

Formvar (11), or to make Formvar substrates thinner, yet strong and free of defects (5); but we have been unable to use them successfully with our regimen.

The appendices are by no means intended to be the last word in negative staining and related procedures, but rather provide workable starting techniques from which each user may develop routines that are suitable for particular job requirements.

Materials and Methods

Virus Cultures and Their Plant Hosts

The viruses used in this study, the virus groups to which they are assigned (19), and their maintenance hosts were: cowpea mosaic virus (CpMV), comovirus, the Sb isolate obtained from G. Bruening, *Vigna unguiculata* (L.) Walp. 'Knuckle Purple Hull'; cucumber mosaic virus (CMV), cucumovirus, the winged bean isolate (17), *Vigna unguiculata* (L.) Walp., 'Knuckle Purple Hull'; virus-like disease of lettuce (LEV), tentative ilarvirus, original isolate from B. Falk, *Lactuca sativa* L., *Cichorium endivia* L., and *Nicotiana x edwardsonii* Christie and D.W. Hall (4); nandina stem-pitting virus (NSPV), proposed closterovirus, original isolate (1), *Nandina domestica* Thunb., 'Nana Purpurea'; peanut stripe virus (PSV), potyvirus, original isolate from J. Demski, *Arachis hypogaea* L.; pepper mottle virus (PMV), potyvirus, the type isolate, *Nicotiana tabacum* L., 'Samsun NN'; sonchus yellow net virus (SYNV), rhabdovirus, the type isolate, *Nicotiana x edwardsonii* Christie and D.W. Hall, (4); tobacco mosaic virus (TMV), tobamovirus, the common type isolate, *Nicotiana tabacum* L., 'Turkish'; tobacco necrosis virus (TNV), tobacco necrosis virus group, isolate from D. Roberts, *Nicotiana tabacum* L., 'Samsun NN'; and tobacco ringspot virus (TRSV), nepovirus, isolate from D. Roberts, *Nicotiana x edwardsonii* Christie and D.W. Hall (4) and *Nicotiana tabacum* L., 'Samsun NN'.

Preparation of the Clarified Viral Concentrates

The basic principles of the CVC procedures are illustrated by the flowchart in Figure 1.

The first procedure listed is the one used most often and is referred to as the "standard CVC procedure." Modifications, described as "variations," follow the standard procedure.

Standard CVC Procedure (See Figure 2 for Flowchart). The initial tissue extraction was done with a solvent system developed by Steere (27). (Note: throughout the procedure, all virus fractions were held in an ice bath except during manipulations and centrifugations.)