Technical methods

A technique for identifying areas of interest in human breast tissue before embedding for electron microscopy

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The ultrastructural examination of normal and abnormal human breast tissue is complicated by the fact that the parenchymal structures cannot be identified readily in fresh tissue. Using conventional techniques the sectioning of blocks of breast tissue, in the search for structures of interest, can be extremely tedious and time-consuming. In this paper a simple technique is described which reduces this task.

Material and methods

To obtain samples of human breast tissue, breast biopsies were sliced and areas of grey/white connective tissue not exceeding 10 mm × 5 mm × 2 mm were excised. The tissue was placed directly in primary fixative which was normally 3% glutaraldehyde in cacodylate buffer, pH 7.2. In the case of retrospective studies of routine diagnostic material the tissue had been fixed in 4% formaldehyde in phosphate buffer.¹ The material was fixed for a minimum for 3 hours. The blocks were then removed and chopped into 60 µm thick sections using a Sorval TC-2 Tissue Sectioner. The tissue sections were washed overnight in cacodylate buffer and processed in the normal way—that is, the material was post fixed in 1% osmium tetroxide in cacodylate buffer for 1 hour. Dehydration was performed through an ethanol series, followed by propylene oxide treatment and overnight impregnation with Araldite.

At this stage, the 60 µm tissue sections were examined with a dissecting microscope. It was found that tissue slices containing parenchymal structures could be readily identified, and those containing normal ducts and lobules were separated from those with altered parenchymal architecture. A representative number of these sections were photographed to record the subgross appearance of the tissue before they were embedded in Araldite using flat bottomed

BEEM capsules for subsequent ultrastructural examination. The remaining sections were placed in 4 cm × 2 cm "Peel-a-way" disposable plastic tissue-embedding moulds and covered with 2 mm of Araldite and polymerised at 60°C.

The light microscope appearance was observed using a 1 µm thick toluidine blue stained sections, allowing areas to be chosen for thin sectioning. The thin sections were stained with uranyl acetate and lead citrate and examined in an AEI Corinth 275 electron microscope.

Results and discussion

This simple technique, which employs existing methods, is based on the fact that the morphology of the structures within the 60 µm thick tissue sections can be readily resolved. The resolution is apparently due to a differential staining of the tissue by the osmium tetroxide enhanced by a slight clearing effect of the Araldite. The connective tissue is uniformly stained light brown while the epithelial cells of the ducts and lobules take up more osmium and appear dark brown (Fig. 1). It has been found

Fig. 1 The subgross appearance of a tissue slice containing normal parenchymal components is shown. Lobules (arrows) can be differentiated readily from lipid droplets (L). × 25.

that 60 µm is the optimal thickness of the tissue slices. The advantage of flat bottomed BEEM capsules is that the tissue slices are embedded flat, thus making it easier to obtain a 1 µm section through the entire face of the slice.

The flat Araldite blocks, containing the extra tissue sections, obtained from the "Peel-a-way"

Fig. 2 The subgross appearance of a lobule exhibiting lobular carcinoma in situ. The lobule has a solid appearance in which individual ductules cannot be identified. $\times 32$.

Fig. 3 Histological appearance of part of a lobule similar to that in Fig. 2 showing the presence of lobular carcinoma in situ. Araldite section ($1 \mu\text{m}$) stained with toluidine blue. $\times 320$.

Fig. 4 Electron micrograph of part of a ductule exhibiting lobular carcinoma in situ. $\times 2500$.

embedding moulds are easy to store. If additional tissue sections are subsequently needed, these can be identified and sawn from the Araldite block and sectioned for light and electron microscopy.

Using this technique the resolution is such that slight variations in the architecture of normal lobules can be observed (Fig. 1) and abnormal parenchymal structures identified for the study of small pathological lesions. For example, the altered architecture due to non-invasive carcinoma (Fig. 2) can be identified and examined. In the case of the

pathological changes, the lesions can be assessed after staining $1 \mu\text{m}$ sections with toluidine blue (Fig. 3). It is found that in all cases there is good preservation of the ultrastructural features of the breast tissue (Figs. 4 and 5). Recently a whole mount technique has been described for the study of mouse mammary glands.² However our technique has three advantages over the whole mount technique:

- (a) it can be applied to larger samples of breast tissue;
- (b) it is easier and less time-consuming to

Fig. 5 Higher magnification of a tumour cell from lobular carcinoma in situ showing the good preservation of the ultrastructural details. $\times 15\,000$.

perform;

- (c) the ultrastructural appearance of the unit membranes is normal rather than exhibiting a "negative" image.

In conclusion, the technique described in this paper is easy to carry out and allows the identification of areas of interest before embedding without the loss of any ultrastructural detail.

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An automated technique for the rapid processing of breast tissue for subgross examination

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In the study of normal and abnormal breast tissue, additional three dimensional information on the parenchyma can be obtained from the subgross examination of breast biopsies. In this paper an automated technique is described for the rapid processing of breast biopsies for subgross examination. It is adapted from methods previously described for subgross examination of whole breasts.^{1 2}

Material and methods

The breast biopsies were fixed in 4% formaldehyde in phosphate buffer³ for a minimum of 6 h. The tissue for subgross examination was cut into approximately 1-2 mm thick slices using a skin graft knife blade.

The slices were placed in wire mesh histokinette baskets and processed in a histokinette using the following schedule:

- 1 Wash in running water 15 min.
- 2 Stain with Delafield's haematoxylin 1 h.
- 3 Wash in running water 1 h.
- 4 Decolourise in 2% acid alcohol (hydrochloric acid/ethanol) 1 h.
- 5 Differentiate by washing in running water 30 min.
- 6 Dehydrate in 95% ethanol 3 h.
- 7 Dehydrate in 99% ethanol 3 h.
- 8 Dehydrate in 99% ethanol 3 h.
- 9 Dehydrate in 100% ethanol 4 h.
- 10 Dehydrate in 100% ethanol 4 h.
- 11 Clear in methyl salicylate 2 h.

To allow washing in running water, the histokinette beakers were adapted with an inlet and outlet which were connected to the water system.

After clearing, the slices were removed from the histokinette and placed in Kapak/Scotchpak heat-sealable pouches. Excess methyl salicylate and air were expelled prior to heat sealing the pouches. The tissue slices can now be examined with a dissecting microscope. Areas of interest can be readily identified and excised for histological examination.

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