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Simultaneous Glutaraldehyde-Osmium Tetroxide Fixation with Postosmication

An Improved Fixation Procedure for Electron Microscopy of Plant and Animal Cells

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Summary. A fixation procedure for electron microscopy is described which includes a simultaneous glutaraldehyde-OsO_4 fixation followed by postosmication. This procedure was found to have considerable advantages in preserving structures of plant and animal cells.

Introduction

A great amount of the present knowledge on cellular ultrastructure is based on electron microscopic studies which employ a sequential fixation first with aldehydes, such as formaldehyde, glutaraldehyde or acrolein, followed by washing and a second fixation step with osmium tetroxide. This sequential "double fixation" has become the classical fixation scheme in EM laboratories since the pioneering work of SABATINI, BENSCH and BARNETT (1963). During the course of fixation experiments designed to avoid some of the disadvantages of this sequential fixation, for example, the disappearance or rearrangement of microtubules when fixed in the cold, frequent myelinization of lipid material, a more dispersed pattern of ribosomes, and a frequently occurring finely granular precipitation, we found a fixation procedure which had some remarkable advantages. This method now serves as our standard fixation for all kind of biological material.

The early attempts of DANNEEL and WEISSENFELS (1965) who used a mixture of OsO_4/glutaraldehyde/phosphotungstic acid, adjusted to pH 7.0, were obviously unsuccessful as can be seen from the micrographs published. Our procedure was developed as a modification of their work and that of TRUMP and BULGER (1966 and also from remarks made by HIRSCH and FEDORKO (1968) who increased the contrast, especially that of membranes, by using a "postfixation" with an aqueous uranyl acetate solution. While DANNEEL and WEISSENFELS as well as TRUMP and BULGER used a relatively high concentration of aldehyde (6.25%) which accelerates the discoloring interaction of both the fixing redox reagents we employed lower concentrations as suggested by HIRSCH and FEDORKO.

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Materials and Methods

Equal parts of ice cold stock solutions of glutaraldehyde (2 or 4%) and OsO_4 (2%), both buffered with 0.1 M cacodylate to a pH of 7.0, were mixed and immediately used for fixing. Fixation times were varied from 30 min to 1 hr. Particular care was taken with temperature conditions so that all fixation and washing steps were carried out by either keeping the fixation vessels in an ice bath or by working in a 5°C cool-room. At these conditions no observable colorization or precipitation caused by reduced osmium compounds takes place. Then after washing the material several times with chilled 0.1 M cacodylate buffer (pH 7.0) a postosmication was performed using 2% OsO_4, also buffered with 0.1 M cacodylate, for 3 hrs at 5°C. Further washing steps, dehydration, embedding in epoxy resins and poststaining of the sections were as conventional.

Results

This fixation method reveals an ultrastructural appearance generally comparable to that obtained after the sequential double fixation; however, the simultaneous fixation with postosmication proved to be superior with respect to: (1) contrast distinctiveness of membranes and other lipid-containing structures; (2) preservation of nucleoprotein-containing structures as polyribosomes and chromatin strands; (3) preservation of cytoplasmic microtubules even after fixing at low temperatures; and (4) the high staining of any polysaccharide-containing structures. Micrographs which document the quality of preservation achieved with this procedure have recently been published in other contexts (e.g. FRANKE and SCHINKO, 1969; FALK, 1969a, b; FRANKE, KRIEN and SAMEK, 1969).

Of considerable importance seems to be the preservation of polyribosomal structure by the method described. Thus, in a great many plant materials, e.g. bean leaves, cells of the algae *Tetracystis* and *Botrydium*, in which after sequential fixation the cytoplasmic and plastidal ribosomes appeared predominantly "free", i.e. unassociated, this procedure, in fact, showed them to exist in the polyribosomal state (FALK, 1969a).

The routine appearance of cytoplasmic microtubules after simultaneous fixation in the cold was another unexpected find which strongly suggests that fixation temperature alone might not be responsible for their preservation but rather the kind of fixation employed (FRANKE, KRIEN and SAMEK, 1969).

No precipitation contaminants have been encountered with the simultaneous fixation, whereas they are frequently observed in material sequentially fixed at room temperature.

Although many more materials will need to be tested to ultimately prove the universal effectiveness of this method, the results so far obtained were excellent with cyanophycean algae, freshwater chlorophycean and xanthophycean algae, chrysophycean algae, fungi, lichens, roots, stems, and leaves of higher plants, mammalian liver, skeletal muscles, adrenal cortex, amphibian ovaries, and a diversity of isolated subcellular fractions. An example of the value of this fixation method was the excellent fixation in *Tetrahymena pyriformis* (WUNDERLICH and FRANKE, 1969), an organism long-known to exhibit peculiar fixation and preservation problems (see e.g. WILLIAMS and LUFT, 1968).

Note added in proof. Other laboratories which were informed by us on this method yielded equally good results [e.g. B. S. C. LEADBEATER and I. MANTON, Arch. Mikrobiol. **66**, 105—120 (1969)].

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