Improved Knife-Holders for Thin-sectioning with Rotary Microtomes.* (20082)

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Various adaptations of ordinary microtomy have been proposed to permit cutting thin sections of tissue for electron microscopy. Pease and Baker(1) described an auxiliary wedge for the Spencer microtome which reduced its advance by one-tenth, and reported that double-imbedded tissues could be sectioned at adequate thinness. Latta and Hartman(2) found that glass knives were preferable to the usual steel microtome knife. Imbedding in *n*-butyl methacrylate has proved the present medium of choice (Newman, Borysko and Swerdlow(3); Palade(4)). The Minot rotary microtome has been modified by the introduction of a reduction gear into its drive so that advances of 0.05 μ per revolution are possible, and measurements of the sections secured with this instrument have indicated they approximate this thickness (Geren and McCulloch)(5). Some experience with the Spencer and Minot (International) thin-sectioning microtomes convinced us that adequate sections were routinely possible only if cut onto a water meniscus, as proposed by Gettner and Hillier(6). However, a major difficulty with the meniscus arose upon the return stroke of the microtome. When the specimen passed the knife edge, the meniscus touched the block and the section, previously cut, adhered to the block and was lifted off the meniscus. Serial ribbons, therefore, could not be cut. Moreover, the greatest variations in thickness occurred when the first few sections were cut after a period of inactivity; thus, the time consumed between sections by the necessary removal and mounting of the sections one at a time became a major concern.

These difficulties were overcome by mounting the knife holder upon ball-bearings so that the knife-edge was rotated away from the block on the up-stroke of the microtome. A cam and lever arrangement, illustrated in Fig. 1-4, accomplishes this motion automatically. The ratios chosen are such as to move the knife edge backwards approximately one-half millimeter. Since any play in the bearings or looseness in the knife-holder assembly would cause chatter marks or inequalities in the sections, care was taken to clamp rigidly all portions of the assembly. Return of the knife-edge to exactly the same point during the cutting cycle is accomplished by loading the bearings with strong springs. With these precautions, chatter marks and thick areas in the section are eliminated.

The sections, when cut, are floated upon 30% acetone. Minor wrinkles and compressions are rectified by the surface action of this drop. If viewed with reflected light through a low power dissecting microscope, the flattening of the sections can easily be observed, and the interference colors produced in the thin sections can be used as a rough but adequate index of their thickness. The sections are mounted directly upon collodion-coated grids of copper mesh. The grid, held in fine forceps, is sunk beneath the surface of the acetone solution, moved under the desired sections and lifted together with them out of the water. When done with the aid of a low-power binocular, positioning of the section upon the grid is possible.

Recently, Hillier(7) has described a technic for sharpening steel knives for thin sectioning. Some experience by one of us indicated advantages of the long cutting edges on steel knives and the larger water reservoir which can be constructed for them (Lansing and Hillier)(8). However, ribbons of sections are not always secured, for the same reasons outlined above for the glass knife. Consequently, a steel knife holder was designed incorporating the rocking mechanism (Fig. 3 and 4). This knife holder is interchangeable with those for the glass knives.

