# A Useful Sectioning Technique

**Maxwell S. Doty**

***Botany Department,***
***Northwestern University, Evanston, Illinois***

It is often necessary to obtain sections of biological materials for microscopic examination through particular minute areas, and in rather precise positions. Often the investigator has just one cystocarp of a red alga, a single sclerotium of a fungus, or a small fragment of a valuable type specimen to section. At other times it may be desirable to section fresh aquatic (either fresh-water or marine) material without killing the cells that may remain intact, as in the examination of *Porphyra* or living leaves.

The object to be sectioned, e.g. a tooth of the fungus *Dentinum*, is placed on a microscope slide in a drop of mucilage just large enough to cover it. A specimen 0.5–1.0 mm on a side is sufficiently large, though smaller or larger pieces can be handled with ease. The mucilage should be allowed to dry until when prodded with a dissecting needle the object is motionless, as is the mucilage fracture around the needle. In general, the more nearly dry the mucilage is the better, but it must not become brittle. If the mucilage becomes too brittle that condition may be alleviated by the addition of about 10% glycerine.

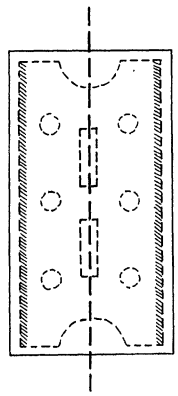

FIG. 1

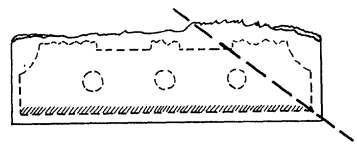

FIG 2

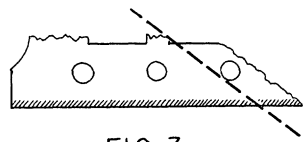

FIG 3

Double-edged razor blades are used for sectioning for several reasons: they are more readily made serviceable, and they have no stiffening edge or back that might obstruct vision or prevent resharpening as shown in Fig. 3. The blades are prepared as follows: While still in the paper package, the blade and the package are broken in half lengthwise (Fig. 1); then one end of a half-blade is broken off obliquely (Fig. 2, broken line) and the paper removed. This procedure produces a pointed blade (Fig. 5) that is very sharp. If a day or so is to elapse between sectioning, it is best to discard the unwrapped blade and start with a newly unwrapped half, as the exposed blades seem to lose their edge. It is essential to have a very sharp cutting edge, and so a further segment of the blade should be broken off parallel to the first "pointing" or oblique break (Fig. 3, broken line) just before sectioning each object.

Sectioning is done on the stage of a dissecting microscope, the stage being nearly at table level, so that there is adequate support for the operator's forearms. A magnification of 10–20 diam is adequate, permitting a sufficiently wide field and ample depth of focus. The object-bearing slide is placed on the microscope stage so that the desired plane of sectioning is oriented about 60° counterclockwise from vertical (i.e., running from about 10 o'clock to about 4 o'clock) in the field of the microscope (Fig. 4), and with the material a little way toward 8 o'clock from the center of the field. Another microscope slide (Fig. 4) is placed over the object as a guide for the razor blade, with the portion of the object to be sectioned half-protruding from under the end of the guide slide. The end of this guide slide should parallel the plane of sectioning and extend to the left (or right for left-handed people). Its right end should be far enough toward 8 o'clock in the microscope field that its end surface can be seen.

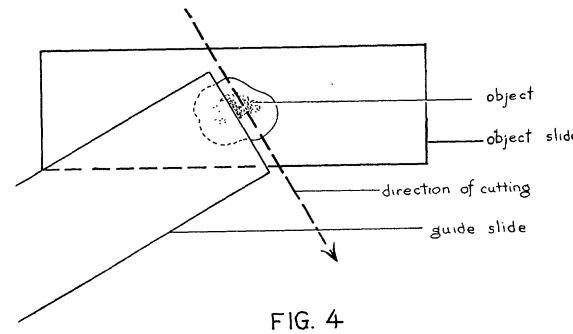


FIG. 4

The guide slide should be held with the left hand and the prepared razor blade in the right hand. Cuts are then made across the mucilage area with the direction of cutting (Fig. 4) from upper left to lower right and with the flat face of the razor blade in contact with the guide slide. The first cuts are made with the razor blade inclined (position 1 in Fig. 5) so that the cutting edge in contact with the object-bearing slide is at a distance

