SUPRAVITAL STAINING WITH METHYL GREEN-PYRONIN

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INTRODUCTION

This simple method was described by Foissner (1). It is an excellent technique for revealing the mucocysts of most ciliates (those of tetrahymenids, however, usually do not stain). Mucocysts are stained deeply and very selectively blue or red, and can be observed in various stages of explosion because the cells are not killed instantly. The nuclear apparatus is also stained. Examples: Fig. 1 - 4.

PROTOCOL

- 1. Pick out desired ciliates with a micropipette and place the small drop of fluid in the centre of a slide.
- 2. Add an equal sized drop of methyl green-pyronin and mix the two drops gently by swivelling the slide.

Remarks: If ciliates were already mounted under the coverslip then add a drop of the dye at one edge of the coverslip and pass it through the preparation with a piece of filter paper placed at the other end of the coverslip.

3. Place a coverslip with vaselined corners on the preparation.

Remarks: Observe immediately. Cells die in the stain within 2 minutes. Mucocysts stain very quickly and many can be observed at various stages of explosion. To reveal the nuclear apparatus, cells should be fairly strongly squashed (=flattened). The preparation is temporary. After 5-10 minutes the cytoplasm often becomes heavily stained and obscures other details.

REAGENTS

1 g methyl green-pyronin (Merck) ad 100 ml distilled water This solution is very stable and can be used for years

LITERATURE CITED

1. Foissner, W. 1979. Methylgrün-Pyronin: Seine Eignung zur supravitalen Übersichtsfärbung von Protozoen, besonders ihrer Protrichocysten. *Mikroskopie* **35**:108-115.



Fig. 1 - 4. Extrusomes stained with methyl green-pyronin. **1.** *Nassula picta*, a nassulid ciliate, secretes a slimy, voluminous, structureless coat when the stain is applied; Ma, macronucleus. **2.** *Phascolodon vorticella*, a cyrtophorid ciliate, secretes a membranous envelope (arrow) when the stain is added. **3.** Mucocysts of *Bursaria truncatella*, a colpodid ciliate. **4.** *Urotricha farcta*, a prorodontid ciliate, discharges trichocyst-like extrusomes which have a darker stained apical granule (arrows).