RESEARCH NOTES

Modification of the Mini-sieve and Prepolymerized Plate Techniques for Use in Electron Microscopy[1]

A. DE GRISSE[2]

The mini-sieve utilized consists of a 2-cm-long glass tube, fitting inside a 3-mm Teflon or polyethylene ring of 1 cm diam (Fig. 1). The ends of the glass tube are polished. A piece of nylon cloth of appropriate mesh (apertures) of 5 to 100 μm is placed between the two rings (Fig. 2), and the glass tube is pushed into the smaller ring (Fig. 3) to form the mini-sieve (Fig. 4). It is placed in a container of the appropriate solution, and small specimens are transferred into it with a Pasteur pipette (Fig. 5, 8). The specimens can be rinsed by transferring the sieve into the rinsing solution (avoiding drying of specimens) or simply by spraying specimens in the sieve with rinsing solution by a pipette (Fig. 6) or siphon (Fig. 7). The specimens can be dehydrated by transferring the sieve to different solutions or starting with the sieve in water (3). Continuous penetration of the specimen by the resin is obtained by transferring the sieve into a bottle containing a mixture of "resin and dehydration solution" and placing the bottle on a slowly rotating sloping disc (5); the sieve rolls inside the rotating bottle, assuring a continuous movement of the mixture (Fig. 8). The resin is changed by placing the sieve on a filter paper until the resin has drained away (Fig. 9). The sieve is then transferred into fresh resin. After the specimens are properly penetrated by the resin, the sieve is opened by separating the two rings, and the specimens are picked off the nylon cloth and transferred to a drop of liquid resin on a prepolymerized plate.

For preparing these plates, resin, in a small plastic container (Fig. 10), is prepolymerized in a layer 0.5 cm thick in the incubator. Four h at 70 C is sufficient when using ERL soft formulation (4). With a pipette, drops of liquid resin are put in rows on the prepolymerized plate (Fig. 11). A specimen is placed in the middle of each drop, and the plate in placed in the incubator for complete polymerization. The drops become firmly attached to the plate, and the specimens are lying parallel to the plate surface. Each specimen is numbered, and the drops are connected by a scratch to facilitate successive examination of the fragments by light microscope. All details can be examined and noted. Small blocks are sawed from the plate, following a desired cutting-angle (Fig. 12).

Picking up fragments of nematodes or other small organisms (rotatoria, spores, or eggs) is not possible when they are in high concentrations of alcohol or acetone, since evaporation of those solutions swirls the specimens. Embedding specimens in agar (7) is helpful, but orientation of the fragments is difficult. Earlier (1, 2), we proposed the use of a small sieve to retain these fragments. The sieve was made of two closely fitting glass rings of differing diameters, between which a nylon cloth was stretched. If the diameters of the two glass rings differ appreciably, the nylon cloth is held too loosely, so that specimens are sometimes sucked between the two rings and lost during rinsing. In contrast, when the rings fit too tightly, they are difficult to separate again.

When the nylon cloth is held between an inner glass-tube and a small flexible teflon ring, it is tightly pressed between the two rings but the sieve can be easily opened.

The orientation of small specimens is very difficult when they are embedded in gelatin capsules. The prepolymerized plate technique (6) makes it possible to orient small specimens, but checking the orientation and quality of the specimens by light microscope with an oil-immersion lens is impossible because the upper layer is too thick. With the drop technique, an oil-immersion lens can be used.

The described mini-sieve permits an almost perfect rinsing of the tissue and

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[2] Laboratorium voor Dierkunde. Faculty of Agricultural Sciences, R.U.G., Coupure 533, B-9000, Gent, Belgium.

