A Device for Staining Tissue Sections for Electron Microscopy. By Lee D. Peachey. (*From The Rockefeller Institute.*)*

It has been the experience of this laboratory that the lead hydroxide stain of Watson (1) gives excellent results in increasing contrast in thin sections of tissue, but that the stain is inconvenient to use because on contact with air it forms an insoluble precipitate which contaminates both the stored solution and the sections.[1] Contamination introduced in transferring the solution from the storage vessel to the staining vessel and the excessive amount of solution wasted in filling even the smallest of ordinary staining vessels can be mentioned as additional shortcomings of common methods of using any staining solutions.

These difficulties can be minimized by the technique reported here which involves the use of a plastic syringe both as an air-free storage container for the staining solution[2] and as a staining vessel. The syringes used are of a disposable variety and are commercially available.[3] They have a capacity of 2.5 cc. and are supplied complete with needles and protective needle covers. Modification of the syringes for staining consists of removing the needle by cutting around the tip of the syringe about 2 mm. back from the needle collar, and boring a staining well about 4 mm. in diameter and about 2 mm. deep in the cut end of the syringe (see Fig. 1). Best results are obtained if the well is reasonably well centered.

The needle cap is used as a dust cover for the staining well and as a CO_2-trap. The latter is prepared by packing the cover with a sodium hydroxide pellet, some anhydrous calcium chloride to reduce the amount of water vapor that gets to the hydroxide, and some cotton. There is a small hole and a cotton plug in the end of the cap as it is supplied, and these are left intact to allow free passage of air into and out of the cap when it is being put into place or removed (see Fig. 1). The assembled syringe can be conveniently mounted on a wood or plastic base with a ¼ - 20 flat-head screw, which fits reasonably well into the unthreaded hole in the plunger of the syringe, holding the whole assembly upright (see Fig. 2).

The assembled device is shown in Fig. 2 along with an unmodified syringe and cap. The syringe is filled with freshly made staining solution, and any air present is ejected so that the solution

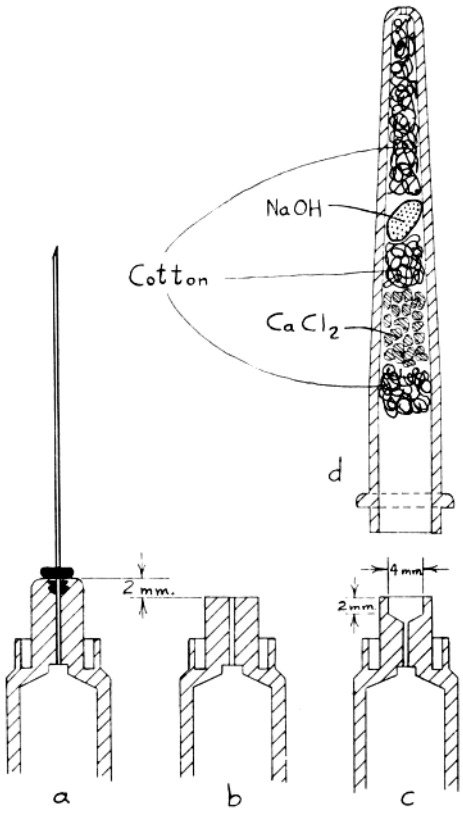

FIG. 1. Sectional views of modification of plastic syringe to staining vessel. *a*. Syringe with needle. *b*. End cut away and needle removed. *c*. Staining well drilled in cut end. *d*. Needle cover modified to dust cover and CO_2-trap as is used for lead hydroxide solution. For solutions that do not react with CO_2, only the plastic cap with a cotton plug is necessary.

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[1] This precipitate is probably lead carbonate, rather than lead oxide, as can be shown by passing 5 per cent CO_2 in air over one drop of the lead hydroxide solution and pure oxygen over another: a precipitate forms only with the CO_2.

[2] The use of a disposable syringe for air-free storage of staining solutions was suggested to the author by Dr. M. L. Watson.

[3] Disposable syringe-needle unit, manufactured by the E. H. Wilburn Corp., Rutherford, New Jersey.

