COMPLETE EXAMINATION OF MASTECTOMY SPECIMENS USING SHEET PLASTINATION WITH EPOXY RESIN

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INTRODUCTION

In Europe, the malignancy exhibiting highest incidence in the female is breast cancer. When one lesion is found, chances are between 13.4 and 41.6 percent that another growth exists in the same organ (1) (2) . Because of this high rate of multicentric occurrence, it is desirable to have a method that permits examination of each mastectomy specimen in its entirety.

Total-specimen examination has become even more important in recent years, given the emphasis now being placed on conservative surgical treatment such as subcutaneous mastectomy and quadrant-part resection. Specimens obtained from procedures such as these require complete histologic examination so that surgeon and patient alike may be assured of complete tumor resection.

Plastination, a method of impregnating tissue with curable polymer, was developed by one of the authors (GvH) and is now widely used for the preparation of teaching specimens (3) (4) (5). A variation of this technique (sheet plastination with clear epoxy) is ideally suited to the preparation of mastectomy specimens (total, subcutaneous or quadrant-part) for comprehensive macroscopic and microscopic inspection. In fact, it is the only method presently available that permits complete histologic scrutiny of such large specimens on a routine basis.

MATERIALS AND METHODS (EPOXY IMPREGNATION)

The first five steps in the process lead to a sliced specimen completely impregnated with uncured clear epoxy. We will list these in sequence and discuss surface preparation and curing as a separate section.

1. Fixation: The intact specimen is placed in 5% buffered formalin for one or two days until fixed hard enough to be cut by a slicing machine.

2. Slicing: The specimen is now cut into 2.5 mm slices using a commercial meat slicer (see Appendix A) . Each slice should be identified by writing the case and slice numbers on blotting paper which is then stapled to the tissue. Slices may be radiographed at this stage if a comparative image is desired. We recommend a Faxitron table machine, using 45 sec exposure at 20 KeV.

3. Staining: The slices are placed in a hemalum staining bath (Appendix B) until they acquire enough color for macroscopic viewing. This usually takes two or three minutes when the bath consists of one part hemalum and three parts distilled water. If the stain is diluted further with distilled water, the slices may remain in the bath up to several hours.

4. Dehydration: The stained tissue is now dehydrated by progressive exposure to increasing concentrations of ethanol. We use 30%,50%,70%,90% and two or three changes of 100%. It is then immersed in a sequence of three baths of 100% acetone and one of 100% methylene chloride. The slices must remain in each bath for a minimum of two hours. This step accomplishes not only dehydration, but also degreasing and saturation with a volatile intermediary solvent (methylene chloride) that will propel the impregnation occuring in the next step.

5. Impregnation: Tissue slices are now placed in a reaction mixture of the epoxy polymer (Appendix C) contained in a vacuum chamber. Pressure within this chamber is reduced at a very gradual rate, permitting the methylene chloride to vaporize at a gentle boil (indicated by slow bubbling at the surface). It should take about four hours to reach full vacuum, after which the tissue is allowed to remain for 12 additional hours at this low pressure. Air is then readmitted to the chamber over a one-hour period. The slices are now completely impregnated with uncured epoxy.

Dehydration of the tissue slices causes some shrinkage and results in a distorted surface. For proper visual inspection, the surface of each slice must now be rendered smooth.

1. Compression: On a large glass plate (45x45 cm) lay a piece of thick plastic foil the same size. This foil must be heavier than ordinary mylar wrap so that it will not tear on removal. On top of this foil spread a layer of the polymer reaction mixture used in Step 5 (above). Now press the tissue slices firmly into the polymer, so as to remove trapped air bubbles. Pour more of the polymer over the tissue and cover them with a second sheet of plastic foil, similarly coated with a layer of polymer. Again, make sure that no air bubbles are trapped between the tissue and this second foil. A second glass plate is now placed on top of the second sheet of foil and the entire assembly is pressed together with strong clamps.

