Improved Method of Embedding with Epoxy Resin 'Quetol 651' for Both Light and Electron Microscopic Observation of Identical Sites in Semi-thin Sections

By

TSUYUKA KUSHIDA and HIROSHI KUSHIDA*

Department of Anatomy, Tokyo Women's Medical College, Shinjuku-ku, Tokyo 162, Japan
*Electron Microscope Laboratory, Keio University, Shinjuku-ku, Tokyo 160, Japan

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Key words: Light and electron microscopy, Semi-thin sections, Embedding medium, Quetol 651, En bloc staining.

Summary: In order to examine semi-thin sections approximately 0.2 µm thick for light microscopy with an accelerating potential of 100 kV by a conventional electron microscope, Quetol 651 as a low viscosity water-miscible epoxy resin was suitable for an embedding medium. Semi-thin sections approximately 0.2 µm thick embedded in Quetol 651 could be examined with an ordinary electron microscope operating at 100 kV.

Tissues were fixed in 2.5% glutaraldehyde with buffered cacodylate at pH 7.4 for 3 hr, postfixed in 2% osmium tetroxide with buffered cacodylate at pH 7.4 for 2 hr and stained with en bloc by 3% aqueous solution of uranyl acetate for 2 hr at 37°C. After dehydration in graded alcohol, they were embedded in the Quetol 651 mixture. Semi-thin sections approximately 0.2 µm thick were cut with glass knives on a conventional ultramicrotome. Semi-thin sections on grids were stained with the Giemsa method and methylene blue-azure II-basic fuchsin. Staining without removal of the embedding matrix was sharp and brilliant. These stained sections were observed under a light microscope. For electron microscopy, they were doubly stained with uranyl acetate and lead salt. Identical sites on such sections could be distinctly examined with an accelerating potential of 100 kV at low magnification (250-1,500 times) using LEM-2000, a combined light and electron microscope. Thus, photomicrographs electron micrographs of identical sites on tissue samples could be compared exactly.

Semi-thin sections 1 µm thick embedded in ordinary epoxy resins for light microscopy have been examined with a light microscope. It was, however, difficult to stain these semi-thin sections approximately 0.2 µm thick with the Giemsa method and methylene blue-azure II-basic fuchsin according to a method of Humphrey. These stained sections could not be examined with an electron
microscope.

Quetol 651 as water-miscible epoxy resin with a low viscosity was suitable for embedding medium in order to examine semi-thin sections with light and electron microscope. Semi-thin sections approximately 0.2 μm thick embedded in Quetol 651 could be examined with an ordinary electron microscope operating at 100 kV.

The present embedding method employed Quetol 651 as epoxy resin, nonenyl succinic anhydride and methyle nadic anhydride as hardener and DMP-30 as accelerator. The mixture of these could be hardened to give excellent cutting properties.

Tissues were fixed in buffered glutaraldehyde, postfixed in buffered osmium tetroxide, washed in a non-phosphate buffer, and then en bloc staining of these tissues should be carried out. After dehydration in graded alcohol, they were embedded in the Quetol 651 mixture. Semi-thin sections approximately 0.2 μm thick could then easily be cut with glass knives on a conventional ultramicrotome. Semi-thin sections on grids were stained with the Giemsa method and methylene blue-azure II-basic fuchsin. Staining without removal of the embedding matrix was sharp and brilliant. These stained sections were observed under a light microscope. For electron microscopy, they were doubly stained with uranyl acetate and lead salt. Identical sites on such sections approximately 0.2 μm thick could be distinctly examined with an accelerating potential of 100 kV at low magnification (250-1,500 times) using LEM-2000, a combined light and electron microscope. Thus, photomicrographs and electron micrographs of identical sites on tissue samples could be compared exactly.

**Materials and Methods**

1. **Specimens**

   Fresh tissues (small intestine, pancreas, kidney and testis) excised from adult male I.C.R. mice were used. The tissue samples were approximately 2×2×1 mm in size.

2. **Fixation**

   The tissue pieces were fixed in 2.5% glutaraldehyde with buffered cacodylate at pH 7.4 for 3 hr at 0°C-4°C, and washed in the buffer for 2 hr. After washing, they were postfixed in 2% osmium tetroxide with buffered cacodylate at pH 7.4 for 2 hr at room temperature, and washed in the buffer for 1 hr.

3. **En bloc staining**

   After washing, they were stained en bloc by 3% aqueous uranyl acetate for 2 hr at 37°C, and washed in distilled water for 1 hr.

4. **Dehydration**

   They were dehydrated in the ordinary way in 50%, 70%, 80%, 90%, 95%, and two changes of 100% alcohol for 30 min each.

5. **Embedding**

   The embedding method used Quetol 651* as the epoxy resin, nonenyl succinic anhydride (NSA) and methyl nadic anhydride (MNA) as hardener and DMP-30 as accelerator.

