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.

The technical assistance of Mrs M Hearn and Mr S McKenzie is gratefully acknowledged. This work was funded by Project Grant G978/336 to TJA from the Medical Research Council.

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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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The subgross appearance of a tissue slice containing normal parenchymal structures. $\times 10$.
(Insert: Detail of the ducts and lobules $\times 20$.)

Results and discussion

The times followed in this schedule gave optimal results in our laboratory. However, they should only be used as a guide and may have to be varied depending, for example, on the pH of the tap water. We have found that the staining time is not critical nor is the decolourisation in acid alcohol. The critical step in obtaining optimum results is the wash subsequent to the acid alcohol and this is the stage which may require variation depending on the reaction of the tissue sample. After acid alcohol the tissue appears pink and as washing proceeds the parenchyma becomes blue in colour. The point at which to stop washing and begin dehydration is when the lobules and ducts are a deep blue colour and the background stroma appears white or has a slight bluish tint.

The samples to be studied histologically after subgross examination are returned to absolute alcohol

and processed for embedding in either wax or plastic. We have found that this technique does not affect subsequent histological staining. Haematoxylin and eosin, alcian blue/periodic acid-Schiff and Feulgen stains have been successfully carried out on the tissue.

The value of the subgross technique is the appreciation that it gives of the three dimensional appearance of the ducts and lobules, including the duct pattern, lobular distribution, and lobular architecture (Figure 1). Furthermore, it allows examination of relatively large tissue samples to identify small atypical areas or regions of altered architecture which can subsequently be removed for histological examination. By applying the criteria described by Wellings *et al.*² it is possible to identify certain types of lesions at the subgross level.

The main advantages of this subgross technique over those described previously^{1,2} are that it is fully automated, only requires 24 h to complete and a number of samples can be processed simultaneously.

The technical assistance of Mrs M McHenry and Miss L Dunlop is gratefully acknowledged. This work was supported by a grant from the Scottish Home and Health Department to TJA.

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Letters to the Editor

Absorption spectra of pigments in vertebrate and non-vertebrate muscle

I enjoyed reading the article by Dr Rinsler in the March issue of the Journal. However, there is one historical inaccuracy which I would like to correct.

I refer to the work of Dr Charles MacMunn, who established the differing absorption spectra of pigments in vertebrate and non-vertebrate muscle in various oxidant states. Dr Rinsler refers to this work as distinguishing the spectrum of myoglobin in muscle from that of circulating haemoglobin. Although it is probable that Dr MacMunn did observe such changes, the pigment he described as "myohaematin" was not myoglobin, but what we would now call cytochromes, in particular reduced cytochrome c.

The description of this work is clearly indicated in the book by David Keilin, published posthumously in 1966.

In fact, Dr MacMunn's clear descriptions of the cytochromes were vehemently denied by Hoppe-Seyler, who attributed MacMunn's findings to bad technique. MacMunn's work preceded the "reductase-oxidase" dispute in the early 20th century—a dispute only finally solved by the rediscovery in the 1920s by David Keilin of MacMunn's original observations.

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Dr Rinsler replies as follows:

Dr Boulton correctly points out in his letter of 7th April an inexactitude in my

very brief note of CA MacMunn's work on pigments.¹ MacMunn described a four-banded spectrum of a material, widely distributed in animal tissues, which behaved as a respiratory pigment. He was unable to isolate this material but called it histohaematin.² It was this material that Keilin called cytochrome in 1925.

MacMunn observed similar spectra in mammalian muscle which he distinguished from haemoglobin and attributed to another unisolated material which he called myohaematin. The lack of pure material led to confusion with myoglobin and the controversy with Hoppe-Seyler. Keilin himself states the "Fischer's opinion that MacMunn's findings were correct was based upon an erroneous identification of myohaematin with myoglobin and on the false belief that what MacMunn had demonstrated was the existence of myoglobin as distinct from blood haemoglobin".³ Anyone who wishes to have a greater understanding of these issues will enjoy reading the account by Florkin and Stotz of the story of "histohaematin" and "cytochrome".⁴

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Comparison of the Dicopac with the conventional Schilling test

The Dicopac is a variant of the Schilling test based upon the simultaneous administration of free ⁵⁸Co cyanocobalamin (Cn-Cbl) and ⁵⁷Co Cn-Cbl combined with human intrinsic factor. Several groups have shown that the ratio, ⁵⁷Co:⁵⁸Co, does not always distinguish patients with pernicious anaemia from those without.¹⁻³ We have experienced similar difficulties in this laboratory and had to perform conventional Schilling tests to clarify the diagnosis in 13 patients ultimately shown to have pernicious anaemia by typical results on the conventional test, megaloblastic anaemia responsive to cobalamin and a low serum cobalamin. The conventional Schilling tests were performed in two parts each using the appropriate capsule provided for the Dicopac test (Radiochemical Centre, Amersham); for part I the ⁵⁸Co Cn-Cbl capsule was given and for part II the ⁵⁷Co Cn-Cbl capsule containing intrinsic factor was given.

The Figure shows our data expressed as the ⁵⁷Co:⁵⁸Co ratio and is presented so that the results obtained by the Dicopac method and by the conventional Schilling test can be compared on a patient basis. In all instances the ratios