Virion Exclusion: The Competition for Grid Sites

Virion numbers discovered by electron microscopy were often considerably higher for the CVC final preparations when compared to the starting exudates than could be expected on the basis of an increase in virus concentration alone. Thus, it seemed probable that these elevated counts owed, at least in part, to the removal of some of the sap constituents which otherwise compete with virions for the available grid sites (18,22). Virion exclusion was therefore investigated to determine the magnitude of this phenomenon and to assess its effect on the counts of particles made from the CVC preparations.

Purified CpMV was obtained from E. Hiebert. Following preliminary clarification steps, the virus had been subjected to approach to equilibrium centrifugation on cesium chloride gradients, and the virus-containing zones were collected. The virions were then precipitated by PEG and resuspended in SP buffer. This suspension was water-clear and free of any detectable impurities. When examined with the electron microscope, virions were seen to be intact and monodisperse and the background was devoid of debris.

The preparation was then diluted with SP buffer to make a stock solution containing 2.5 mg/ml as determined by its optical density at 260 nm. Samples of this stock solution were mixed vol:vol with (I) SP buffer (control), (II) the supernatant from a centrifuged extract of healthy cowpea (1 g of leaf tissue was triturated in 2 ml SP buffer, expressed through cheesecloth, and the expressate was centrifuged at 12,200 × *g* for 5 min), (III) the supernatant from a healthy extract of tobacco that had been prepared in the same manner as II, and (IV) which was the same as II except that the expressate was stirred vol:vol with *n*-butanol just prior to centrifugation and the aqueous phase was collected and used in the mixture. Specimen grid mounts were made with these products, and were washed and stained with uranyl acetate as outlined in Appendix 2. Particles were counted and the results of these counts are presented in Table 2, Experiment 1.

Addition of either cowpea (II) or tobacco (III) sap to the stock solution of virus reduced the observed number of virions to less than 10% of control (I). However, when cowpea sap was clarified with *n*-butanol prior to adding it to the virus solution (IV), the number of virions tabulated was 80% as great as control (I) (see Figure 5).

In another test, cowpea mosaic virus was concentrated from infected cowpea tissue using the standard CVC procedure. Samples were then taken and used to prepare the following mixtures: (*a*) a sample from the Step 7 (final) supernatant was mixed vol:vol with SP buffer (control); (*b*) a sample from Step 1 (expressate) was centrifuged as in Step 3, but the solvent treatment at Step 2 was omitted, and the supernatant was then mixed vol:vol with SP buffer; and (*c*) a sample