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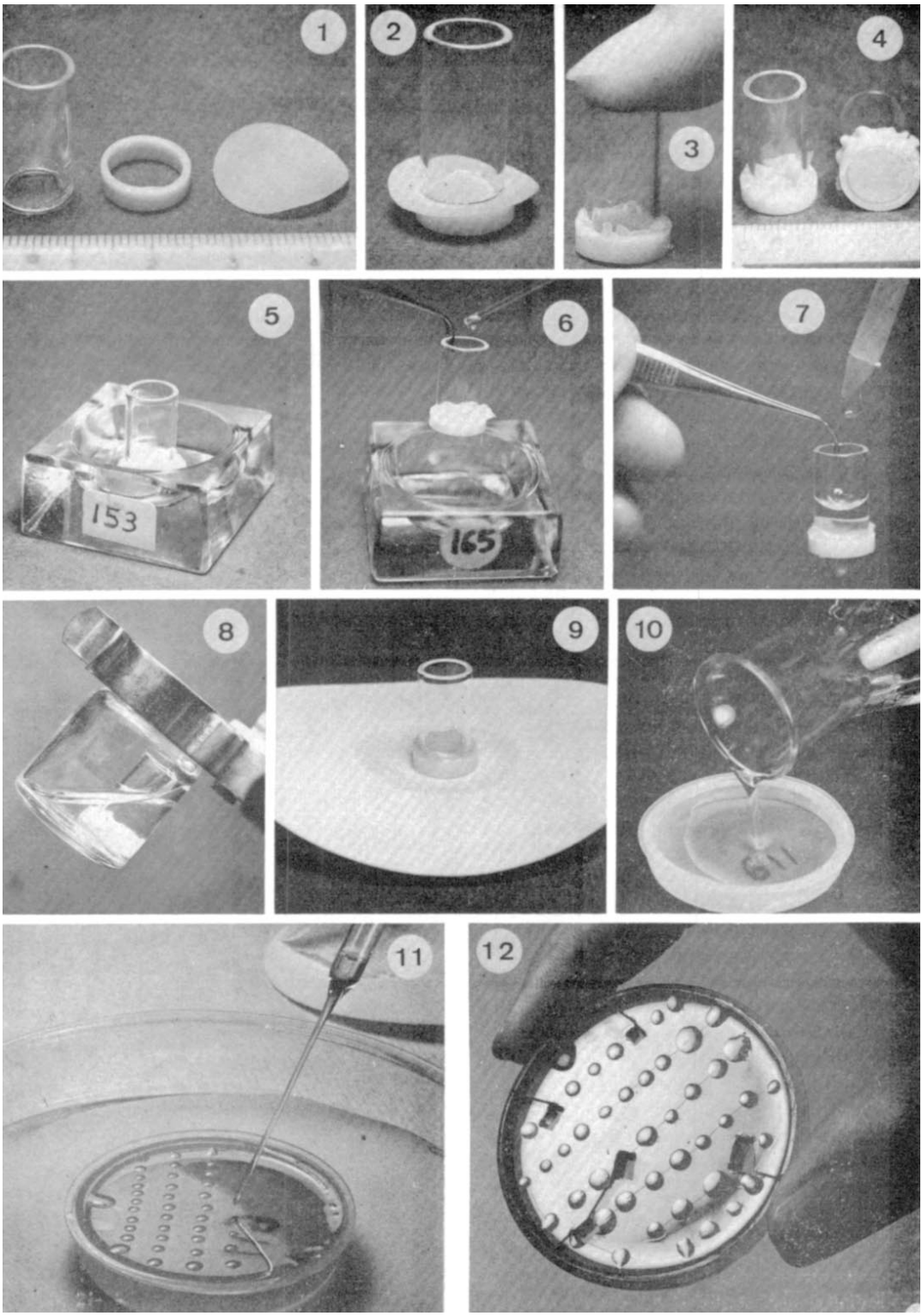
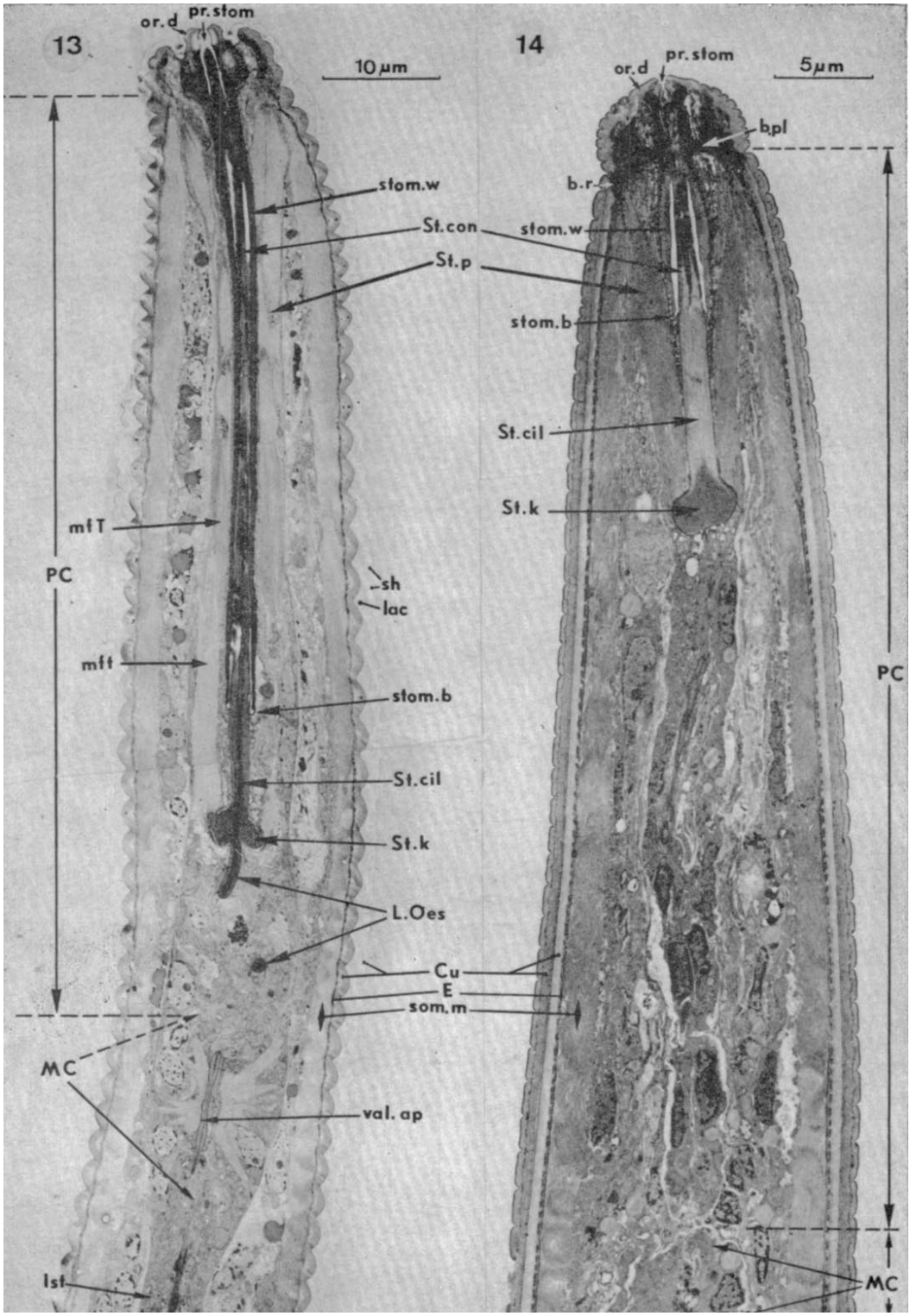