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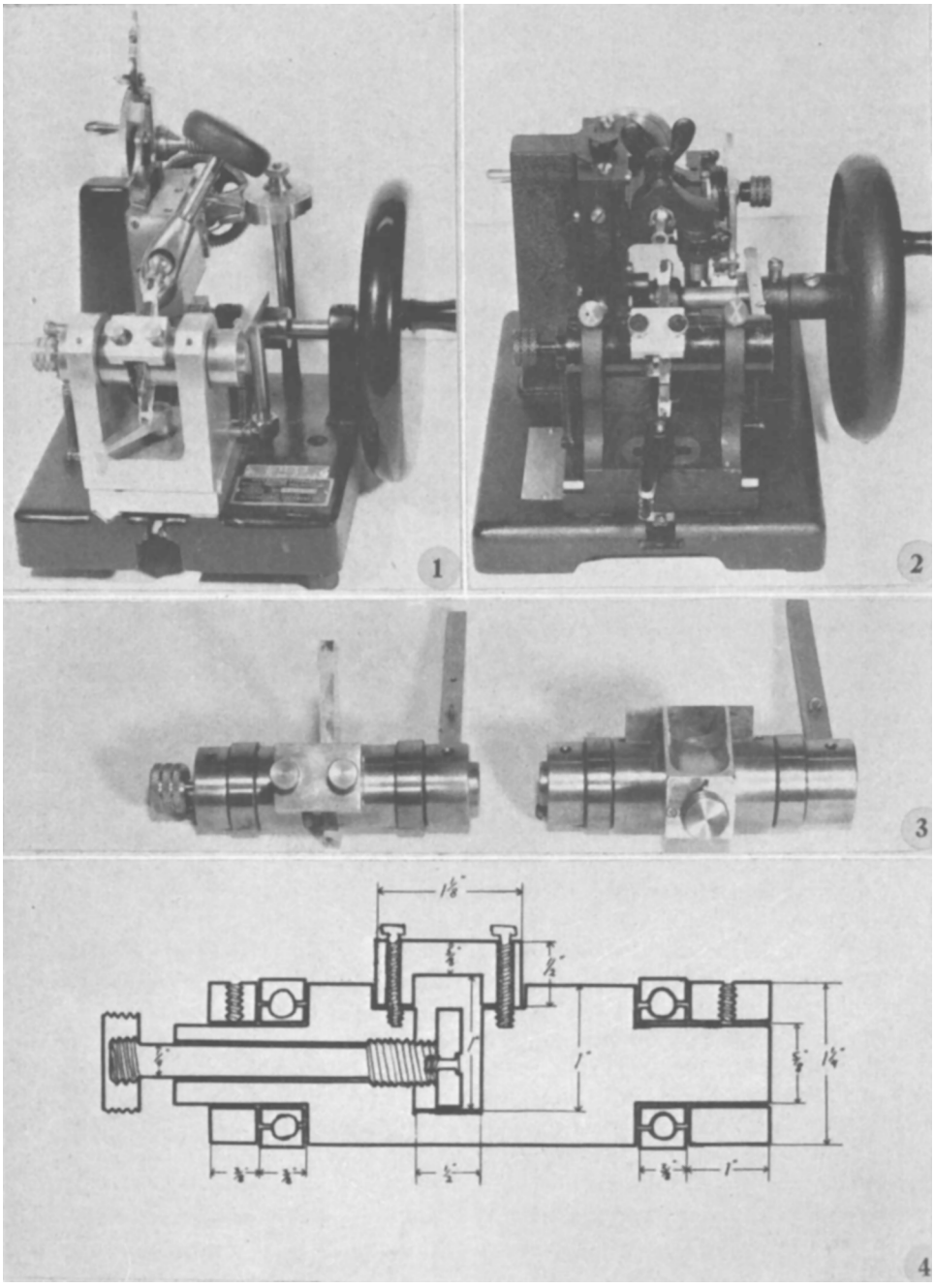

FIG. 1. Minot rotary microtome, adapted for thin-sectioning by the International Equipment Company and modified by the addition of the rocking holder for glass knives described in this article. The cam and lever arrangement, and the adjustable stop to control the excursion of the knife edge are shown.

FIG. 2. Rotary microtome manufactured by Bausch and Lomb, modified for thin sectioning by introducing a worm and pinion gear into the drive mechanism and by adding the rocking holder for glass knives.

FIG. 3. Holder assemblies for glass knives (left) and steel knives (right). On the glass knife, a water-trough is constructed by building up a dam of paraffin. For the steel knife, a

metal trough is employed, sealed to the knife blade by a rubber gasket. The larger metal trough permits longer ribbons of sections to be prepared.

FIG. 4. Scale drawing of the glass knife holder illustrated above. The material is brass, except for the ball bearings which are available commercially.

