Introduction

Chemically fixed tissue should be thoroughly washed with a buffer solution before introducing a post-fixative or a dehydrating agent. This step minimizes the possibility of a reaction between various chemical reagents. It is also recommended that sucrose be added to the buffer to maintain the same osmolarity as the fixative.

Organic dehydration

Dehydration is the complete removal and replacement of all water in the sample with a solvent which is miscible with the final embedding media. Ethanol and acetone are the two most commonly used organic dehydrating agents. Shrinkage, commonly associated with dehydration, can be minimized by using either dehydrant in increasing concentrations. Cooling the dehydrating agent can reduce some cellular extraction, but care must be taken to eliminate possible water uptake (via condensation) in the higher concentrations. The time allotted to each successive increment should assume good exchange of the solutions but excessive dehydration will cause extraction of cellular materials. Finally, some embedding media are not or only partially miscible in either alcohol or acetone. This necessitates the introduction of an intermediary transitional solvent which is miscible with both dehydrating agent and embedding media. The two most frequently used transitional solvents are styrene with acetone or propylene oxide with alcohols.

Dehydration with water - miscible resins

Early attempts at developing water mis