

Variation G. At Step 1, 0.1% sodium sulfite and 0.01 M EDTA were added to SP buffer. This solution was also used to resuspend the PEG pellet at Step 6. This variation was used for PSV.

Variation H. The extraction solution used in Step 1 was 0.1 M Tris-HCl, pH 8.4, that contained 0.1 M magnesium acetate, 0.04 M sodium sulfite, and 0.001 M magnesium chloride (14). This solution was also used at Step 6 to resuspend the pellet. Step 2 was omitted, and no solvents were used. A subroutine was inserted following Step 3. A 10% (w/v) aqueous solution of activated charcoal was stirred into the supernatant from Step 3. The charcoal mixture was allowed to stand for 5 min and then centrifuged at $12,200 \times g$ for 5 min. This supernatant was used to continue the procedure at Step 4. This variation was used for SYN V.

Electron Microscopy

A droplet of test solution was applied to a carbon top-coated, Formvar-clad electron microscope grid, which was then washed and stained in the manner described in Appendix 2 ("Negative Staining of Virions"). Bacitracin (10) was usually not needed in the washing solutions, as there was sufficient protein present in the CVC preparations to provide good wetting. However, the negative stain, usually uranyl acetate, always contained bacitracin. Grid preparation was as described in Appendix 2.

The stained grids were examined in a Hitachi H-600 electron microscope. The numbers of particles were determined at magnifications that presented a manageable number of particles in the viewing field. Magnification was kept constant for any given experiment, but varied from one experiment to another.

Electron micrographs were made on 35-mm film as described in Appendix 3.

Results

Electron Microscopy

The data pertaining to the CVC trials are summarized in Table 1. Utilization of the CVC procedure resulted in increased virion populations as determined by electron microscopy (Figure 3). Varying amounts of clarification of the CVC extracts were realized throughout the procedure, but only at Steps 4 through 6 was there actual concentration of virions, achieved by resuspending the virus-containing PEG pellet from Step 6 in a smaller volume than that of the original extract. The degree of virus increase is given as a ratio comparing the number of particles counted on specimen grids prepared from the CVC preparations to the numbers seen on grids