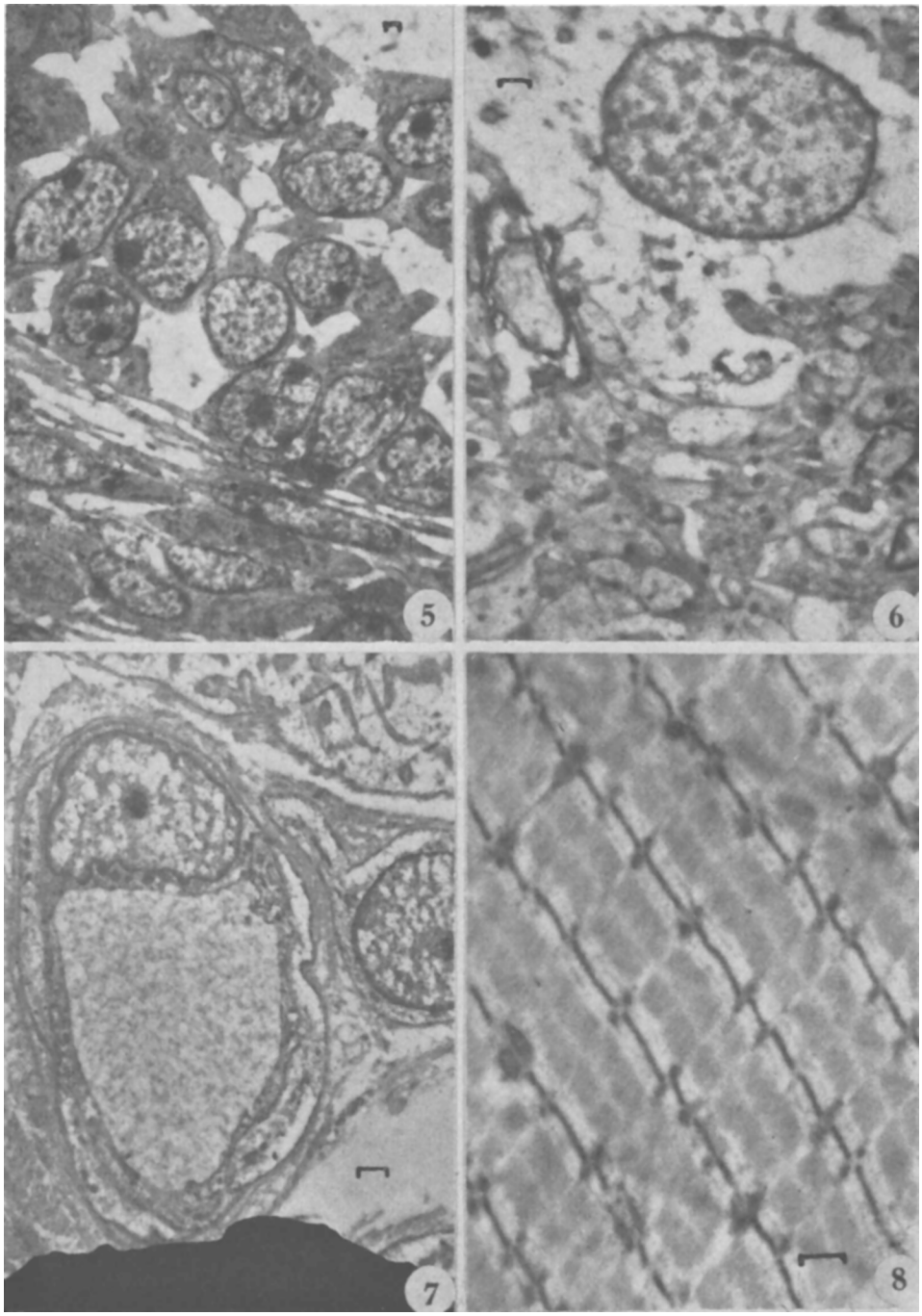

FIG. 5. Electron micrograph of a section through an ovarian follicle in a 5-day-old guinea pig. Liquor folliculi is shown in the upper right corner, granulosa cells occupy the middle portion of the field, and thecal cells are at the bottom. This section was cut with a glass knife.

FIG. 6. Electron micrograph through the spinal cord of a new-born mouse. In the upper portion, a cell and its nucleus are present. The lower portion of the picture represents cell-processes and fibers. The age of the mouse accounts for the fact that little myelin is seen. This section was cut with a steel knife.

FIG. 7. Electron micrograph of a capillary from the tongue of a cat. The section was cut with a glass knife.

FIG. 8. Electron micrograph of a section through the gastrocnemius muscle of a mouse. The section was cut with a glass knife.

All sections illustrated on this plate were from tissue fixed by Palade's buffered osmic acid procedure and imbedded in butyl and methyl methacrylate mixed in a ratio of 3:1. The black line on each photograph represents one micron.

In practice, adequate sections have been secured with either knife. Longer ribbons can be cut with the steel knife. On the other hand, glass knives are quickly interchangeable and our impression is that with the microtome advances available the thinnest sections possible are more readily cut with the glass knives.

A Minot microtome, manufactured several years ago by the Bausch and Lomb Co. was also modified for thin sectioning. A reduced rate of advance was obtained by introducing a worm and pinion gear with a ratio of 100:1 into the advance mechanism. The knife holder was also modified as described above. Fig. 2 illustrates the arrangement of the various components in this instrument.

Both microtomes, modified as described, have given good service. The instrument manufactured originally by the International Company routinely cuts ribbons at thicknesses of 0.05 μ and above. The Bausch and Lomb instrument similarly produces ribbons, but in this case the lower limit of thickness is 0.025 μ. For most purposes sections approximately 0.1 μ thick are adequate. However, some structures encountered in sections of mammalian tissues are quite dense and the thinner sections offer a great advantage. Epidermis, hair and nail, for example, require very thin preparations.

Fig. 5-8 are electron micrographs of sections cut with these instruments. They illustrate the uniformity of thickness which can be obtained, the freedom from chatter marks, and the adequacy of the sections for resolving detail without its being obscured by overlying structures.

Summary. A modification of the knife holder for thin-sectioning microtomes has been described. A cam and lever arrangement, timed with the cutting cycle, advances the knife edge to the cutting position on the down stroke of the instrument and withdraws the knife edge approximately one-half mm on the return stroke. This modification permits the routine cutting of serial ribbons of uniform thinness. The ribbons are flattened upon the surface of an acetone-water meniscus and can be lifted directly on to suitably coated wire grids for electron microscopy. Sections as thin as 0.025 μ have been prepared routinely.

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