

table. Although the UF staining solution can be used in combination with any of the procedures described in Appendix 2, it is most useful with virus preparations that have undergone some degree of purification. UA will delineate the gross morphology of virions better than UF, but UF is peerless in the elucidation of virion ultrastructure.

**Wetting Agents for Negative Stains.** A great variety of additives have been suggested as wetting agents for negative staining, but the mainstay for many years has been highly purified BSA (Fraction V,  $5\times$  crystallized). In spite of the popularity of BSA, bacitracin is certainly superior to it in every respect. (a) Bacitracin is a much smaller molecule than BSA and is not resolved at high magnifications, while BSA produces a distinct and distracting pebbly background. (b) Bacitracin is a more efficient wetting agent than BSA, and may therefore be used effectively at lower concentrations. (c) Bacitracin is compatible with all of the commonly used negative stains, including the highly acidic UA, and to some degree with UF, whereas BSA is incompatible with these stains.

Bacitracin may coagulate if added to acidic solutions in dry form, and for that reason it is best to have aqueous stock solutions available. The degree to which proteinaceous wetting agents may compete with virions for grid space has not been studied, but it seems a wise precaution to abstain from adding either BSA or bacitracin directly to virus solutions. For a thorough discussion on the use of bacitracin as a wetting agent for negative stains, consult Gregory and Pirie (10).

**Washing Grids with Bacitracin.** Grids mounted with purified virus preparations may be virtually unwettable, especially at high dilutions, because the sap components that normally confer wettability to the grid surface have been removed in the purification process. If the washing solutions are repelled by the grid substrate, the final results may be unacceptable, even when there is a wetting agent in the stain and the stain seems to spread normally. The grid surface may appear to have a large number of large, angular, dark objects present, and the virus itself may stain poorly, or not at all. We have found that, in these situations, excellently stained grids can be produced by adding bacitracin to the washing solutions, and especially the final water wash. For washing purposes we have used concentrations of bacitracin ranging from 100–300  $\mu\text{g/ml}$  successfully, and the highest concentration has consistently produced the best results. For reasons given in the previous section, we have not added the bacitracin directly to the virus preparations.

**Storage of Negative Stains and Wetting Agents.** General practice is to prepare the stains complete with wetting agent, refrigerate them, and to retain them either for a set period of time or until contaminating growths appear in them. So, although the principal