

## Brief Notes

**Thin Sectioning: Details of Techniques.** BY DOROTHY GELBER. (*From the Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York.*)\*

The study of cytological fine structure with the electron microscope, has as an important prerequisite, the ability to cut consistently good quality thin sections. Satisfactory techniques for doing this are well known in many laboratories and an excellent review of the subject has recently been published by Farquhar (1). However, some of the details of the practice of microtomy are not in the literature and it is the purpose of this note to describe those which have been used successfully in this laboratory to obtain both single and serial sections (2-4).

### *Microtomes*

Conventional reciprocating microtomes with special attachments for cutting thinner sections have been generally discarded in favor of specially designed microtomes. Many of these have been described (5-13) and a few are commercially available. Three types have been used in our work and will be compared. These are two Porter-Blum models which have been used extensively, and the Sjöstrand microtome with which our experience has been somewhat limited.

*1. Porter-Blum Thermal Advance Type.*—This instrument is simply constructed; its cantilever arm is mounted between pivots which in our instrument are oiled

\* Received for publication, November 16, 1956.

and maintained under considerable pressure. No further attention has been required during constant use for more than two years.

The following are the advantages of this instrument over the later model using automatic advance: During sectioning when a change of thickness is desired only a change in the period of heating the cantilever arm is necessary; it is not essential to stop in order to change the rate of advance. Very thin (blue-white) sections are more consistently obtained because the light may be switched on for a fraction of a second at each stroke, thus causing the minutest amount of controlled expansion which may be maintained for a prolonged period of time. In order to obtain very thin sections with the mechanical advance, the setting must sometimes be brought to zero which is not satisfactory because after cutting a few sections the block fails to advance anymore.

*2. Porter-Blum Automatic Advance.*—This is the only Porter-Blum microtome now available,<sup>1</sup> but it may be converted to the thermal advance type by removing the cover from the cantilever arm, setting the advance control to zero and providing an incandescent lamp as a source of thermal energy. With the automatic advance little experience is necessary to obtain thin sections. In theory the operator merely sets

<sup>1</sup> Ivan Sorvall, Inc., Norwalk, Connecticut.

the automatic advance for the thickness desired. Since there is no danger of contraction during the time the block is retrimmed, the knife reoriented, or sections picked up, readvancing the knife to the block is not necessary and sectioning can be resumed immediately.

3. *Sjöstrand Microtome*.—This instrument<sup>2</sup> utilizes an eccentric rotation of the block about an axis perpendicular to the knife edge. The block surface thus remains in the vertical plane, whereas in cantilever instruments the knife edge must be positioned more precisely so that it is tangential to the arc of motion.

The instrument is equipped with convenient adjustments such as an easily oriented chuck and a screw drive for minute lateral adjustments of the knife. It is motor driven, thus requiring less attention from the operator. In general, however, it has not been found to be superior in performance to the more simple Porter-Blum instrument. Bringing the block to rest directly opposite the knife and adjusting the knife to its exact cutting position is very difficult. The heat capacity of the built-in heater hinders constant and rapid control of section thickness. In the Porter-Blum thermal expansion microtome radiant heat from a distance provides faster and more reliable control. The rate of block motion is adjustable but constant throughout the cycle; in passing the knife at proper speed the rest of the cycle is too slow. Unless great care is exercised in insulating the microtome from its driving motor, harmful vibration may be transmitted.

#### *The Glass Knife*

The type of knife originally described by Latta and Hartmann (14) has been

<sup>2</sup> Manufactured by LKB Produkter, Stockholm, and distributed by Ivan Sorvall, Inc., Norwalk, Connecticut.

adopted as the most suitable type for thin sections. When commercially available knives were found unsatisfactory, strips of green-tinted lime plate glass,  $\frac{1}{4}$  inches thick and  $1\frac{1}{2}$  inches wide, were obtained as material for preparing knives in the laboratory. The cutting edge, *E*, is prepared from the narrower side of the strip (see Text-fig. 1).

By means of a steel disc glass cutter and pliers, *P*, knives of the shape indicated in Text-fig. 1 were broken from the strips. A cutter may be used to make about 200 knives before becoming too dull. In scoring, a metal ruler is held flat against the glass strip at a  $45^\circ$  angle and sufficient pressure is applied to the cutter to actually fracture the surface. The score mark, *S*, is made only about  $\frac{1}{3}$  of the way across the glass strip as indicated in Text-fig. 1. Application of turpentine, kerosene, castor oil, or other wetting agents to the score mark has not been found to be helpful. Neither has the use of cork or felt as a backing material facilitated the breaking of knives. A strong table top is all that is required.

