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Short Technical Communication

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An Improved Pyridinated Silver Carbonate Method which Needs Few Specimens and Yields Permanent Slides of Impregnated Ciliates (Protozoa, Ciliophora)[1])

Eine verbesserte Pyridin-Silberkarbonat-Methode für Ciliaten (Protozoa, Ciliophora), die wenig Material benötigt und zu Dauerpräparaten der Infraciliatur führt

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With 5 figures

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Summary

The improved method is simple and yields good results especially with ciliates which have a firm pellicle and are able to stand formalin fixation. The ciliates are fixed in formalin (ca. 4 %), then mixed with Fernandez-Galiano's solution, impregnated on a hot plate, mounted in a glycerol-albumin mixture, and dried in an incubator at 60° C. Subsequently, the preparation is stabilized in a mixture (9 : 1) of isopropyl alcohol (98 %) and pure formalin (ca. 37 %), then dehydrated and finally mounted in synthetic resin. All steps can be carried out on slides. The advantages of this improved method are: 1. You get permanent slides. 2. You can work with few or even single specimens. The permanent slides are usually not so excellent as the ,,fresh" impregnations but they are good enough to see important details.

Zusammenfassung

Die verbesserte Methode ist einfach auszuführen und liefert gute Ergebnisse, besonders bei solchen Ciliaten, die eine feste Pellicula haben und daher mit Formalin fixiert werden können. Die Ciliaten werden in Formalin (ca. 4 %) fixiert, mit Fernandez-Galiano-Lösung versetzt, auf einer Heizplatte imprägniert, in Eiweißglyzerin eingebettet und im Wärmeschrank bei 60° C getrocknet. Anschließend wird die Präparation in einem Gemisch (9 : 1) aus Isopropylalkohol (98 %) und konzentriertem Formalin (ca. 37 %) stabilisiert, danach entwässert und in Kunstharz eingebettet. Alle Schritte können auf Objektträgern durchgeführt

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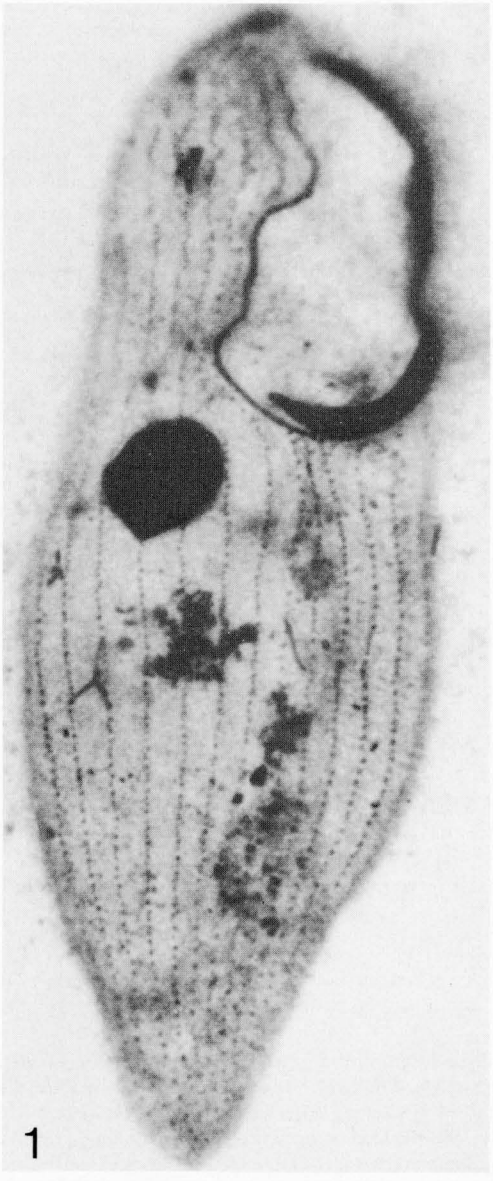
Examples for impregnations achieved by the described method.

