

ultrastructure. Common practice is to prepare fresh solutions of UF whenever they are needed and to use them without a wetting agent. Other techniques, such as exposure to a glow discharge plasma, may be used to render the grids hydrophilic.

These difficulties tend to discourage the use of UF. We have found however, that the principal limitations of UF can be overcome, and that solutions of UF can be both stored and combined with a wetting agent for negative staining applications. UF is soluble in methanol (methyl alcohol) and stable in methanol solution for several months if it is protected from light. Stir the desired quantity of methanol with an amount of UF powder that exceeds the solubility limit of the solvent. Arrange to do the stirring in darkness. If a magnetic stirrer is used, it can be completely covered with a box, or the container can be wrapped. Check occasionally to see if more UF powder should be added, and continue stirring for about 1 hr. Absolute saturation is not necessary, and probably not even desirable. The aim is to produce a concentrated stock solution, and the solubility of UF in methanol is probably at best 4% at 20°C (solubility of UF powders varies from one supplier to another).

When the stirring is completed, allow the solution to settle for a few minutes, and then transfer aliquots to small test tubes without disturbing the sediment. Seal the tubes tightly and store in a light-tight container at room temperature until needed. This stock solution should range in color from a deep canary yellow to orange. It will remain free of precipitates for several months, and thereafter a white precipitate will gradually form. But, since this precipitate will settle as it forms, it will cause no problems, and the stock solution can be kept in use until it becomes too weak to stain properly.

The negative staining solution should be prepared from the UF stock solution just prior to use. Put a few drops of stock solution in a small beaker, the well of a spot test plate, or a similar small container and dilute it dropwise with water until the color is close to that of a similar quantity of UA used for comparison. If a wetting agent is needed, then after the correct color has been achieved, carefully swirl in a drop of bacitracin solution (300 $\mu\text{g/ml}$) for every 6 or 7 drops of stain (the exact final concentration of bacitracin is not critical). If the solution turns cloudy after the bacitracin has been mixed in, discard it and start anew. Do not add the bacitracin solution to undiluted UF stock solution, as it will surely precipitate. Use the stain within a few minutes of preparation.

The procedure is simple, and fresh UF stain can be made from the stock solution in a few seconds. Often, satisfactory grids may be prepared without the addition of a wetting agent, but the bacitracin works well for the staining of those grids that are otherwise unwet-