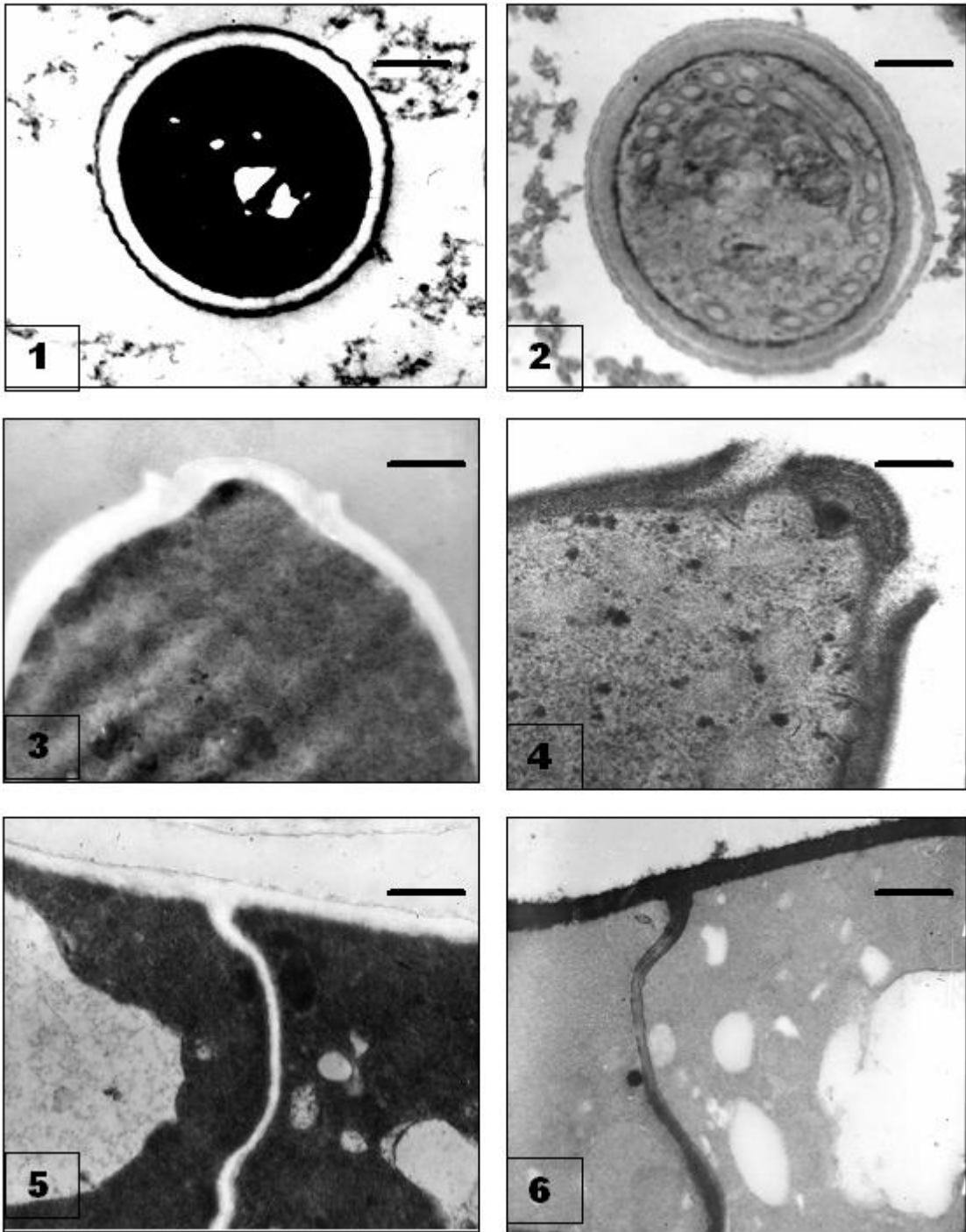


USE OF RUTHENIUM TETROXIDE IN ELECTRON MICROSCOPY STUDIES OF CELL WALLED MICROORGANISMS Orlando Torres-Fernández, Laboratorio de Microscopía, Instituto Nacional de Salud, Bogotá, Colombia. E-mail: otorresf@ins.gov.co

