problem with refrigeration is that storage time is limited because of contamination with various organisms, some stains, such as UA, tend to gradually precipitate at low temperatures, which results in a decline in stain concentration. Some stains, such as PTA, can be made up and adjusted for pH, complete with wetting agent, and then be freeze-dried in aliquots; which will reduce waste and preparation time. However, other stains are not amenable to such treatment.

An alternative storage technique, and one that has general application to stains, wetting agents, buffers, and washes, is to pass these solutions through sterilizing filters into sterile containers, and to store them all at room temperature. We have found it convenient to use membrane sterilizing filters, of the type that is mounted on a hypodermic syringe, to prepare suitable quantities of these solutions. If the quantities needed are anticipated, then there need be little waste. As an example, we pass negative stains through filters with a 0.2-μm pore size, store aliquots of 2–3 ml in small sterile test tubes, and keep them in a dark cabinet at room temperature. Adjustments to pH and the addition of wetting agents are made prior to filtration.

Negative Staining of Virions

Materials Needed. *Grids with fine mesh, 200–400, 75 × 300, or 100 × 400, for example.* Copper grids are most commonly used, and are available with one side rhodium-plated for purposes of orientation. The grids should be clad with a plastic substrate such as Formvar that is coated with carbon.

Double-sided (adhesive on both sides) cellophane tape.

Blotting paper cut into squares of approximately 2.5 inches (6.5 cm). This paper is available in large sheets or may be found as individual ink blotters.

Pasteur pipettes of the 5.75-inch (15-cm) length, with 1-ml rubber bulbs.

Small beakers for mixing and diluting. Generally, disposable plastic beakers of 5-, 20-, and 50-ml capacities are sufficient; although, of course, glass beakers may be used.

Washing solutions. Quite often a buffer used for the final resuspension of the virus is used. Water, or an aqueous solution of bacitracin is used as the final wash before staining.

The following items are needed only when crude extracts such as leaf dips are to be mounted and stained: (*a*) glass microscope slides (an economy grade will do); (*b*) razor blades; (*c*) wooden toothpicks (the round type work best); and (*d*) extraction buffers.

If the virus to be mounted is known, a buffer known to be compatible with that virus should be used. Otherwise, a general purpose