FIGS. 1-12. *1)* Inner glass tube, outer teflon ring, and nylon cloth. *2-4)* Assembling of the mini-sieve. *5)* Sieve in container with desired solution. *6)* Rinsing of the tissues with a Pasteur pipette. *7)* Rinsing of the tissues with a siphon. *8)* Sieve in rotating bottle. *9)* Sieve on filter paper. *10)* Preparing a resin-plate. *11)* Putting drops on the plate. *12)* Polymerized drops on plate.



FIGS. 13-14. Longitudinal section through stylet and procorpus and metacorpus of esophagus. *13) Hemicycliophora* sp. *14) Scutellonema cavenessi.*

penetration of the resin and manipulations of the smallest organisms. The drop technique permits transverse as well as longitudinal sections to be cut through very small structures such as the stylet of phytoparasitic nematodes (Figs. 13, 14). The success of this drop technique is illustrated in the longitudinal section, through the stylet, 71 μm long and 1 μm in diam, of *Hemicycliophora* sp. (Fig. 13).

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ABBREVIATIONS USED

b. pl.	= basal plate of cephalic framework
b.r.	= basal ring of cephalic framework
Cu.	= cuticle
E.	= epidermal tissue
Ist.	= isthmus of esophagus
lac.	= lacune
L. Oes.	= lumen of esophagus
M.C.	= metacorpus of esophagus
m.f.T.	= thick myofilaments of stylet protractor (electron-dense region)
m.f.t.	= thin myofilaments of stylet protractor (electron-transparent region)
or.d	= oral disc
P.C.	= procorpus of esophagus
pr.stom.	= prestoma
sh	= sheath
som.m.	= somatic muscle
St.cil.	= cylindrical part of stylet
St.con.	= conical part of stylet
St.k.	= stylet knot
Stom.b	= base of the stoma
Stom.w.	= stomatal wall
St.p.	= stylet protractors
val.ap.	= valve apparatus in M.C.