2. Draining: While still clamped, this multilayered "sandwich" is now placed over a pan to permit draining of excess polymer. Care must be taken to drain only the excess, not material impregnating the slices. We have achieved good results by placing the sandwich at a slight angle (about 10 degrees). At this angle we find that the impregnating resin remains in the tissue and only the excess is removed. Using a greater angle causes polymer to drain out of the tissue, resulting in surface irregularity around the surgical margins. Draining should be continued for about 2 days.

3. Curing: The fully drained sandwich,still firmly clamped, is now placed in an oven, previously heated to 50°C. After 24 to 48 hours at this temperature the epoxy will be fully cured. The sandwich may now be opened and the tissue slices removed.

RESULTS

Without magnification it usually possible to clearly distinguish skin, fat, gland, connective tissue blood vessels, tumor, cysts and lymph nodes. Thus, a preliminary screening can be accomplished using only the naked eye.

Magnification (up to 100X) permits detailed inspection in three dimensions by simply picking a field and adjusting the microscope through a sequence of focal planes. In this manner, a ductal carcinoma can be followed, in continuity, over several slices, from its origin at a terminal duct to its surface manifestation at the nipple.

Hemalum stains only the nucleus of the cell, hence only nuclei can be observed. This permits preliminary evaluation of at least two important diagnostic criteria of malignancy, nuclear pleomorphism and mitosis. If closer inspection is required, thinner sections may be cut from the questionable area of the slice and restained with hematoxylin and eosin for routine histopathological evaluation.

DISCUSSION

Until the development of plastination, two methods for screening breast slices had been reported: radiographic examination and saturation with wintergreen oil. The radiographic technique was described by Egan (6) and further refined by Lagios (7). Marcum (8) and Wellings (9,10) advocated immersion in wintergreen oil.

We have compared radiographic imaging with conventional pathologic examination for the screening of sliced subcutaneous mastectomy specimens (11) and have found that the former is three times more liable to detect residual cancer.

The plastination method herein described is judged to be even more sensitive than radiography, particularly when the specimen contains an abundance of dense fibrous connective tissue. Also, it must be considered that this method permits total, three-dimensional, visual inspection of the surgical margins of a specimen, in continuity. Insofar as we know, such versatility is unique to plastination.

Using the wintergreen oil technique, it is necessary to prepare 100 to 500 thin slices in polyethylene bags (9) . This is much more time-consuming than plastination (which requires only 10-15 slices) and limits the amount of magnification that can be employed. Also, this method involves serious problems regarding storage and the objectionable odor of the clearing medium.

SUMMARY

A method is described for the complete macroscopic and microscopic examination of mastectomy specimens. The entire specimen is cut into 2.5 mm slices, stained and plastinated with clear epoxy. This renders each slice so transparent that it can be examined both grossly and at a magnification of 100X or greater. Thinner sections (4-10 microns) may be cut from a plastinated slice if an area requires even closer inspection. The entire process, including macroscopic and microscopic screening takes approximately seven days. Thus, the method is quite practical for use as a diagnostic regimen.

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APPENDIX

A. SLICING MACHINE

We use and recommend a Bizerba slicing machine. This instrument consistently leaves a smooth surface on each slice, a matter of importance for close inspection. The machine is available from: Bizerba, D-7460 Balingen, West Germany.

B. HEMALUM STAINING BATH (MAYER)

Hematoxylin (Merk #4305) ----------	1 gram
distilled water ----------	1 liter
Sodium lodate (Merk #6525) ----------	0.2 gram
Potassium aluminum sulfate (Merk #1047) — (after 24 hours)	50 grams
Chloral hydrate (Merk #2425) ----------	50 grams
Citric acid (Merk #242) ----------	1 gram

C. POLYMER REACTION MIXTURE*

Biodur E12 Epoxy Resin —	1 kilogram
Biodur El Hardener -----	300 grams

* Biodur products obtainable from:

Biodur
Jahnstrasse 8 D-
6900 Heidelberg
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