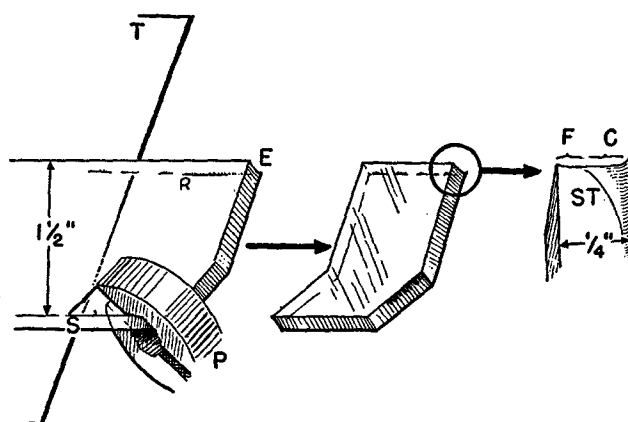
In breaking off the knife, the glass strip is placed flat on the table, *T*, perpendicular to the table edge. The inner end of the score mark should coincide with the edge of the table. The pliers, held at approximately  $45^\circ$  to the table edge, are brought up to the point where the score mark and the edge of the table meet. By applying a rapid, downward stroke to the pliers, the knife is broken off. The line along which the knife breaks is not straight with the score mark because the score mark extends only about  $\frac{1}{3}$  of the way and the fracture tends to follow the shortest path to the edge. Thus the angle at the cutting edge becomes  $70-80^\circ$  instead of  $45^\circ$ . It should be emphasized that the length and angle of the score mark and

the angle the table edge makes with the glass strip are of extreme importance. If the table edge is made parallel to and placed directly beneath the score mark, the knife broken is a more perfect parallelogram with a smaller angle at the cutting edge, but the edges are of poor quality, containing small striations that are difficult to see with the microscope unless the bottom illumination is weak. Grossly, the knife edge should appear straight except for

which they are washed with cold tap water and dried with a towel, except for the area near the cutting edge, and used immediately. If the cutting edge is exposed to the air too long the acetone solution fails to wet it properly. It can be reconditioned, however, by the cleaning solution.

#### Sectioning

"Scraping" would probably be a better term for the process of making thin



TEXT-FIG. 1. Schematic diagram demonstrating the method of breaking glass knives and the appearance of the cutting edge. See text for details.

*T*, table upon which knife is broken. *E*, knife edge. *P*, pliers used to break off knife. *R*, ridges on long narrow side of glass strip. *ST*, single striation extending from the cutting edge. *C*, part of knife edge used for cutting sections. *F*, part of knife edge used for facing the block.

an elevation at the extreme right (see Text-fig. 1). Upon examination in the light microscope (magnification  $50\times$  to  $100\times$ ) no more than one striation, *ST*, leading away from the edge must be present. The sharpest part of the knife, *C*, is between the striation and the elevation to the right. The portion to the left of the striation, *F*, is used for trimming the face of the block. Approximately 80 per cent of the knives broken are satisfactory. Before sectioning, the knives are soaked in chromic acid cleaning solution for at least 5 minutes, after

sections, because the block is actually not cut, but instead a thin film is scraped from its surface. The ability to scrape a thin film depends, of course, on the radius of curvature of the edge being extremely small. The film is compressed and sharply bent as it comes in contact with the advancing knife, but if it flows smoothly from the edge it will straighten out on the surface of a wetting liquid. The uniformity of film thickness then depends on the smoothness of the knife edge and not on its acuteness. Experience has indicated that

smooth glass edges occur more often when the angle is only slightly less than 90°. Cutting processes have already received considerable attention (15-19).

In placing the knife in its cutting position, it should be oriented so that the side facing the block is very nearly vertical (within 5-10°), thus forming almost a tangent to the arc described by the motion of the block in the Porter-Blum microtome.

The number of sections that can be cut before a new knife is needed depends on the thickness of the sections and the nature of the tissue. The softer the block and the thinner the sections, the longer will be the life of the knife.

With the Porter-Blum thermal expansion microtome, a 60 watt bulb is used to heat the duraluminum rod. The bulb is at a distance of 1 to 3 inches from the rod. For cutting thin sections the bulb is switched on for a second or less before each passage of the block across the knife. While sectioning, the room temperature should be kept nearly constant. Although the rate of motion through most of the cycle is immaterial the block should pass the knife slowly.

The size and shape of the face of the block are important in preserving the knife edge. For maximum sectioning efficiency the face of the block should be rectangular with dimensions of approximately 0.6 mm. by 0.4 mm. By placing the rectangular block with its short side parallel to the knife edge the usefulness of the knife may be prolonged because in this way more new parts of the cutting edge are available as the knife is shifted along its cutting length.

