A Simple Method for Cutting Sections in the 0.5 to 1 μ Range, and for Sections of Chitin

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With one plate (fig. 2)

SUMMARY

Tissues fixed and stained with osmium/ethyl gallate often require sectioning at 0.5 to 1 μ. In the method described they are double-embedded in agar and 'ester wax' (Steedman), cut on a film of water on the coverslip, and mounted in gum containing ethyl gallate. Prepared safety razor blades are recommended. This method is useful also for cutting serial sections of insect cuticle.

The osmium/ethyl gallate method for histological fixation and staining (Wigglesworth, 1957) gives such intense coloration in most tissues that sections must not exceed 2 to 3 μ; and in tissues such as the liver, where the cells are densely packed with mitochondria, sections must be only 0.5 to 1 μ thick if a confused mass of superimposed mitochondrial granules is to be avoided.

In the development of this staining method the sections were usually cut in polyethylene glycol wax and mounted individually. The purpose of this note is to describe a simple procedure by which serial sections in the 0.5 μ range can be prepared.

The methods and the embedding media for section cutting in the 1 μ range have been fully reviewed by Gettner and Ornstein (1956) and I have made extensive use of their article. The embedding medium employed is the 'ester wax' of Steedman (1947) as supplied by British Drug Houses Ltd. (B.D.H.); equally good results are obtained with the modified 'ester wax' of Chesterman and Leach (1956).

The tissue is first embedded in 5% agar (Haarlov and Weis-Fogh, 1952) and the agar block then embedded in ester wax. The agar improves the elasticity of the block and reduces compression. It is also convenient for orientation and it increases the affinity for water so that the ribbon spreads rapidly and steadily as soon as it is moistened.

The standard Cambridge rocking microtome has been used. In this model one tooth on the milled wheel gives an advance of 0.42 μ; this is as thin as is generally useful for the light microscope. Tolerably complete series have been cut in dry ribbons at 0.85 and 0.42 μ, but it is more convenient to cut directly on to a moist film on the coverslip.

Special care is needed to prepare a standard microtome knife to cut at this thickness without scratches. It is much simpler to use safety razor blades.

prepared and mounted by a slight modification of the method of Sjöstrand (1956), which is described in the appendix to this paper.

Sections prepared by this method have been figured in some recent papers on the insect nervous system (Wigglesworth, 1959 a, b).

**PROCEDURE**

*Fixation.* Small pieces of tissue, 1 to 2 mm in thickness, are fixed in 1% osmium tetroxide in isotonic veronal buffer at pH 7.2 (Palade, 1952) for 4 h. They are then rinsed in distilled water.

*Staining.* The tissue is transferred to a saturated solution of ethyl gallate (progallin A of Nipa Laboratories) in 0.25% cresol (as a preservative) for 16 to 24 h.

![Fig. 1.](image)

**Embedding in agar.** The tissue is placed in 5% agar at 55 to 60° C for 1 h or longer. The agar is then allowed to set at room temperature and it is at this stage that the object is orientated.

This is conveniently done as follows (fig. 1, A). Pieces of brass rod with square cross-section (7 to 9 mm) are cut into lengths of 10 to 15 mm. The metal block so formed is bound with adhesive cellophane tape so that this
projects 3 mm above the surface to form the embedding trough. For easy removal it is advisable to cut the free end of the tape and bend it over to form a small tab; and also to apply a square of cellophane tape to the floor of the trough.

The metal block is heated by standing on the embedding bath and the trough is filled with agar containing the specimen. The specimen is now orientated under the binocular with the face that is to be cut lying against the floor of the trough, and the agar allowed to set.

The cellophane tape is removed and the agar block trimmed until it is rather larger than the block that will finally be cut.

**Embedding in ester wax.** The agar block is passed through the following series (half-an-hour in each): ethyl alcohol 30%; 50%; 70%; 70% alcohol plus cellosolve, 2:1; the same, 1:2; pure cellosolve (3 changes); cellosolve plus ester wax, equal parts; pure ester wax, at least two changes. It is left overnight at 55 to 60° C in the final ester wax.

The agar block is now placed in the same type of embedding trough as before, with the face that is to be cut resting on the surface. Since the specimen was oriented in the agar no further orientation is necessary. The trough is filled with ester wax and cooled rapidly by standing in a Petri dish in a fast stream of cold water, with the usual precautions to avoid the inclusion of air (Steedman, 1947).

**Dry sections.** The block is mounted and trimmed so that the agar extends to the surface on all sides. (If a margin of ester wax alone is left, it will restrict flattening on that side.) The sections are cut slowly as with pure ester wax.

As with pure ester wax the sections are apt to become separated and scattered during flattening, when several rows are placed on one slide. It is therefore helpful to prepare the slide as follows. First smear with glycerine-albumen. Then wrap a wisp of cotton wool round the points of a fine forceps, dip the point in yellow soft paraffin, trace out on the slide the margins of the area to be occupied by the sections, and divide this by horizontal lines to form a compartment for each row. Lay the ribbon in its compartment; apply a small drop of 20% alcohol to one end of the compartment and let it flow below the ribbon to the other end. Immediately the drop reaches the ribbon this unfolds smoothly and steadily. Any excess fluid is removed with a pipette and final flattening and drying completed on the warm plate. The sections shown in fig. 2, A–D were cut dry in this way.

**Sections on a moist film.** When cutting sections at 1 μ or less it is simpler to collect the ribbon directly on to a film of water. A half-coverslip measuring 11 by 22 mm is used. A mound of 'plasticine' is built up on the knife holder to such a level that a coverslip placed on it and resting just below the edge of the blade, will be horizontal (fig. 1, b).

