J. Bernal, personal communication, and our unpublished observation). Virion integrity is dependent upon protein–RNA interactions, and the virions are partially permeable, making them subject to degradation on exposure to low concentrations of ribonucleases ***(17)***. In addition, the virions are not stable to freezing. Hence, all steps of virus purification are done at 4°C. The half-life of purified virions is about 2 wk to 1 mo. Long-term storage of cucumoviruses is most reliable in the form of viral RNA, which is highly infectious, and very stable at temperatures of –20°C. Alternatively, many strains may be stably stored as virions in buffer C, plus 50% glycerol, at –20°C.

The method for virus purification described here is based on that published by Lot et al. ***(18)***, with modifications for PSV as in **ref. *19***, for TAV as in ***(20)***, and for the universal buffer system as in ***(3)***. *Nicotiana tabacum* (tobacco), *N. clevelandii*, or *N. benthamiana* are suitable hosts for propagation of most cucumoviruses, although some strains of PSV do not infect tobacco. CMV may also be propagated in *Cucurbita pepo* (zucchini squash), or *Cucumis sativus* (cucumber), and PSV may be propagated in *Vigna unguiculata* (cowpea). The RNA extraction method is based on that of Palukaitis and Zaitlin ***(21)***. RNA is very susceptible to degradation by ribonucleases. **Caution:** All glassware used for RNA should be baked overnight at 160°C, and gloves should be worn to prevent contamination by ribonucleases found on the skin.

Purification of cucumoviruses involves thoroughly grinding infected tissue in a buffer appropriate to the particular virus, followed by a chloroform extraction, and high-speed centrifugation through a sucrose cushion. Virus pellets are then resuspended and contaminants are removed by a low-speed centrifugation, followed by a second high-speed centrifugation. For very pure virus, a sucrose gradient may be utilized, but for most purposes the sucrose cushion is sufficient. The buffers given in **Subheading 3.1.** are standard buffers for CMV purification. Other buffers used for other cucumoviruses, as well as a universal buffer system, are given in **Subheading 4**. Viral RNA is readily purified from virus by the addition of SDS, and three extractions with phenol:chloroform.

2. Materials

2.1. Virus Purification

All buffers should be used at 4°C. Buffers are given for CMV. (*See* **Notes 1–4** for buffers for other cucumoviruses.)

1. CMV buffer A: 0.5*M* sodium citrate, pH 7.0, 5 m*M* EDTA, 0.5% thioglycolic acid (stored in liquid form at –20°C, and added just before use).
2. CMV cushion I: 0.5*M* sodium citrate, pH 7.0, 5 m*M* EDTA, 10% sucrose.
3. CMV buffer B: 5 m*M* sodium borate, pH 9.0, 5 m*M* EDTA, 2% Triton X-100.
4. CMV cushion II: 5 m*M* sodium borate, pH 9.0, 5 m*M* EDTA, 10% sucrose.
5. CMV buffer C: 5 m*M* sodium borate, pH 9.0, 5 m*M* EDTA. Autoclave.