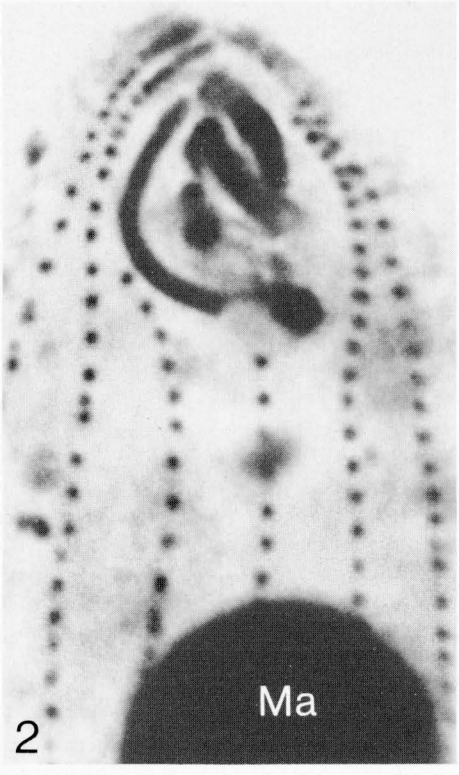
Fig. 1: Blepharisma americana, *ventral view.*

Fig. 2: Tetrahymena pyriformis, *ventral view of the anterior region with oral apparatus and macronucleus (Ma).*

Figs. 3, 4: Paracolpoda steinii, *right and left polykinety. Note the excellent resolution of these very small (about 5* µm) structures.

Fig. 5: Paracolpoda steinii, *total view. Note the paired basal bodies and the oral apparatus.*

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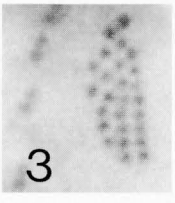

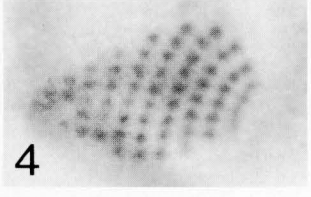

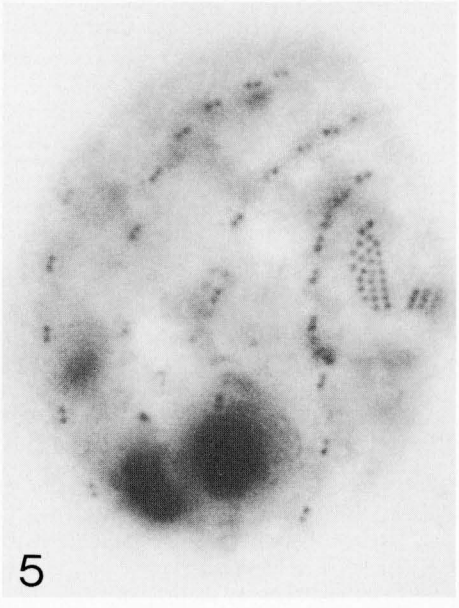

werden. Die Vorteile dieser verbesserten Methode sind: 1. Man erhält Dauerpräparate. 2. Es kann mit weniger oder sogar mit einzelnen Individuen gearbeitet werden. Die Dauerpräparate sind in der Regel nicht so hervorragend wie die „Frischpräparate", die wichtigen Details sind aber ausreichend gut erkennbar.

Introduction

The silver impregnation technique of Fernandez-Galiano (1976) is used more and more frequently (e. g. Puytorac et al., Foissner) and represents a valuable supplementation to the silver impregnation methods of Chatton-Lwoff (1936), Foissner (1976, 1982), and Tuffrau (1967). But this method has two disadvantages: It requires much material and it is impossible to obtain permanent slides. These inconveniences can be overcome by the following method.

