Introduction

Fixation is an attempt at stabilizing biological systems for viewing in a vacuum with minimal distortion to their cytomorphology and cytochemistry. Biological electron microscopy generally employs two methods for obtaining these results, chemical and/or physical fixation. While chemical fixation remains the most common method of specimen preservation being less expensive, physical techniques (mainly cryo) are gaining in popularity. Certain procedures combining chemical and physical fixation are both useful and advisable.

Chemical fixation

Chemical fixation prepares the cell for a whole series of rather traumatic events imposed by the physical characteristics of the electron microscope, (i.e., improved resolution, extreme vacuums and the intense heat of the electron beam). Ideally, the reagent selected as a fixative should transform the viscous colloidal protein solution (protoplasm) into a stabilized material. Simultaneously, the spatial relationship of all organelles should be maintained; the phospholipids, which form the framework of the cell, should be stabilized and the remaining chemical constituents rendered insoluble. This rather large undertaking probably contributes to the popularly held notion that “the perfect fixative” has yet to be developed. There are, however, a number of fixatives, which are capable of performing at least some of the desired effects, provided appropriate steps, are taken to adjust certain physiological parameters to approach the cell’s natural milieu.

Rate of penetration

Determines the speed at which the reagent effects the cell’s structure and function. The rapidity with which this is accomplished is paramount to good fixation. High pressure freezing stabilizes the cellular structure in milliseconds. Formaldehyde penetrates the cell faster than glutaraldehyde. For further reading see “Principles and Techniques of Electron Microscopy Biological Applications” M. A. Hayat.

Buffers

Are used to maintain the desired pH regardless of the chemical nature of the fixative selected. Most animal cells are fixed at a pH of 7.3 - 7.4. Certain highly hydrated tissues prefer a more

Temperature

Fixation in this lab is at room temperature or for cells in culture at the temperature of the incubator. In some labs, fixation is initiated at a low temperature (e.g. 4OC) and allowed to slowly rise to room temperature. It is thought that reduced temperatures diminish “leaching” of cellular components and aid in preserving some enzymatic activity. At lowered temperatures, enzymatic activity is reduced, thus preventing catabolism. At room temperature, fixation occurs more rapidly and the cytoskeleton does not depolarize.

Size of sample

1.

preparations immediately upon completion of physical fixation. For example, certain unfixed fungi are capable of withstanding the rigors of scanning electron microscopy with no apparent effect on their viability.