THE SILVER CARBONATE METHODS

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INTRODUCTION

Because of the large quantity of cells needed for the basic Fernández-Galiano (2) technique, Augustin et al. (1) proposed a modification which requires only few specimens and may also yield permanent slides. Although the results are highly variable with all modifications, the method is worthwhile because it works very quickly and often produces excellent impregnations, especially with hymenostomes (e. g., *Tetrahymena*, *Paramecium*), prorodontids (e. g., *Prorodon*, *Urotricha*), nassulids (e. g., *Colpodidium*, *Nassula*), colpodids (e. g., *Colpoda*, *Bryometopus*, *Platyophrya*) and heterotrichs (e. g., *Stentor*). Fixation is by formalin, which means that the shape of the cells is poorly preserved and even destroyed (cell bursts) in some species (e. g., most hypotrichs). The cells swell strongly during the preparation process but become very soft and are thus easily flattened between the slide and the coverslip. This makes photographic documentation easy but may result in interpretation errors. The silver carbonate methods reveal the infraciliature and certain cortical and cytoplasmic structures, especially the kinetodesmal fibres and the nuclear apparatus. Several other modifications have been suggested (3, 4). The silverlines in most cases do not stain. Examples: Fig. 1 - 6.

PROTOCOL

1. Place 1 droplet (about 0.05 ml) of a rich ciliate culture or even single specimens on a slide.
   Remarks: Slide need not be grease-free. Its middle third should be delimited by lines drawn with a greasy finger-tip or a wax crayon to prevent solutions from spreading over the whole slide.
2. Add 1-2 drops of formalin (about 4 %) and fix for 1-3 minutes. Mix organisms with formalin by circular motions of the slide.
   Remarks: The duration of this step may greatly influence the results. Species with a firm pellicle (or resting cysts) usually need to be fixed longer (3 minutes or more) than those with a more fragile pellicle (1 minute or less). Some species cannot be fixed well with formalin and cells may even burst. For these fixation with osmium tetroxide vapours (place inverted slide with ciliates for about 1 minute over a 4 % osmium tetroxide solution in a fume hood) is sometimes useful. Fix as usual with formalin after osmium treatment.
3. Add 1-3 drops of Fernandez-Galiano's fluid to the fixed ciliates, without first washing out the formalin, and mix by circular motions of the slide for 10-60 seconds.
   Remarks: The amount of Fernandez-Galiano's fluid needed depends on many unpredictable factors (e. g., amount and concentration of fixative, size of drops, kind of species, composition of sample fluid). 1-3 drops usually work well. The same holds for the reaction time (10-60 seconds). The trial and error method must frequently be used to obtain best results!
4. Place slide on a pre-heated (60 °C) hot-plate and leave until the drop, which will be rather large, turns golden brown (like cognac). This usually takes 2-4 minutes and the slide must be kept in constant circular motion during this time. As soon as the drop appears cognac-coloured, check impregnation with the compound microscope. Replace the slide on the hot-plate if impregnation is still too faint; if it is already too dark repeat procedure,

starting with step 1, but vary amount of Fernandez-Galiano's solution and/or impregnation time etc.

Remarks: The correct impregnation time depends on many factors which are difficult to control (size of drops, temperature, kind of species...). The amount of pyridine and silver carbonate in the Fernandez-Galiano fluid is especially important. Add some drops of pyridine and/or silver carbonate solution to the Fernandez-Galiano fluid if impregnation is repeatedly too faint, i. e. cannot be intensified by prolonged heating. Fix ciliates in 2-3 drops of formalin instead of 1-2 drops if impregnation is too faint. Ciliates from old cultures, ion-rich fluids (e. g., sewage, soil) or anaerobic biotopes frequently impregnate poorly. For these impregnation sometimes improves if they are washed prior to fixation (fluid from sample and distilled water 1:1).

5. Interrupt impregnation by removing the slide from the hot-plate and by adding 1 drop of fixative (sodium thiosulfate).

   Remarks: The preparation is now ready. Augustin et al. (1) describe a method for obtaining permanent slides. Their quality is, however, often not as good as with wet (fresh) preparations, which are thus usually preferred for investigation and photography. Pick out the well impregnated specimens with a micropipette, place them on a clean slide and cover with a coverslip. For good pictures the drop with the selected specimens should be very small so that cells are compressed between the slide and the coverslip. Excess fluid may be removed from the edge of the coverslip using a piece of filter paper. The impregnation need not be fixed with sodium thiosulfate if the investigation is undertaken immediately. The impregnation is stable for some hours when stored in a moist chamber. I recommend that the cells be compressed between the slide and coverslip immediately after the impregnation since silver precipitates may occur with time in the reaction fluid.

