second droplet of diluted antiserum and allow it to remain standing on the grid for 1 hr. Make the droplet as large as practical without forcing the droplet to spread, and cover it with the lid of a Petri dish. Check the grid occasionally, adding buffer if necessary, to insure that the droplet does not dry.

Wash the grid with 30–35 drops of SP buffer (if a Pasteur pipette has a 1-ml bulb attached, this would be one full pipette).

Remove all but a thin film of buffer, wash the grid with 1 drop of prepared antigen, and apply a large droplet of the antigen. Cover, and allow the antigen to remain on the grid for 3 hr. Check frequently, and add buffer if it is necessary to prevent the grid from drying.

Wash the grid with 30–35 drops of SP buffer.

Wash the grid with 10 drops of distilled or deionized water.

Wash the grid with several drops of uranyl acetate, removing all but a thin film of stain, and then allow the grid to dry.

2. The Decoration of Virions with Antibodies. Particles are attached to the grid surface using any of the procedures above, washing as called for, continuing with the steps below following the final washing with buffer, but prior to the water wash and staining steps. The Derrick procedure may be used to enhance virion numbers, but the final resolution and contrast of the decorated virions will be better if a nonserological method is used to attach the virus particles to the grids. The best decoration will be realized using purified virus, but virions obtained by one of the CVC procedures will often serve nearly as well.

Apply a droplet of an appropriate antiserum, diluted 1:20 with SP buffer, to the grid (virions have already been attached to the grid and it has been washed with buffer) and allow it to remain for 5 min.

Wash the grid with 20 drops of SP buffer.

Wash the grid with 10 drops of water.

Wash the grid with 2 or 3 drops of UA, allowing it to dry after having removed all but a thin film of the stain.

Problems with Negative Staining.

Most failures to obtain clean, uniform, well-stained specimen grids result from inadequate washing. It is necessary to provide both a vigorous flow and to use sufficient quantities of the washing solutions. It is far better to wash the grids too much than to wash them too little. Use at least 1 ml of washing solution (add the droplets so that they fall on the grid rather than simply flow onto it). Change the blotting paper frequently enough to insure that it is able to readily absorb the solution, and draw the paper far enough away from the grid to promote a vigorous flow of the washing solution across the