(*see* Chapter 26). If the two genome components are encapsidated in particles which differ in their sedimentation coefficient, then it is possible to obtain preparations of virus particles enriched in either of the two RNAs by sedimentation through 10–40% sucrose in 0.07*M* phosphate buffer, pH 7.0. Further purification may be necessary, particularly if the RNA-2 component of the virus in question is in the lower size range. The M component, containing RNA-2, may be subjected to a second centrifugation through a sucrose gradient; RNA-1-containing B particles may be purified by isopycnic centrifugation in cesium chloride ***(19)***. RNA may then be extracted from pooled fractions containing the appropriate component. The preparations of RNA-1 and RNA-2 thus obtained may then be further purified by a final electrophoretic separation through agarose or polyacrylamide gel. To obtain a sample of RNA-1 without contaminating RNA-2, a preliminary particle separation will almost certainly be necessary.

10. A mixture of pronase and SDS has been used by some in RNA isolation (**Subheading 3.2., step 1**). However, if infectious RNA is required, the use of a proteolytic enzyme is not recommended. There is a possibility that the 5'-terminal VPg will be removed from the viral RNAs.
11. It is possible to use a prepared phenol:chloroform mix in **Subheading 3.2., step 2**. However, my experience is that a two-stage procedure as described gives better results.
12. The addition of a small quantity of silicone grease (**Subheading 3.2., step 4**), which collects at the interface between the organic and aqueous phases during centrifugation, greatly facilitates the collection of the aqueous layer without contamination.

References

1. Murant, A. F. (1981) Nepoviruses, in *Handbook of Plant Virus Infections and Comparative Diagnosis* (Kurstak, E., ed.), Elsevier/North Holland, Amsterdam, pp. 197–238.
2. Roberts, I. M. (1988) The structure of particles of tobacco ringspot nepovirus: evidence from electron microscopy. *J. Gen. Virol.* **69,** 1831–1840.
3. Murant, A. F., Taylor, M., Duncan, G. H., and Raschke, J. H. (1981) Improved estimates of molecular weight of plant virus RNA by agarose gel electrophoresis and electron microscopy after denaturation with glyoxal. *J. Gen. Virol.* **53,** 321–332.
4. Mayo, M. A., Harrison, B. D., Murant, A. F., and Barker, H. (1973) Cross-linking of RNA induced by ultra-violet irradiation of particles of raspberry ringspot virus. *J. Gen. Virol.* **19,** 155–159.
5. Greif, C., Hemmer, O., and Fritsch, C. (1988) Nucleotide sequence of tomato black ring virus RNA-1. *J. Gen. Virol.* **69,** 1517–1529.
6. Demangeat, G., Greif, C., Hemmer, O., and Fritsch, C. (1990) Analysis of the in vitro cleavage products of the tomato blackring virus RNA-1-encoded 250K polyprotein. *J. Gen. Virol.* **71,** 1649–1654.