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Permanent Light Microscopy Slides of *Eimeria nieschulzi* Oocysts

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Flotation techniques commonly used in recovering coccidian oocysts from fecal debris for examination and study cause plasmolysis and collapse of the inner oocyst wall on prolonged contact with saturated salt or sugar solutions (Davis, 1973, *in* Hammond and Long (eds.), The Coccidia, Univ. Park Press, Baltimore, p. 411–458). Although the coverslip can be sealed with Vaseline to prevent drying, oocysts only retain their structural integrity in these solutions for short periods (~12 hr or less). Therefore, slides obtained by flotation techniques have limited usefulness for studying oocysts, especially when the number of parasites is small. We describe here two methods for the preparation of light microscopy slides of *Eimeria nieschulzi* oocysts.

Suspensions of sporulated oocysts were treated prior to fixation by six different methods: 5% CO_2 at 37 C for 30 min, 1 hr, 2 hr; 30% hypochlorite solution at 22 C for 1 hr; Lillie solution (Humason, 1972, Animal Tissue Techniques, Freeman, San Francisco, p. 31) at 22 C for 24 hr; or 4% EDTA (Hayat, 1972, Basic Electron Microscopy Techniques, Van Nostrand, New York, p. 23) at 22 C for 24 hr. Each treated oocyst suspension was then fixed by the following agents for 18 hr at 22 C: 10% (v/v) aqueous acrolein; 4% formalin; 3 or 15% (v/v in 0.2 M cacodylate buffer) glutaraldehyde with and without postfixation in 2% (w/v in 0.2 M cacodylate buffer) OsO_4 for 2 hr; and Karnovsky's fluid (Karnovsky, 1965, J Cell Biol **27:** 137A–138A) with and without postfixation in 2% OsO_4 (as above).

A suspension of oocysts from each of the treatment-fixative combinations was collected on a 1 mm^2 piece of 0.45 mμ Millipore filter (Millipore Filter Corp., Bedford, Mass.) and covered with a thin layer (~1 mm) of 1.5% (w/v) Bacto-agar (Difco Laboratories, Inc., Detroit, Michigan) at 45 C. Samples from each combination were stained at 22 C for 6 to 8 hr with the following: 5% aqueous (w/v) acid fuchsin; 0.1% aqueous (w/v) acridine orange; 1% aqueous (w/v) alcian blue; 1% aqueous (w/v) gentian violet; 1% aqueous

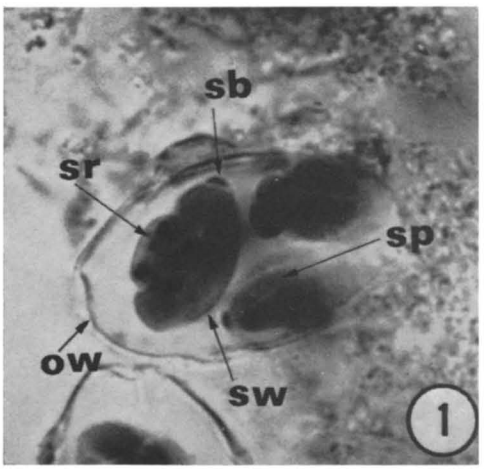

FIGURE 1. Photomicrograph of sporulated *Eimeria nieschulzi* oocyst, × 2,300. Prepared by Method 1 (see text); note sporocyst showing Stieda body and sporocyst residuum. Abbreviations: ow, oocyst wall; sb, Stieda body; sp, sporozoite; sr, sporocyst residuum; and sw, sporocyst wall.

(w/v) methylene blue-azure II; Paragon Multiple Stain (Paragon C and C. Co., Inc., 190 Willow Avenue, Bronx, New York); 2% aqueous (w/v) safranin O; and 0.5% aqueous (w/v) thionin. The samples were dehydrated in ethanol and two changes of propylene oxide and embedded in one of the following: Araldite-502; Epon-812; Epon-812 : Araldite-502 (60 : 40); Glycol methacrylate; or Spurr's medium (Spurr, 1969, J Ultrastruct Res **26:** 31–43) using standard infiltration techniques (Hayat, 1970, Principles and Techniques of Electron Microscopy: Biological Applications, Vol. 1, Van Nostrand-Rienhold, New York, p. 145–179). Samples were placed on 25 × 75 mm glass microscope slides and a 22 mm^2 glass coverslip was affixed with media which was polymerized at 45 C for 18 hr.

Two combinations of methods were found to be superior to produce permanent light microscopy slides of *E. nieschulzi* oocysts: 1. Oocysts treated with 5% CO_2 at 37 C for 30 min, fixed in 15% glutaraldehyde at 22 C for 18 hr, postfixed in 2% OsO_4 at 22 C for 2 hr and embedded in Epon-812. 2. Oocysts treated with 5% CO_2 at 37 C for 30 min, fixed in Karnovsky's fluid at 22 C for 18 hr, postfixed in 2% OsO_4 at 22 C for 2 hr and embedded in Spurr's medium.

Oocysts treated by these two methods were morphologically similar to normal oocysts when examined by light microscopy. The percent shrinkage in oocysts and sporocyst dimensions from treatment by the two methods was $\leqslant 11\%$, 15 months after polymerization.

Although OsO_4 was not always necessary as a fixative, postfixation increased cellular detail and oocyst contrast by acting as a structural stain especially to the Stieda body and sporocyst residuum (Fig. 1). The sporozoites were also stained by the osmium.

None of the dyes used stained oocyst structure; however, the agar retained some stain and provided a background for the oocysts. The Millipore filter was rendered transparent by the propylene oxide during the dehydration process.

Oocysts embedded in Epon-812 and Spurr's media had 91% and 89% intact sporocysts, respectively, with good to excellent cellular detail. Spurr's medium became dark yellow in color after polymerization but Epon-812 hardened colorless. All media tested caused moderate bubble formation during polymerization.

The methods described here allow for the preparation of light microscopy slides of coccidian oocysts and thus, provide a durable sample and record for research and teaching. These methods used only one species, *E. nieschulzi*; however, it is likely that use of other coccidian oocysts would produce similar results.

Millipore filters have been utilized as a solid substrate for cell cultures and viruses in ultrastructural studies (McCombs et al., 1968, J Cell Biol **36:** 231–243). The methods described here have modified this procedure for use in light microscopy. In addition, these methods may be used to embed coccidia for electron microscopy instead of pelleting the organisms with tissue culture cells (Speer and Duszynski, 1975, J Protozool **22:** 476–481).

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