REAGENTS

a) Fixative for organisms (stable for a long time)
   0.1 ml formalin (HCHO; commercial concentration, about 37 %)
   ad 10 ml distilled water

b) Fernandez-Galiano's fluid (prepare immediately before use; components can be stored and must be mixed in the sequence indicated. The mixture must be slightly milky. If stored in brown flask it can be used for some hours. Keep away from sunlight. Make up a fresh fluid when no more impregnation can be achieved)
   0.3 ml pyridine (C_5H_5N; commercial concentration)
   2-4 ml Rio-Hortega ammoniacal silver carbonate solution
   0.8 ml proteose-peptone solution
   16 ml distilled water

c) Fixative for impregnation (stable for several years)
   2.5 g sodium thiosulfate ($Na_2S_2O_3$)
   ad 100 ml distilled water

d) Rio-Hortega ammoniacal silver carbonate solution. Preparation (the ratios are important!): 50 ml of 10 % aqueous silver nitrate solution are placed in a flask; 150 ml of 5 % aqueous sodium carbonate (Na_2CO_3) are added little by little under constant stirring; add 25 % ammonia (NH_3), drop by drop, until the precipitate dissolves, being careful not to add an excess; finally add distilled water up to a total volume of 750 ml. The solution is stable for several years.

e) Proteose peptone solution (long term stability if not colonized by bacteria and/or fungi; discard dull solutions)
   96 ml distilled water
   4 g proteose-peptone (bacteriological; sprinkle powder on the surface of the water and allow to dissolve without stirring).
   0.5 ml formalin (HCHO; for preservation)

LITERATURE CITED

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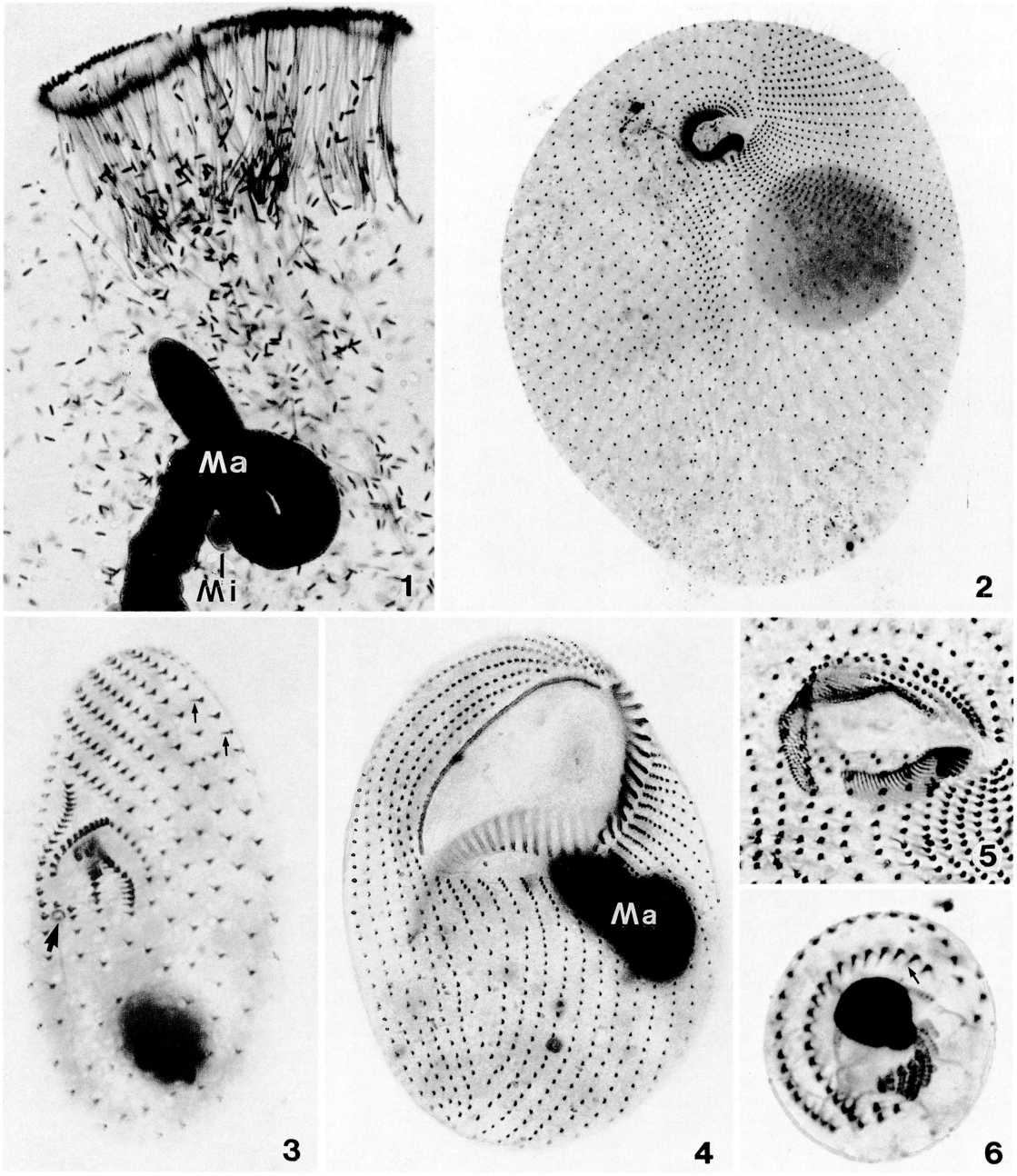

Fig. 1 - 6. Ciliates prepared with the silver carbonate protocol described. **1.** *Epispathidium terricola*, a haptorid ciliate; oral region showing many short and long extrusomes. **2, 5.** *Colpoda cavicola*, a colpodid ciliate; ventral view and detail of oral apparatus, length about 150 µm. **3.** *Colpodidium caudatum*, a nassulid ciliate; ventral view, length about 40 µm. Small arrows mark well impregnated kinetodesmal fibres; large arrow points to the excretory pore of the contractile vacuole. **4, 6.** *Bryometopus sphagni* and *Kreyella minuta*, bryometopid colpodids; ventral views, length about 100 µm and 20 µm. Arrow in figure 6 marks well impregnated transverse fibre. Ma, macronucleus; Mi, micronucleus.