   The following mixture was recommended for this embedding:

<table>
<thead>
<tr>
<th>Material</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quetol 651</td>
<td>35 ml</td>
</tr>
<tr>
<td>NSA</td>
<td>54 ml</td>
</tr>
<tr>
<td>MNA</td>
<td>11 ml</td>
</tr>
<tr>
<td>DMP-30</td>
<td>1.5-2.0 ml</td>
</tr>
</tbody>
</table>

   This mixture set in about 24 hr at 60°C yielding clear, light yellow blocks. It was suitable for cutting approximately 0.2 μm sections.

* Sold by Nissin EM Co. Ltd., Esupowaru Ichigaya Bldg., 40-10 Tomihisa-cho, Shinjuku-ku, Tokyo 160, Japan; and Ted Pella Inc., P.O. Box 510, Tustin, Calif. 92680, U.S.A.
After dehydration, the following infiltration procedure was appropriate. n-Butyl glycidyl ether (n-BGE, QY-1) was employed as auxiliary to infiltration. All steps were carried out on a shaker at room temperature.

1) 100% alcohol/n-BGE (1:1) 30 min
2) n-BGE 30 min
3) n-BGE/Quetol 651 mixture (1:1) 1-2 hr
4) Quetol 651 mixture 2-3 hr
5) Quetol 651 mixture 2-3 hr
6) Embed in gelatin (or polyethylene) capsules

Gelatin capsules were filled with the fresh Quetol 651 mixture, and the specimens were introduced. Curing was achieved in about 24 hr at 60°C.

6. Trimming and Sectioning

After removal of the gelatin capsule, the block was placed in a holder and observed under a dissecting microscope to aid precise trimming. The block face was carefully trimmed to a size of approximately 2×2 mm with a clean, sharp razor blade. The trimmed block was cut with a glass knife of approximately 10 mm in width on an ultramicrotome (Porter-Blum MT-1 or MT-2B). The sections were generally cut approximately 0.2 μm in thickness.

After cutting, a chloroprene rubber coated grid without supporting membrane was pressed gently over the floating sections in a trough. The excess water on the grid was blown off with a quick blast of clean dry gas from a freon gas spray. The sections were allowed to dry at room temperature. They adhered to the grids during staining and washing.

7. Staining

The sections were stained with the Giemsa method and methylene blue-azure II-basic fuchsin. For the Giemsa method, the staining solution was prepared by mixing the Giemsa solution produced by Merck Co. and a phosphate buffer (pH 6.4) in a ratio of 1:3 in volume. The semi-thin sections were stained with this solution for 30 min at 55°C. For another staining, they were stained with aqueous methylene blue-azure II solution for 30 min at 55°C and then basic fuchsin solution for 5 min at room temperature according to the method of Humphrey. Staining without removal of the embedding matrix was sharp and brilliant.

For staining the sections on grids, a method employing a short piece of polyethylene tubing as a grid-holder was used to facilitate handling of the grids. The grids were firmly inserted into small slits in the polyethylene tubing and pieces of the tubing were attached to sharp glass rods of a cap. The sections were stained with aqueous solutions without removal of the embedding matrix.

The grids with sections were placed into a staining solution for a given length of time. After staining, they were washed with distilled water and treated with another solution in the case of double staining without drying. After washing with distilled water, the pieces of polyethylene tubing were removed from the glass rods, rinsed carefully with a stream of distilled water directed along the axis of the tubing, and then dried under a gentle jet of freon gas applied in the same direction.

For electron microscopy, the sections were doubly stained with uranyl acetate and lead salt as usual.

8. Microscopic Examinations

The stained sections were observed under a light microscope. Identical sites on such sections, approximately 0.2 μm thick, were examined with an accelerating potential of 100 kV at low magnification using LEM-2000, a combined light and electron microscope. Electron micrographs where prepared at low magnification (250–1,500 times) and enlarged photographically.
Results and Discussion

Semi-thin sections approximately 0.2 \( \mu m \) thick embedded in ordinary epoxy resins for light microscopy have not been examined with an electron microscope. In order to examine semi-thin sections approximately 0.2 \( \mu m \) thick embedded in epoxy resins for light microscopy with an accelerating potential of 100 kV by a conventional electron microscope, it was important to use specific embedding medium. Quetol 651 as a low viscosity water-miscible epoxy resin was suitable for embedding medium, since it was easy in embedding, infiltration, sectioning, staining for both light and electron microscopy. Therefore, this resin was characterized by low electron scattering. Semi-thin sections approximately 0.2 \( \mu m \) thick embedded in Quetol 651 could be examined with an accelerating potential of 100 kV by a conventional electron microscope.

The present embedding method used Quetol 651 as epoxy resin, NSA and MNA as hardener and DMP-30 as accelerator. Quetol 651 is an ethylene glycol diglycidyl ether, an epoxy resin of low viscosity. It is a light-colored epoxy resin with a viscosity of 15 cps at 25°C. It is readily miscible with water, alcohol, acetone, \( n \)-butyl glycidyl ether, etc. It acts as a dehydrating agent, and combines chemically in any cured epoxy resin formulation.

