EMBEDDING AID FOR COVERSLIP MOUNTS IN ELECTRON MICROSCOPY*

By B. R. NEBEL, M.D., and O. T. MINICK

(From the Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois)

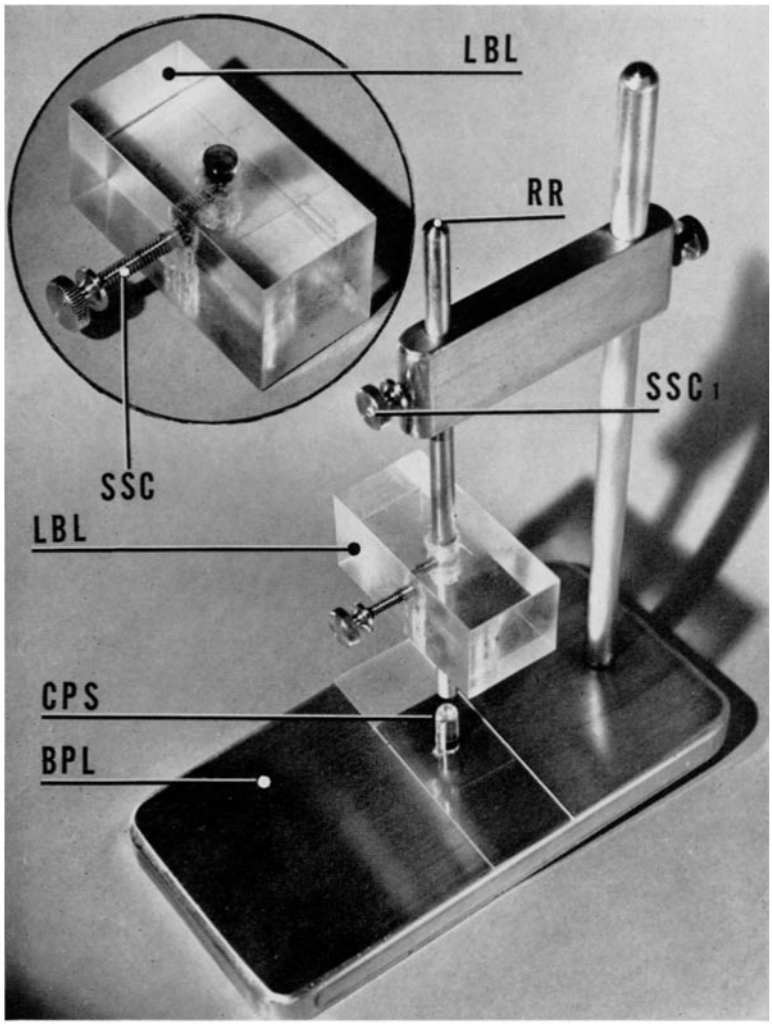
Borysko and Sapranauskas (1) and independently Gay (2) have described a method of making coverslip preparations of biological material from which small selected cell groups or single cells may be transferred to methacrylate for sectioning for electron microscopy. In both procedures it is suggested that the selected material viewed under light and phase microscopy be covered with a capsule containing partially polymerized methacrylate for final polymerization and hardening. The essential aim is to place a capsule filled to the brim with partially polymerized methacrylate open end down on the selected cell or cell group, so that the latter can be in the center of the (future) block. The present authors found it difficult accurately to invert and position the capsule with reference to a single cell or a small group of cells in a single cell layer. Such positioning is readily accomplished by the use of the auxiliary equipment described here, which can be built at low cost (about 4 shop hours).

Text-fig. 1 shows the apparatus used. The insert shows a lucite block (*LBL*) containing a central canal drilled to hold loosely a capsule filled to the brim with partially polymerized homogeneous methacrylate. The capsule is held by the set-screw (*SSC*) so that the open end projects about 0.15 mm. above the surface of the lucite block. Block and capsule are now placed on the stage of a light microscope with the condenser removed. The coverglass mount (1, 2), in which the methacrylate is also partially polymerized, is inverted and observed in contact with the methacrylate in the capsule under the light microscope. It is now easy to arrange the essential material centrally over the surface area of the prospective block by gently shifting the coverglass.

This done, the coverglass may be "tacked" to the capsule in the desired position by running a small amount of partially polymerized methacrylate under the coverglass. The inversion can now be executed without hesitation; surface tension will hold the coverglass in place, while the entire assembly shown in the insert of Text-fig. 1 is turned upside down by hand. Next the coverglass preparation with the attached lucite block is placed coverglass down on a slide which rests on the baseplate (*BPL*). The set-screw (*SSC*) of the lucite block is loosened, and the ramrod (*RR*) is positioned and lowered until its cupped end engages the closed end of the capsule. After the set-screw

* Work performed under the auspices of the United States Atomic Energy Commission.

(SSC_1) is tightened, the preparation may be considered secure, and finally the lucite block may be elevated out of reach of the capsule (*CPS* in the figure).



TEXT-FIG. 1. Assembly of apparatus. Lucite block (*LBL*), baseplate (*BPL*), ramrod (*RR*), capsule (*CPS*), set-screws (*SSC*), and (SSC_1). The insert shows the lucite block and set-screw in greater detail.

The ramrod is then lifted, freeing the capsule, and the base slide, coverslip, and capsule are removed to an oven for final polymerization.

After 48 hours at 60° the coverglass may be removed from the methacrylate surface with a razor blade, after brief cooling of the coverglass and after plac-

ing a drop of acetone at the periphery of the open end of the capsule. After proper trimming of the final block no difficulty is experienced in finding the selected cells for sectioning.

REFERENCES

1. Borysko, E., and Sapranauskas, P., *Bull. Johns Hopkins Hosp.*, 1954, **95,** 68.
2. Gay, H., *Stain Technol.*, 1955, **30,** 239.