If the face of the block becomes large, the quality of sections is affected.

In order to cut serial sections, the face of the block is trimmed to dimensions of approximately 0.3 mm. by 0.1

mm. In contrast to routine sectioning, the block is placed in the chuck so that the longer side is parallel to the knife edge. This procedure lengthens the area along which any two sections come in contact with each other and thus increases the opportunity for mutual attachment; consequently, ribbons with more sections can be formed.

In both serial and routine sectioning, it is suggested that the block be trimmed in an abrupt or short pyramidal shape to furnish support to the face of the tissue block sectioned. Also, this is especially advantageous in serial sectioning, since the area of the face of the block may be enlarged in changing from serial to routine sectioning by merely removing several thick sections.

A boat (20) made of aluminum or copper is sealed to the top of the knife as is indicated in Text-fig. 2. The boat is shallow and flared at the top to facilitate mounting the sections on grids.

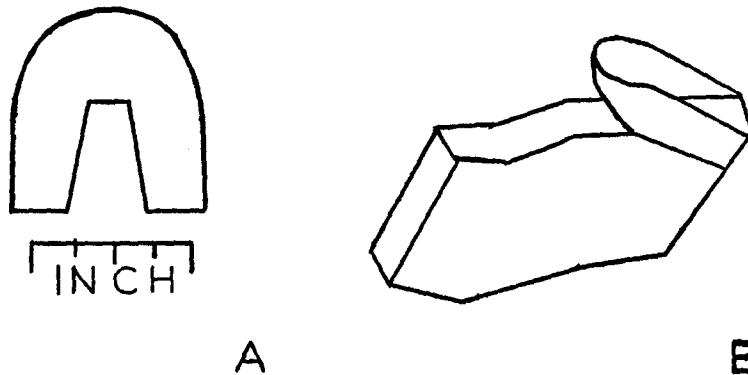
The paraffin used in fastening the boat to the knife must be heated until it fumes. This precaution insures a better seal. Before attaching the boat to a fresh knife, the boat is washed free of paraffin and other contaminants in running hot water and then rubbed with a clean dry towel.

In order to maintain the proper liquid level the tip of the boat must reach the tip of the knife edge. The level of the liquid in the boat is critical for satisfactory flattening out of sections. Although the meniscus must not be high enough to wet the block as it passes, the liquid must wet the cutting edge of the knife. Acetone (20 to 30 per cent) is effective as a wetting agent.

In mounting the routine sections on the copper grid, a needle and a sharp pointed curved forceps are used; the needle is needed to separate and select

the sections and to manipulate them in the boat. The forceps are used to hold the grids. Both instruments must be perfectly clean. Contact of the instruments with the skin should be avoided for this can cause a layer of grease to float on to the surface of the liquid in the boat. In order to be certain that the section is centered on the grid, the following procedure is used. Slightly more than half the screen is dipped below the liquid surface at a small angle to the horizontal. The section is placed

is submerged. After the proximal section of the ribbon is attached to the grid, it is retracted from the liquid very slowly to prevent a break in the ribbon. By this method up to 40 serial sections can be placed on one grid. These long ribbons are often curved, thus making examination on straight-slitted grids difficult. Ribbons are of course curved when the top and bottom sides of the block are not exactly parallel. However, ribbons have been obtained containing up to 29 sections which have been suf-



TEXT-FIG. 2 A and B. A, pattern of boat made from thin aluminum sheet. B, diagram of the aluminum boat attached to the knife.

so that one side of it touches the un-submerged portion of the center of the screen. This attaches the section to the grid, which is then retracted from the liquid.

*Serial Sectioning.*—In mounting serial sections, Sjöstrand grids<sup>3</sup> which contain a series of parallel slits are used. In order to align a ribbon satisfactorily along a slit, the ribbon in the boat is placed perpendicularly to the knife edge. The grid with its slits oriented in the same direction is dipped into the liquid at a slight angle until most of it

<sup>3</sup> Manufactured by Smethurst High-Light, Ltd., Bolton, Lancaster, England.

ficiently straight for examination on one slit of a Sjöstrand grid. By way of example, 12 serial sections are shown in Figs. 1 A to 1 L. In order to see the sections in the boat a stereoscopic binocular microscope ( $\times 12$ ) is needed.

The color of the sections in order of increasing thickness are blue-white, white, straw, orange, purple, blue, green, and red.

