APPENDIX 2

Negative Staining Techniques

Negative Stains and Wetting Agents

A satisfactory negative stain must dry without crystallization, be reasonably inert chemically, be sufficiently dense to electrons to lend good contrast to biological specimens (which are usually electron transparent), be soluble in water or a solvent miscible with water, and preferably be compatible with a suitable wetting agent (see Figures 9 and 10 for a comparison of stains and wetting agents). Negative staining results may be influenced by the source and age of the chemicals.

Phosphotungstic Acid (PTA). PTA is the most frequently used negative stain for electron microscopy of biological extracts. Desirable features of PTA include high contrast, ready solubility in water over a wide range of pH values, and compatability with bovine serum albumin (BSA), a commonly used wetting agent. The undesirable features of this stain include an incompatability with some proteins, a susceptibility to electron beam damage, a hygroscopic nature, and a failure to delineate fine structure as well as other stains, such as the uranyl stains (see Figures 9 and 10). The usual method of preparation used for PTA is to adjust a 2% aqueous solution to pH 6.5 to 7.0, and add Fraction V BSA to a concentration of 250 μg/ml. If bacitracin is used instead of BSA, then resolution will be increased slightly, and the background will be smoother and cleaner. PTA may also be used at lower pH values to minimize its destructive effect on some proteins.

Uranyl Acetate (UA). This stain is superior to PTA in many respects, and if it is properly applied, it may be used in most situations as the stain of choice. UA has not usually been used for crude extracts because of its extremely low pH value, which causes many sap components to coagulate and cannot be altered without causing the stain to precipitate. This low pH value also makes UA incompatible with BSA. As plant saps curdle in the UA staining solution, it cannot therefore be used to make leaf dips in the traditional way, i.e. by extracting the tissue with the stain itself. However, if the tissue is extracted with buffer, water, etc. before adding the stain and the extract is applied to the grids, which are subsequently washed, then the UA can be applied to these grids without causing damage, and smooth, well stained grids will usually be produced, exhibiting good contrast and specimen detail.

Grids prepared with UA usually have a more pleasing overall aspect than do other stains. UA is far more stable in the electron