slide as before, but use some 95% ethanol to clean it more thoroughly, and submerge it deeply enough in the detergent solution to cover the area from which the film will be produced. Allow it to remain in the detergent solution for at least 5 min. Drain the slide vertically on filter paper, and prop it up to air dry. It will take 15 min or longer to dry, but of course several slides can be processed together. When the slide is dry, polish it with tissue to remove the powdery surface residue. At this stage, the slide should look clean and shiny and have a waxy surface. Submerge the slide into Formvar solution and proceed as described before, except that it will probably not be necessary either to scrape the edges of the slide or to breathe on the film to release it.

7. Carbon Coating the Grid Substrates. The exact set-up used for carbon coating depends on the particular evaporator used, but some general precautions may be useful.

Plastic substrates such as Formvar require a stabilizing coating to keep them from squirming and tearing when bombarded by electrons, as in the beam of an electron microscope. Carbon films will disperse heat rapidly and are remarkably transparent to electrons, as well as being chemically inert; thus, they are admirably suited as coatings for stabilizing Formvar and other plastic substrates. Although any substance in the beam path of an electron microscope will reduce illumination and decrease contrast, the relative electron transparency of carbon permits fairly heavy applications to be used. Any loss of contrast and brightness due to carbon coating may be recouped by keeping the thickness of the plastic substrate to the absolute minimum required to provide enough mechanical support to span the areas between the grid bars.

Grids used with negative stains generally require more carbon than those used to support ultrathin sections, replicas, or metal-shadowed particulate specimens. The extreme contrasts encountered when illuminating negatively stained grids produce enormous differentials that stress the plastic and cause distortion and tearing of the substrate. Thin carbon coatings may be insufficient to deal with this situation. For improved stability, we apply a carbon coating that may be regarded as excessive by others. We expected to sacrifice some resolution to achieve stability, but surprisingly, the resolution seems as good as with thinner carbon coatings. Resolution in electron microscopy of negatively stained virions is severely limited by the characteristics of negative stains; so other factors such as the electron scattering caused by the density of the specimen and its supporting medium are generally of lesser importance in achieving good resolution. This argues in favor of applying the carbon heavily enough to achieve a substrate that is stable in the electron beam.