The upper surface of the coverslip is smeared with glycerine-albumen, and the lower surface with yellow soft paraffin.

A section or two sections are cut dry and left adhering to the edge of the blade. (This is essential in order that the water shall be separated from the front of the block. If the front of the block or the upper surface of the section
is allowed to get wet the section will be picked up when the block moves up again behind the blade.)

About 8 or 10 drops of distilled water containing 0.1% sodium chrom-glucosate (D. W. Haering & Co., San Antonio, Texas) as an anti-corrosive are placed on the coverslip and swept up to the edge of the knife and below the newly cut sections, with a fine camel's hair brush. The sections will probably spread on the water; and on cutting more sections these will spread into an even ribbon with little compression.

If it is desired to place a second length of ribbon on the coverslip, the first ribbon is cut through with a mounted bristle, displaced to one side, and cutting resumed. The operations are best done under a dissecting microscope.

The sections shown in fig. 2, E–G were cut on a film of water in this way.

Mounting. After draining off excess fluid the slides or coverslips are dried on the warm plate to complete the flattening of the sections. They are then immersed briefly in xylene, taken down to 30% alcohol, and mounted in Farrant's medium (or other aqueous gum medium) containing ethyl gallate in solution. The slide is immediately placed on filter paper with the coverslip downwards and pressed very firmly to displace as much of the mountant as possible.

The staining is completely permanent and is improved by leaving on the warm plate exposed to the sun.

Results

Sections stained with osmium / ethyl gallate. Fig. 2, A–D shows sections of mouse liver cut dry at 2 μ, 1.25 μ, 0.85 μ, and 0.42 μ. In the 2 μ section there is a confusion of super-imposed mitochondria. At 0.42 μ there is only a single layer of mitochondrial granules, many of which are cut through. If the sections are less than 0.42 μ the contrast is reduced and they allow no increased resolution in the light microscope. For visual examination, as opposed to photography, sections at 0.85 μ are very satisfactory for this tissue.

Fig. 2, E–G shows sections of the proximal convoluted tubules in the mouse kidney after cutting on a film of water at 2 μ, 0.85 μ, and 0.42 μ. Here again, the 0.85 μ sections are satisfactory for visual examination, but even at this thickness the densely packed palisade of mitochondria overlap and obscure one another in photographs.

For tissues which are not so rich in mitochondria, such as the intestinal epithelium, sections cut at 2 μ are thin enough for many purposes.

Sections stained by other methods. The same methods of embedding and...
section cutting at any thickness can be employed after other fixatives, and any standard stains can be used. Some of these stains colour the agar film but can be removed by brief treatment with acid alcohol.

Sections of chitin. Double embedding in agar and ester wax has proved very useful for cutting sections of insect cuticle. Fig. 2, H shows a 6 μ horizontal section of the posterior region of the head in the bug Rhodnius prolixus, where the neck is invaginated into the front of the thorax. Fig. 2, J shows the same in transverse section. This is difficult material to cut; it is unusual, by other methods, to obtain the almost unbroken series with the epidermal cells closely adherent to the cuticle, which are readily obtained by this method.

APPENDIX

Preparation and mounting of the blade

As already pointed out it is possible to cut sections by the methods described with an ordinary microtome knife with a good edge. But it is simpler to use safety razor blades. Blades of the American 'Schick' or the English 'Pal' or 'Personna' type, measuring 3.75 cm × 8 mm, have been used; and these have been prepared by methods closely based on the procedures described by Sjöstrand (1956) and by Gettner and Ornstein (1956). I have made some small modifications and it may be useful briefly to describe the methods.

Fig. 1, B shows the brass holder for sharpening the blades. This measures 3 × 7 cm. It is of the type described by Sjöstrand but is provided with a series of flat-sided steel pegs of different lengths so as to give facet angles of 25°, 30°, 40°, and 50°. (In the figure the 40°-pegs are in use.) By the addition of suitable washers below the pegs it is possible to obtain any facet angle from 25° to 55°. For soft material a facet angle of 35° has generally been used; for harder material an angle of 50°.

To prepare a facet angle of 35°, the 30°-pegs are inserted and the blade is ground down by the method of Gettner and Ornstein on plate glass or on a ground glass stone, by using their lapping fluid consisting of Linde A synthetic sapphire polishing powder (Linde Air Products Co., 30 East 42nd Street, New York 17, N.Y.) 1 volume, in 2 volumes of the following solution: distilled water 10, glycerol 8, acetone 2. After washing the blade and the stone, polishing is continued with the same lapping fluid containing Linde B powder. Finally, the blade and holder are washed again, the facet angle is increased to 35° by the insertion of the appropriate washers beneath the pegs, and the blade is very gently polished on another sheet of plate glass with a lapping fluid consisting only of distilled water 6, glycerol 12, acetone 2, with no powder. This gives a very small 35° facet at the margin of the 30° edge. The whole procedure requires only about 10 minutes.

The blade holder for section cutting is illustrated in fig. 1, c, d; the drawings are self-explanatory. The mild steel support is shaped like a microtome blade with a 20°-angle. A wedge-shaped thickening with a 10°-angle is left in the middle and bears two small pegs which fit into the slots in the blade. When the blade is in position and held firmly by the facing piece applied to its surface, it will be inclined at an angle of 30°. With a facet angle of 50° on the blade, this will allow for a clearance of 5° (30°-35°) between the block and the surface of the facet.

I am indebted to Mr. James Higgins for his help and skill in making holders and carriers for the blades as described above.
REFERENCES

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