Steps in Procedure

1. Place 1 drop (ca. 0.05 ml) of a rich culture or even single ciliates on a slide.[3])
2. Add 1 drop of formalin (ca. 4 %) and fix for about 2 minutes (but very fragile ciliates should be fixed only a few seconds, otherwise they will burst).
3. Add 1 drop of Fernandez-Galiano's solution[4]) and mix 10–60 seconds (length of time depends on the material).
4. Put the slide on a hot plate, taking care that the temperature does not go up to more than 60° C: Hold the slide at one end and keep it in constant circular motion for about 1–3 minutes until the liquid takes the color of cognac (yellow-brown).
5. Interrupt impregnation by removing the slide from the hot plate.
6. Collect impregnated ciliates promptly (to prevent rough silver precipitates) with a pipette and place them on another slide into a small drop of glycerol-albumin.[5])
7. Mix impregnated cells with glycerol-albumin (be careful with large ciliates!). Remove superfluous glycerol-albumin.
8. Control microscopically whether impregnations are good.
9. Put good slides into an incubator at 60° C for about 1/2 to 1 hour (air-drying over night is not recommended because the impregnations begin to fade).
10. Stabilize slides for about 10 minutes in a mixture (9 : 1) of isopropyl alcohol (98 %) and (pure commercial) formalin (ca. 37 %).
11. Dehydrate in isopropyl alcohol (100 %) in two steps for 10 minutes each.
12. Dip slides in xylene in two steps for 5 minutes each.

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[3]) Use different pipettes for each operation.

[4]) Make it up immediately before use; mix in the following sequence:

a) 0.3 ml pure pyridine (Merck, no. 9728).

b) 4.0 ml Rio-Hortega ammoniacal silver carbonate solution. Preparation (the ratios are important!): 50 ml of 10 % silver nitrate are placed in a flask; 150 ml of 5 % sodium carbonate are added little by little, the solution being stirred in the process; add a solution of ammonia, 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. Wilbert (1983) also attained excellent results using commercial silver carbonate.

c) 0.8 ml proteose-peptone solution. Preparation: dissolve 4 g proteose-peptone (bacteriological, Merck no. 7229) in 100 ml distilled water; add 5 drops of formaldehyde (ca. 37 %) for conservation.

d) 16 ml distilled water.

This mixture must be obviously milky-dull. Then it can be used for some hours. Keep away from direct sunlight. Make up a fresh solution when no more impregnation can be achieved.

[5]) It is not necessary to wash the specimens between steps 6 and 7. We use the same glycerol-albumin as for protargol silver impregnation. If there is a rather large amount of impregnated specimens and solution on a slide you should divide up the material.

13. Mount in synthetic resin.

14. Results: Following this method, good impregnations and permanent slides are regularly obtained (e. g., see figs. 1–5). The same structures are revealed as with the original method.

Discussion

In old cultures there is sometimes such a reducing environment that the liquid darkens strongly when the ammonium silver carbonate solution is added. FERNANDEZ-GALIANO (1976) stated that a few drops of very diluted solution of potassium permanganate (0.10 % w/v) could avoid this inconvenience. We confirm that potassium permanganate prevents a strong darkening of the liquid, but the ciliates usually do not or very weakly stain. Thus we tried $FeCl_3$ (0.1 % w/v) in the ratio 1 : 1 and proceeded as above. But cells did not stain, either.

The fixation of hypotrichs is still inconvenient. The modified procedure with a mixture (1 : 1 : 1) of ethanol (80 %), formalin (15 %), and glycerol (5 %), as suggested by FERNANDEZ-GALIANO (1976), has also been tried, but neither a good fixation nor an impregnation could be achieved. Although formalin fixation is not ideal for hypotrichs, it yields at least some workable results if you fix the ciliates only for a very short time (about 10 seconds).

We suppose that the impregnations obtained with our method are very permanent because our oldest preparations which have been achieved 6 months ago are still unchanged.

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