection with HPV-77 virus (B); and an uninfected control (A).



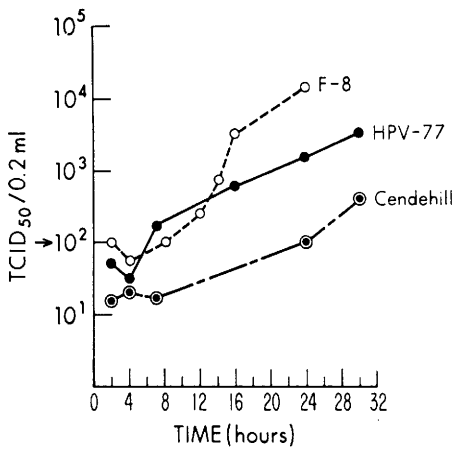


FIG. 2. Growth curves of rubella virus in cultures of nonrheumatoid synovial cells. The input titer is indicated by the arrow.

multiply within the synovial membrane of

those recipients who develop arthritis post-vaccination. The growth characteristics of rubella in cultured synovial cells may reflect the degree of attenuation vis-à-vis growth in synovial membranes, and perhaps could be used to select strains of virus which would be unlikely to cause synovitis following immunization.

1. Cooper, L. Z., Ziring, P. R., Weiss, H. J., Mattern, B. A., and Krugman, S., *Amer. J. Dis. Child.* **118**, 218 (1969).

2. Grayzel, A. I., and Beck, C., *J. Exp. Med.* **131**, 367 (1970).

3. Hamerman, D. J., Janis, R., and Smith, C., *J. Exp. Med.* **126**, 1005 (1967).

4. Reed, L. J., and Muench, H., *Amer. J. Hyg.* **28**, 493 (1938).

5. Marcus, P. I., and Carver, D. H., *J. Virol.* **1**, 334 (1967).

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