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## EXPLANATION OF PLATE 99

FIG. 1. Micrographs of 12 serial sections of an Ehrlich ascites tumor cell containing mitochondria, endoplasmic reticulum, and Golgi apparatus. The following is of interest. Two mitochondria are cut secantly in *A*. The upper one shows distinct internal double membranes. In *B* and *C* three of these membranes are attached to the upper periphery, but do not reach the lower membrane. In *D* two reach the lower periphery and the space between the membranes seems to be continuous with the cytoplasmic matrix. The open connection is closed again in *E* and in *F* appears the last secantal section of this mitochondrion. Similarly in the lower mitochondrion the inner double membranes change their attachment points from section to section and change in number. A new mitochondrion appears in *I* which is shown in *K* and *L* to be part of the lower. In place of the endoplasmic reticulum with attached Palade granules, visible in the sections *A* to *F*, the later sections show thinner membranes representing the Golgi apparatus without granules.

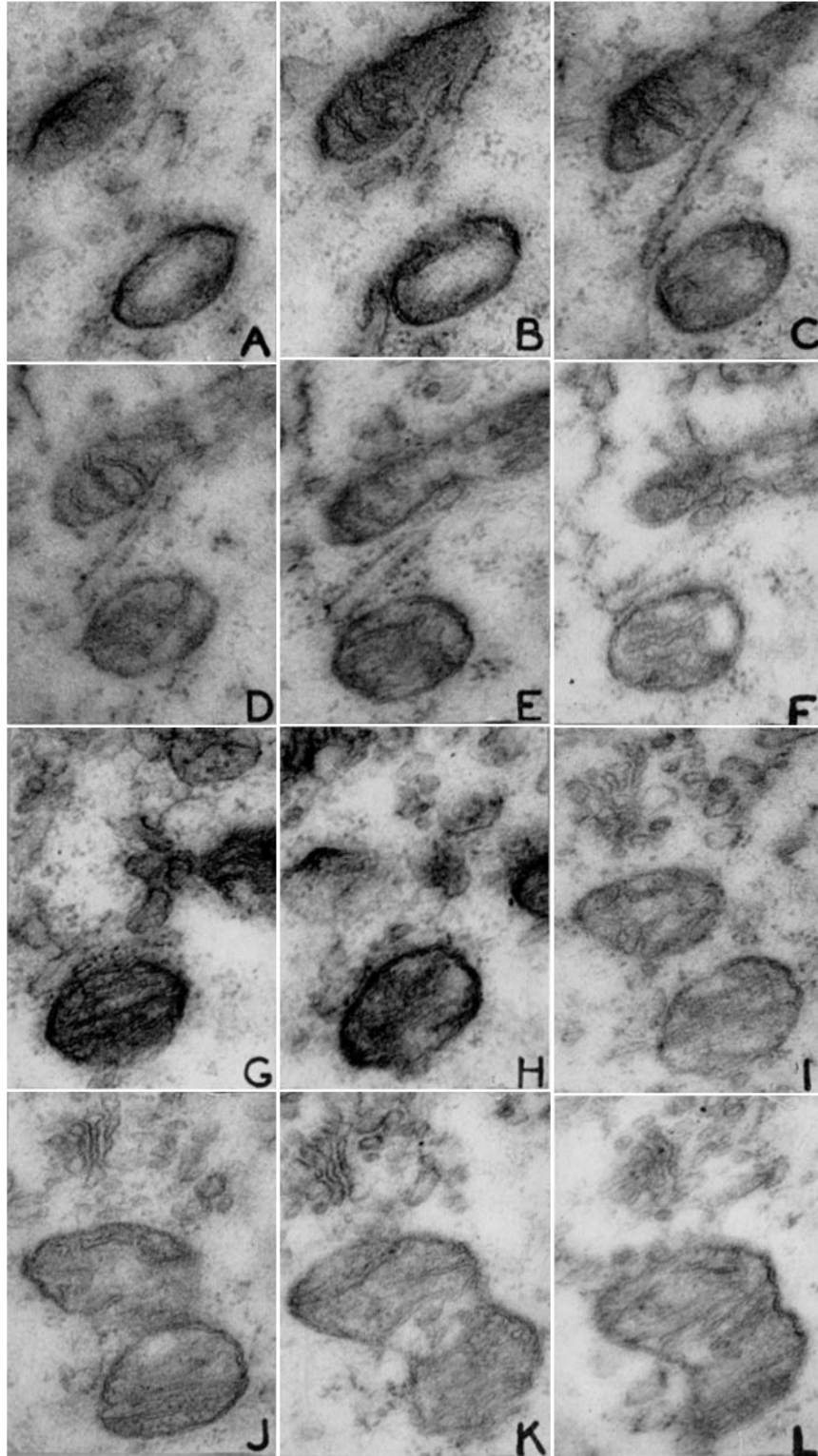


FIG. 1

(Gelber: Thin sectioning: details of techniques)