The Quetol 651 mixture set in about 24 hr at 60°C. It had a low viscosity, and was therefore easy to handle. It penetrated readily and completely into the specimen, producing a homogeneous block from which approximately 0.2 \( \mu m \) sections could be easily cut. The final hardness of the blocks could be adjusting by altering the ratio of Quetol 651-NSA and Quetol 651-MNA mixtures to suit the specimen. This mixture of Quetol 651, NSA and MNA was suitable for sectioning at 20°C. A gelatin capsule with a flat bottom was appropriate for flat embedding of a specimen.

The tissues were fixed in buffered glutaraldehyde, postfixed in buffered osmium tetroxide, and washed in a non-phosphate buffer. For, when washing in phosphate buffer, uranyl phosphate was precipitated with uranyl acetate. They should be carried out with en bloc staining by aqueous uranyl acetate, since the contrast in the images of the specimens for electron microscopy could be improved by en bloc staining.

Sections approximately 0.2 \( \mu m \) thick could be easily cut with glass knives on a conventional ultramicrotome. The sections on grids were allowed to dry at room temperature. They adhered to the grid during staining and washing.

For staining sections on grids, a method employing a short piece of polyethylene tubing as a grid-holder was suitable to facilitate handling of the grids. Staining with the Giemsa method and methylene blue-azure II-basic fuchsin, without removal of the embedding matrix, was sharp and brilliant as illustrated in Figures 1A, 2A, 3A and 4A. Figures 1A and 2A depict mouse small intestine, Figure 3A depicts mouse seminiferous tubules and Figure 4A depicts mouse pancreas. These approximately 0.2 \( \mu m \) sections were doubly stained with uranyl acetate and lead salt after the Giemsa method or methylene blue azure II-basic fuchsin. Figures 1B, 2B, 3B and 4B show electron micrographs of identical sites to the sections shown in Figures 1A, 2A, 3A and 4A, respectively. Figures 1C, 2C, 3C and 4C are enlargements from Figures 1B, 2B, 3B, and 4B, respectively. Photomicrographs of identical sites on the tissues could thus be compared exactly.

In Figures 1B and 2B, the specific tissue contrast was based on the selected color of the dye at the light microscopic
level. In Figure 3B, the specific contrast at the intercellular boundaries distinguished each cell and the cytoplasmic components were clearly recognizable. In Figures 1C, 2C, 3C and 4C, the resolution of the electron microscope was so high that the cytoplasmic components, particularly mitochondria, Golgi apparatus and endoplasmic reticulum, could be readily identified. The cellular structure of the striated border of intestinal epithelial cell, the desmosomes of the seminiferous epithelium of the testis and the endoplasmic reticulum of the pancreas, were also clearly demonstrated under the electron microscope. Images from both en bloc and double staining showed a distinctly improved contrast.

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References

Explanation of Figures

Plate I

Fig. 1A. Mouse small intestine fixed in 2.5% buffered glutaraldehyde, postfixed in 2% buffered osmium tetroxide, stained with en bloc and embedded in a Quetol 651 mixture. 0.18 μm section stained with the Giemsa method. ×840.

Fig. 2A. Mouse small intestine fixed in 2.5% buffered glutaraldehyde, postfixed in 2% buffered osmium tetroxide, stained with en bloc and embedded in a Quetol 651 mixture. 0.18 μm section stained with methylene blue-azure II-basic fuchsin. ×470.

Fig. 3A. Mouse seminiferous tubule fixed in 2.5% buffered glutaraldehyde, postfixed in 2% buffered osmium tetroxide, stained with en bloc and embedded in a Quetol 651 mixture. 0.18 μm section stained with the Giemsa method. ×840.

Fig. 4A. Mouse pancreas fixed in 2.5% buffered glutaraldehyde, postfixed in 2% buffered osmium tetroxide, stained with en bloc and embedded in a Quetol 651 mixture. 0.18 μm section stained with the Giemsa method. ×840.
Plate I

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Plate II

Figs. 1B, 2B, 3B and 4B. Electron micrographs of identical sites to the sections in Figs. 1A, 2A, 3A and 4A, respectively. These sections stained doubly with uranyl and lead salts after the Giemsa method or methylene blue-azure II-basic fuchsin staining. $\times 800$, $\times 720$, $\times 840$ and $\times 840$ respectively.
Plate III

Figs. 1C and 2C. Electron micrographs, enlarged from Figs. 1B and 2B, respectively. $\times 9,500$ and $\times 9,000$ respectively.
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Plate IV

Figs. 3C and 4C. Electron micrographs, enlarged from Figs. 3B and 4B, respectively. $\times 1.700$ and $\times 6.000$ respectively.