buffer or water should be used. Phosphate buffers are generally suitable for a wide range of viruses. Molarities in the 0.05 to 0.1 range with a pH range of 6.5 to 7.5 are suitable.

Negative stains. PTA, UA, and UF as prepared above are recommended, but other stains such as ammonium molybdate may be used.

The Staining Procedure (See Figure 11)

Attach a length of the double-coated cellophane tape to a microscope slide, parallel to the long axis of the slide and overlapping one side (the right side for right-handed workers) by 2–3 mm. If the tape is longer than the slide, it will conveniently anchor the slide in place. We mount the slide on a large piece of filter paper so that spills will be absorbed, and to provide a writing surface to label the grids. Position the grids along the length of tape, slightly overlapping the bottom surface of each grid on the upper surface of the tape, pressing it hard enough to barely hold it in position. Too firm an attachment will make the grid very difficult to remove without causing severe damage to the grid or to the substrate. Do not crowd the grids along the tape as cross-contamination with the contents of neighboring grids is a distinct danger. Seven to eight grids/slide is reasonable, but experience and personal preference will dictate the exact number.

Mounting the Virions on the Grid. *CVC preparations or purified virus suspensions.* Apply a droplet to the grid and allow it to stand for 1 min. There is usually no benefit to be obtained by longer standing, except perhaps in the case of very dilute suspensions. Conversely, there are usually no problems associated with long standing, as the grids are washed of excessive material before staining.

Leaf dips. The term leaf dip originally applied to the practice of dipping the cut edge of leaf into a negative stain droplet placed directly on a specimen grid, and then removing all but a thin film. Nowadays, however, it generally refers to any procedure that negatively stains crude leaf extracts, no matter how they are derived. The method we have normally used is to place a small piece of leaf blade 6–8 mm^2 in 5–6 drops of buffer on a microscope slide which has been polished clean, and chop it a number of times with a razor blade. The idea is to expose a great number of cut edges to the buffer. To facilitate this, the leaf piece may be chopped with a number of parallel cuts while holding it in place with a toothpick, and then rotating the tissue through 90° for further chopping. If gentle vertical chopping is used rather than a slicing motion, the leaf tissue will often hold together instead of yielding many tiny bits of tissue—which will need to be coped with when drawing up the extract. (If, in