A TECHNIQUE FOR THE SIMULTANEOUS DIRT-FREE LEAD STAINING OF SEVERAL ELECTRON MICROSCOPE GRIDS OF THIN SECTIONS

D. F. PARSONS and E. B. DARDEN, Jr. From the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. This Laboratory is operated by Union Carbide Corporation for the United States Atomic Energy Commission

The staining of thin sections of Osmium tetroxide (OsO_4)-fixed biological materials with the lead hydroxide preparation of Watson (5) or with semisaturated solutions of lead subacetate (1) represents a considerable advance in electron microscope technique. The contrast of the image viewed in the electron microscope is so far enhanced that accurate focusing is now possible at appreciably higher magnifications. The contrast and detail visible in the micrograph also appears much improved, without the introduction of obvious artifacts. For routine work, a serious handicap is the formation of a precipitate of lead carbonate over a large proportion of the section area. The method described by Peachey (4) considerably reduces this precipitate but allows only one grid to be stained per syringe. In the method to be described, as many as six grids can be stained simultaneously without any precipitation of lead carbonate.

The apparatus for the filtration of the lead-staining solution and the staining of the sections is illustrated in Fig. 1A. To prepare the lead solution the wire mesh support table is removed and a 3-cm. by 11-cm. test tube stood inside the lower part of the apparatus. A length of polythene tubing is pushed to the bottom of the test tube and a 5-cm. diameter filter funnel and fine pore filter paper are arranged as illustrated in Fig. 2. The polythene tubing is passed through port *c* (Fig. 1A) and connected to a No. 19 needle.

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The rubber stopper is inserted lightly into the hole. A brisk flow of nitrogen, humidified by bubbling through water, is passed through the apparatus for 5 minutes. Port *c* is then opened and the funnel filled with the lead solution by means of a pipette.

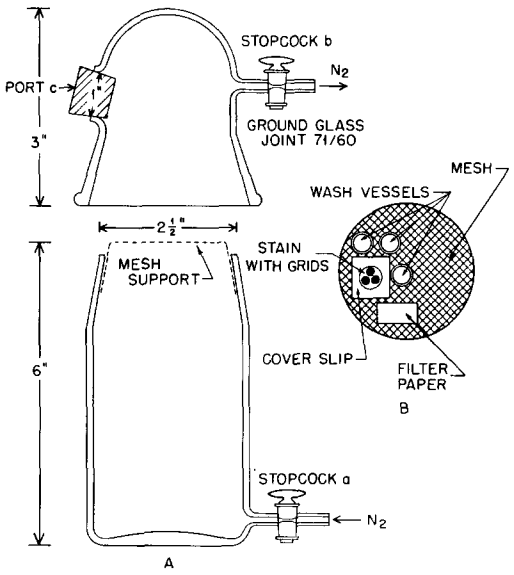

FIGURE 1

Apparatus for the lead staining of thin sections in an atmosphere of nitrogen. *A*. Sectional view of glass vessel and mesh support. *B*. Arrangement of coverslip, wash vessels, and filter paper on mesh support.

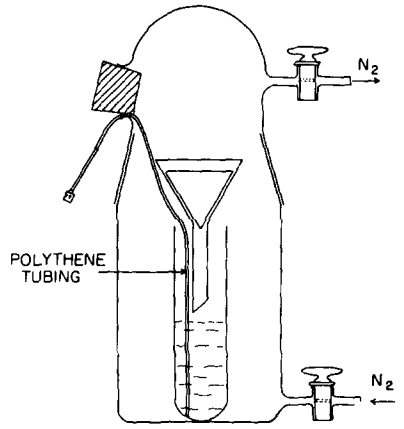

FIGURE 2

Arrangement of apparatus for the filtration of lead-staining solutions in an atmosphere of nitrogen.

Port *c* is again closed until sufficient filtrate (which should be crystal clear) has collected. The filtrate is withdrawn into a 10 or 20-cc. syringe which has previously been rinsed out with boiled glass-distilled water. The syringe is fitted with a 2-inch No. 26 needle, and the needle point is pushed into a piece of cork. A supply of gently boiling, glass-distilled water is kept at hand. A 20-cc. syringe is filled with hot water from beneath the surface by means of a long needle and attached polythene tubing. Small bubbles of steam are expelled, and a 2-inch No. 26 needle fitted and the end closed with a piece of cork.

Fig. 1A illustrates the apparatus for staining the sections in the absence of air. A brisk flow of tank nitrogen, humidified by bubbling through water, is passed through the apparatus. The mesh support table is adjusted to a convenient height for the manipulation of grids with forceps through port *c*. A brisk flow of nitrogen is passed, *c* is opened, and three wash vessels (diameter 10 mm., height 7 mm.), a clean ⅞-inch-square coverslip, and a piece of filter paper are placed on the mesh table as shown in Fig. 1B. The vessels are next filled with water from the syringe, and one or several drops of the lead stain are placed on the coverslip. The first drop from each syringe is rejected. The grids are floated gently on the drop of stain solution with the section side downward. While the nitrogen flow is being reduced, port *c* is closed, and the gas now escapes through stopcock *b*. The forceps are wiped free of stain. After the desired period of staining, the nitrogen flow is increased while port *c* is being opened. The grids are dipped successively for 1 or 2 seconds in each of the three wash vessels and then blotted on the filter paper. The grids are held with the forceps at the edge only, to avoid picking up a drop of stain. Port *c* is closed and the grids are allowed to dry for a few minutes in a moderate flow of nitrogen.

Several precautions are necessary to ensure freedom from a small amount of lead precipitate and other dirt. The coverslips are washed in ethyl acetate and cleaned thoroughly before use. The wash vessels are cleaned occasionally in nitric acid. During blotting, only the edge of the grid should touch the filter paper, and only papers that have not been acid-treated should be used.

This technique also appears to be suitable for the staining of sections with potassium permanganate without the necessity of using a citrate wash solution (3). The lead hydroxide staining technique of Dalton (2) appeared while this publication was in preparation. In our hands it has given clean preparations with a moderate intensity of staining. This method may be preferable for lead hydroxide staining although the time required to complete staining is longer, and more manipulations are involved. Since, however, a precipitate-free lead subacetate solution in boiled distilled water cannot be prepared, filtration and staining in a carbon dioxide–free atmosphere are necessary to obtain clean preparations consistently.

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