The two-step fixing procedure, glutaraldehyde followed by osmium tetroxide (OsO_4), continues to be the preferred method for electron microscopy (EM) studies in biological samples. Ruthenium tetroxide (RuO_4) is a neglected fixative because it decomposes rapidly and has poor tissue penetration. However, RuO_4 has been used as a fixative in ultrastructural studies of stratum corneum from skin, where it is able to fix lipid intercellular layers invisible by EM using OsO_4 . These layers contain saturated lipids which constitute the dermal permeability barrier (1). Some microorganisms, mainly microsporidia (Protozoa) and fungi possess resistant and impermeable chitinous cell walls. The microsporidian spore wall (MSW) and the fungal cell wall (FCW), viewed by EM, appear to be transparent when postfixed with OsO_4 (2,3). In this work the effect of OsO_4 and RuO_4 postfixing on MSW and FCW ultrastructure was compared. Two microsporidian species were found infecting aquatic insect larvae (Trichoptera and Diptera-Simuliidae). From each larvae species, minute fragments of infected adipose tissue were studied. *Saccharomyces cerevisiae* yeasts, maintained in distilled water at 4 °C for several weeks, and *Penicillium* sp. mycelia, growing on decaying tangerine, were also used for this study. All samples were first fixed for 24 h in 3% glutaraldehyde. Three groups of samples from each microsporidian and fungal species were separately postfixed in the following solutions: (a) 1% OsO_4 , 1 h; (b) 0.2 % RuO_4 , 1h; c) 1% OsO_4 1 h, followed by 0.2% RuO_4 , 1 h. All fixatives were buffered in 0.13 M phosphate buffer. The same buffer was used for rinsing after each fixation step. The samples were then dehydrated in a graded ethanol series and embedded in Epon-Spurr mixture. Thin sections were first examined unstained, then stained with uranyl acetate and lead citrate and examined with a Zeiss EM 109 electron microscope. Both MSW and FCW showed inner translucent wide layer bordered by a thin electron-dense layer (Figures 1,3,5) when they were postfixed with OsO_4 . In the samples fixed with RuO_4 or OsO_4 followed by RuO_4 , MSW and FCW were observed to be filled with an electron opaque material (Figures 2,4,6). This was the prominent feature showed by RuO_4 postfixing. Some additional ultrastructural differences between microsporidia species or fungi species were observed. RuO_4 postfixed samples required no grids staining to make visible the ultrastructural features of MSW or FCW. The electron transparency of these structures may be due to deficient fixation by conventional methods with OsO_4 . Microsporidia and fungi are structurally and phylogenetically related microorganisms. The polysaccharide chitin is the main component of their cell walls (4,5). As RuO_4 is a stronger oxidizing agent than OsO_4 it probably reacts better with chitin molecule hydroxyl groups. The MSW and FSW also contain saturated fatty acids (5,6). Non- OsO_4 -fixed saturated lipids would be embedded within chitinous structure of the cell wall as barrier lipids with function similar to that of saturated lipids from the stratum corneum of the skin. Because OsO_4 fixes unsaturated lipids whereas RuO_4 reacts with both saturated and unsaturated molecules (1), the electron opacity observed on the MSW and FCW following postfixing with RuO_4 may be explained by the ability of this fixative to react with chitin and saturated lipids. Differences in the electron density in each of the cell wall layers and ultrastructural differences between distinct microsporidian and fungal species suggest different polysaccharide and lipid distributions. These findings might be very useful for diagnosis, taxonomy, and studies on cell wall composition, as well as for evaluating the effect of microbicidal agents.

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Figures 1-2. Microsporidian spores of Trichoptera larvae. **Figures 3-4.** Budding yeasts of *Saccharomyces cerevisiae*. **Figures 5-6.** *Penicillium* sp. hyphae. Figs 1, 3, 5. Samples fixed with OsO₄. Figs 2, 4, 6. Samples fixed with RuO₄. Scale bars: Fig 1 = 0.50 μm ; Fig 2 = 0.45 μm ; Fig 3 = 0.30 μm ; Fig 4 = 0.38 μm ; Fig 5 = 0.54 μm ; Fig 6 = 0.75 μm .