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Materials and Methods for EM Samples

(from Dykstra, M.J. 1993. *A Manual of Applied Techniques for Biological Electron Microscopy*. Plenum Press, NY. 257p.

Materials and Methods for Routine TEM Preparation

Tissues were excised, cut to 1 mm thickness in at least one dimension and placed in McDowell's and Trump's 4F:1G fixative (1) for 1 hr to several months at 4^{o} C. After 2 rinses in 0.1 M sodium phosphate buffer (pH 7.2), samples were placed in 1% osmium tetroxide in the same buffer for 1 hr at room temperature. Samples were rinsed 2 times in distilled water and dehydrated in an ethanolic series culminating in two changes of 100% acetone. Tissues were then placed in a mixture of Spurr (2) resin and acetone (1:1) for 30 min, followed by 2 hr in 100% resin with 2 changes. Finally, samples were placed in fresh 100% resin in molds and polymerized at 70^{o} C for 8 hrs to 3 days. Semi-thin (0.25-0.5 um) sections were cut with glass knives and stained with 1% toluidine blue-O in 1% sodium borate. Ultrathin (70-90 nm) sections were cut with a diamond knife, stained with methanolic uranyl acetate followed by lead citrate and examined with a transmission electron microscope.

1. McDowell, E.M., and B.F. Trump. 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med., 100:405-414.
2. Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res., 26:31-43.

Materials and Methods for Routine SEM Preparation

Tissues were excised and placed in McDowell's and Trump's 4F:1G (1) fixative for 1 hr to several months at 4^{o} C. After 2 rinses in 0.1 M sodium phosphate buffer (pH 7.2), samples were dehydrated in a graded ethanol series to 100% ethanol at which time they were critical point dried with liquid CO_2. Finally, they were mounted on specimen stubs with colloidal silver, sputter-coated with gold-palladium and examined with a scanning electron microscope.

1. McDowell, E.M., and B.F. Trump. 1976. Histologic fixatives suitable for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med., 100:405-414.