A COMMON SOURCE OF DIFFICULTY IN HIGH-RESOLUTION RADIOAUTOGRAPHY

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In high-resolution radioautography, a common source of difficulty in the use of the loop method for applying Ilford L-4 emulsion (1) is an inhomogeneous distribution of water in the emulsion. This inhomogeneity may be due to bad environmental conditions during transportation. It is easily cured by melting the entire bottle of emulsion once before use. Our standard procedure for the preparation of electron microscope radioautigraphs, slightly modified from that described in reference 1, is now as follows:

Specimen screens are attached by a small portion of their edge to a small piece of double-coated masking tape (Scotch Brand No. 400) fixed to a microscope slide (Fig. 1).

When starting a fresh bottle of L-4 emulsion, the bottle is placed into a 45°C bath. As soon as the content is completely liquified, it is mixed thoroughly and allowed to gel. 15 g of this emulsion is weighed, then melted at 45°C in 15 ml of distilled water in a 250-ml beaker, with gentle stirring to avoid bubbles. The emulsion is placed in an ice bath for 1–5 min, then a bath at 20°C (room temperature). It should become quite viscous in about 10 min and remain in a state favorable for preparing films for an additional 10 min.

A loop of copper wire (Fig. 2) is dipped in the emulsion and withdrawn slowly, forming a thin film in the loop. This film should gel almost immediately. The loop is touched to the surface of the slide; the gelled film falls on the grids and adheres to them very firmly. L-4 emulsion tends to vary slightly in its gelling properties, and the schedule of melting and cooling may have to be modified accordingly. If the film does not gel rapidly or if electron microscopic examination reveals gross uneveness in the distribution of silver halide crystals, the time of cooling in the ice bath should be increased slightly. When the viscosity of the emulsion becomes so high that a film cannot be formed, the emulsion can be melted at 45°C and the process repeated with a shorter cooling time in the ice bath. After a few cycles of melting and gelling, the emulsion becomes lumpy and should be discarded. In the electron microscope, the preparation should appear as a tightly packed monolayer of silver halide crystals. Frequent electron microscope control of the emulsion layer obtained should be a routine part of the procedure.

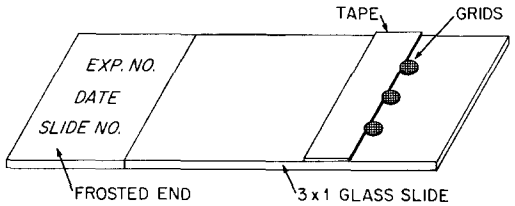

FIGURE 1 Attachment of specimen screens to a glass microscope slide.

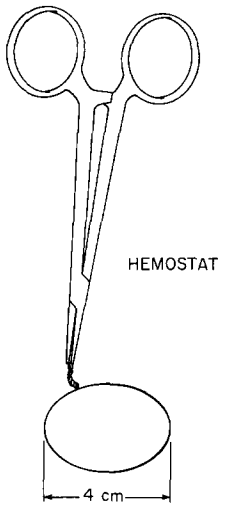

FIGURE 2 Copper wire loop used to form a film of emulsion.

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REFERENCE

1. CARO, L. G., and R. P. VAN TUBERGEN. 1962. High-resolution radioautography. I. Methods. *J. Cell Biol.* **15**:173.