grid. Symptoms of poorly washed grids include densely stained backgrounds and unsharp imaging. In general appearance they are dark, murky, and obscure, and in extreme cases may have a cracked appearance, much like a dry mud flat.

Crystals occur in some plant saps, but others seen on negatively stained grids may result from incomplete removal of buffer prior to staining because of the inadvertent omission of the water washing step, or to crystals of stain that formed at the tip of a Pasteur pipette on standing. These stain crystals are produced by evaporation and suggest that the stain has been held in the pipette too long, usually overnight. The remedy for this problem obviously is to replace both stain and pipette. Stain crystals are generally very dense, but with the exception of those produced from UF, they may not be immediately recognizable as such. UF crystals may often be elongated hexagons.

Another factor preventing the uniform staining of specimen grids is poor wettability of the grid surface. Although the incorporation of a wetting agent such as bacitracin into the final staining solution will usually insure a uniform stain deposit, alone it may be insufficient to produce good wettability. Grids that contain artifacts, those that have little or no background stain, or those on which are discovered fewer virions than are expected may suffer from this problem. The best cure we have found for this problem is to incorporate bacitracin into the washing solutions (at a rate of 300 μg/ml).

However, bacitracin or other proteinaceous wetting agents probably should not be added at an earlier stage because of possible grid site competition with virions. Grids mounted with crude preparations such as from leaf dips will only suffer from poor wettability in unusual cases; routine washing with bacitracin solutions need only be used for purified virus preparations or for CVC preparations of outstanding clarity. This problem of grid wettability is most severe when the samples are highly dilute.

A problem with similar symptoms is not caused by poor wettability, but rather by the presence of substances that interfere with staining. If these are used in the procedure they must be sufficiently removed to prevent unwanted side effects. Examples of such substances are PEG, sucrose, and Triton X-100. Indeed, Triton could prove to be very useful in the CVC procedure if subsequently it could be removed. When Triton X-100 is used for full scale purifications, then centrifugation on density gradients seems to remove it sufficiently for electron microscopy. Other contaminants, such as cesium chloride, solvent traces, and buffers, may present no staining problems whatsoever, if the grids are sufficiently washed prior to staining.