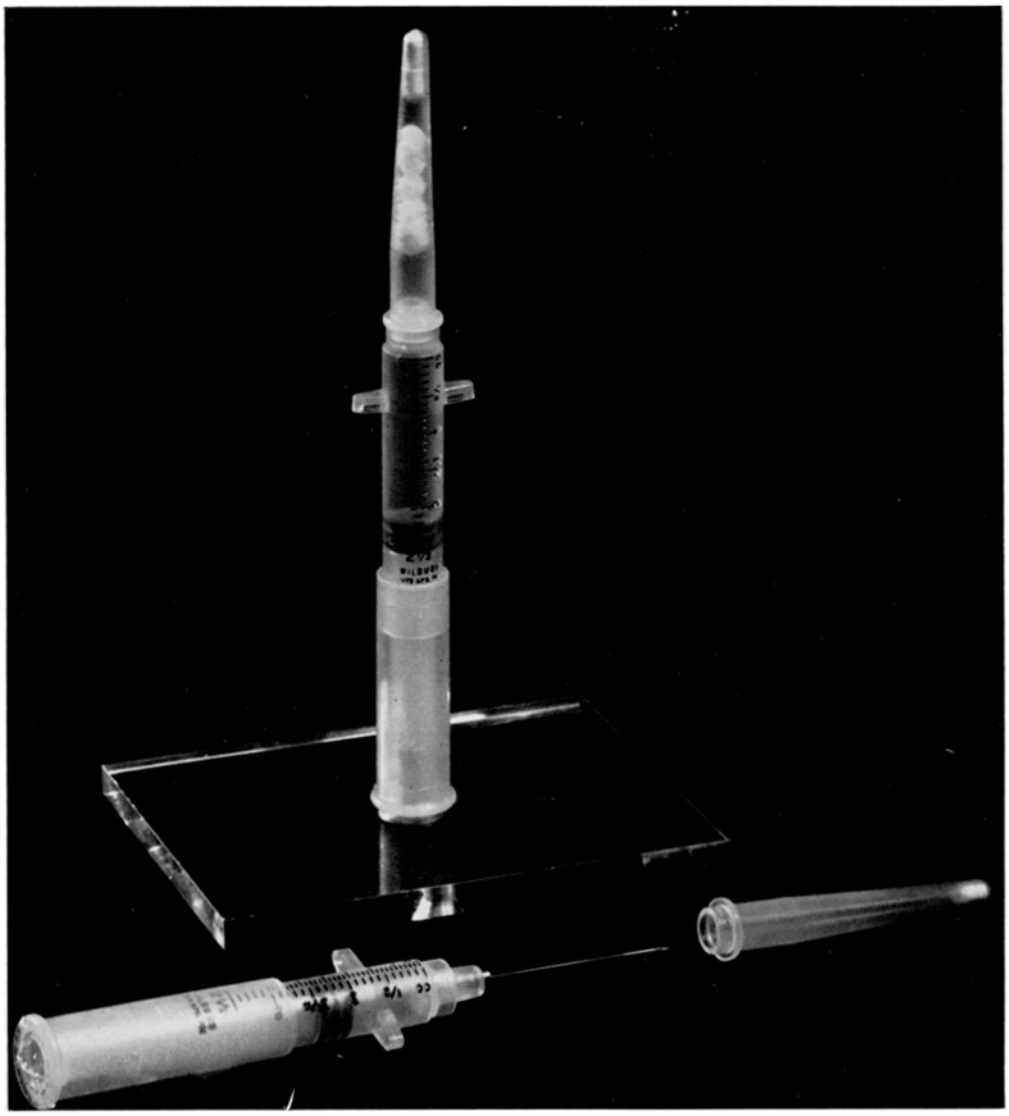

FIG. 2. An unmodified syringe and a completed, filled syringe mounted on a plastic base with a CO_2-trap cap in place.

completely fills the syringe cylinder and the small hole leading to the staining well. The well should then be wiped clean, and the cap put in place. The whole assembly can then be stored for several weeks without noticeable precipitate formation. The CO_2-trap may become moist and should be repacked occasionally. Additional syringes can be filled and stored without CO_2-traps by sealing the staining wells with wax, which can easily be removed when desired.

For use of the staining syringe, the cap is removed and a drop of staining solution is pressed up into the well, forming a convex surface. The surface is wiped clean by pulling a piece of lens tissue across it, the grid with the sections to be stained is quickly floated face-down on the surface, and the cover is put into place. After the staining has progressed for the desired length of time, the cap and the grid are removed and the grid is rinsed and blotted dry. The staining well should

be wiped clean and the cap put into place when finished. The well should be wiped clean and fresh staining solution pressed into it for each grid stained.

This device has been found to ease greatly the difficulties inherent in the use of lead hydroxide stain for thin sections, and has resulted in much cleaner preparations than previously attained. There is no difficulty in storing the solution or in transferring it to the staining vessel since the storage and staining vessels are integrated into one. Since the amount of staining solution needed to fill the well is small (about 0.025 cc.), fifty or more grids can easily be stained with one filling of the syringe. The method as described above can also be used to good advantage with staining solutions other than lead hydroxide that also form precipitates on contact with CO_2, such as the barium hydroxide solution of Watson (1). Obviously, the device without the CO_2-trap is useful for any staining solution which does not react with air.

Reference

Watson, M. L., Staining of tissue sections for electron microscopy with heavy metals. II. Application of solutions containing lead and barium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 727.