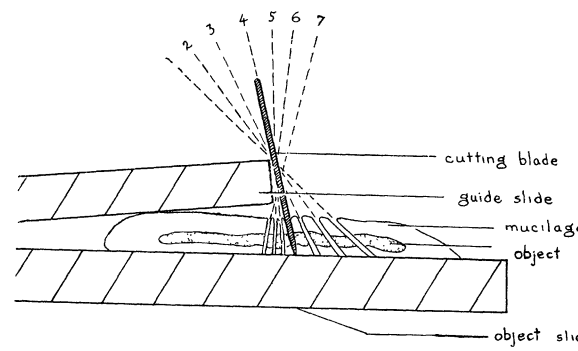


FIG 5

from the end face of the guide slide. Cuts are made clear through the mucilage and object to the glass. With successive cuts the razor blade is brought more nearly erect (positions 1 through 4 in Fig. 5). These cuts bring the razor blade more nearly perpendicular and nearer and nearer to the end face of the guide slide. This procedure controls the thickness of the sections. By the time sufficient sections have been cut to bring the razor blade nearly perpendicular the operator has the feel of the particular object-mucilage mount and of the razor blade. The material should be so placed that the critical sections can now be made by continuing the process of slicing and straightening up the blade with each successive slice (positions 4 through 7 in Fig. 5). The best sections, i.e., the thinnest and most perpendicular, are obtained, as a rule, just when the razor blade is brought perpendicular (position 5 in Fig. 5) and past perpendicular (positions 6 and 7 in Fig. 5) by slanting the razor blade to cut under the guide slide edge.

After slicing is completed through the desired area, the guide slide is removed and a drop of water or dilute glycerine is placed over the mucilage-impregnated sections. In a minute or two the sections will float free, or nearly free, as the mucilage softens. The thick, unsectioned portions of the object may be removed with a dissecting needle and the remaining sections spread about. The sections may then be stained or treated in any manner which may be required.

The technique works equally well with fresh, dried, or preserved materials. Moreover, a section may be obtained with a high degree of success, with almost no equipment, and with a minimum of experience when just one pin-point object is available. Sections have been obtained easily and consistently of materials that are somewhat less than 5 μ thick.

## Effect of a Folic Acid Antagonist on Hormonally Induced Changes in the Rat Prostate

**Herbert Brendler**

***Brady Urological Institute, The Johns Hopkins Hospital, Baltimore***

A definite relationship has been demonstrated in the chick between estrogen utilization and folic acid requirement (*1*). That this relationship is not based simply on a general impairment of body growth has been shown by the fact that optimal oviduct responses to stilboestrol occur in riboflavin- and pyridoxine-deficient chicks. In a more recent development, Hertz has found that the tissue growth response of the chick oviduct to stilboestrol can be blocked by the administration of a folic acid antagonist, presumably by competitive displacement of essential folic acid (*2*). This explanation is supported by the observation that the inhibitive effect can be completely reversed by the administration of excess folic acid.

In the course of an investigation into growth mechanisms of the rat prostate,[1] the foregoing findings raised several questions: 1. The use of a folic acid antagonist to inhibit the *stimulating* potential of an estrogen had been clearly shown in the chick. Could the usual *depressant* effect of estrogen on the rat prostate be nullified in a similar manner? 2. If such were the case, was there a possibility that the folic acid antagonist possessed androgenic characteristics *sui generis?* 3. If the antifolic principle did not inhibit the usual effect of the estrogen on the prostate, the probability was good that the blocking action was directed only at that function of the biological activity of the estrogen concerned with tissue growth. If so, might it not therefore inhibit androgen-stimulated growth as well?

TABLE 1

| Exp. | Type of rat* | No. used | Average rat wt. in g | Mode of treatment | Average prostate wt. in mg† |
|---|---|---|---|---|---|
| | Intact adult | 7 | 201 | α-estradiol | 190 |
| 1 | " " | 7 | 231 | α-estradiol and aminopterin | 373 |
| | Intact adult | 5 | 277 | aminopterin | 469 |
| 2A | Castrate adult | 5 | 238 | aminopterin‡ | 89 |
| | Intact immature | 5 | 62 | ..... | 33 |
| | " " | 5 | 61 | testosterone | 59 |
| 2B | " " | 7 | 54 | aminopterin | 28 |
| | " " | 3 | 59 | testosterone and aminopterin | 53 |
| | Castrate adult | 5 | 223 | testosterone‡ | 380 |
| 3 | " " | 5 | 202 | testosterone‡ and aminopterin‡ | 406 |

* The adult rats used in these experiments were of the Sherman strain, and ranged in age from 90 to 120 days. The immature rats were obtained from Carworth Farms (Wistar) and were approximately 30 days old.

† Combined weight of anterior (ventral) and posterior lobes, except in Exp. 2B, where only anterior lobes were used.

‡ Begun 24 hr postcastrationally.

The results of experiments designed to test the above hypotheses are summarized in Table 1. It will be seen that: 1. The use of the folic acid antagonist[2] partially,

[1] This study is being done in part under an American Cancer Society grant, recommended by the Committee on Growth of the National Research Council, and in part by a grant from the National Brewing Company. The author would like to extend his thanks to Dr. William Wallace Scott for his kind assistance in this work.

[2] Aminopterin (4-amino pteroyl glutamic acid), a product of the Lederle Laboratories, was kindly furnished by Dr. E. B. Schoenbach, of the Johns Hopkins School of Medicine, who also supplied much valuable information derived from his experiences with the compound. The daily dose used in these experiments was 0.2 mg/kg body weight in aqueous solution. Subcutaneous injections were given for 6–7 days, and the animals were sacrificed 24 hr after the last injection.