CORRESPONDENCE

Storage of sliced breast biopsy specimens

In their review of the laboratory handling of impalpable breast lesions Drs Armstrong and Davies emphasise the need to be able to identify individual slices of the specimen during storage in case further blocks of tissue are required.¹ Their method of placing each slice into a separate compartment of a plastic bag clearly achieves this aim but is quite time consuming.

In our laboratory, once blocks of tissue have been taken, the remaining slices are strung on to suture material in the same order as on the laboratory x-ray picture of the slices. Once the ends of the suture are knotted the slices will remain in that order and can be easily identified if further blocks are required. The "necklace" of slices can then be stored in plastic bags or specimen pots according to local practice.

Depending on the number of slices, one packet of suture material will be adequate for several specimens. 3/0 silk (salvaged from theatre) has proved quite satisfactory and does not damage the tissue. We have found this technique to be quick, effective, and relatively cheap.

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- 1 Armstrong JS, Davies JD. Laboratory handling of impalpable breast lesions: A review. *J Clin Pathol* 1990;44:89-93.

Use of whole mount sections for reporting mammographic specimens

Armstrong and Davies are correct to highlight the advantage that large sections have in showing the resection margins of mammographic specimens. We take issue concerning the claim that the sections are of inferior quality to the standard sized sections, require expensive equipment, and specialised skills. In Worthing we have been reporting mammographic specimens using whole mount sections for more than two years, based on the method described by Gibbs.¹ After initial teething troubles we had no difficulty in perfecting this method.

The tissue slices are processed overnight in a Miles VIP tissue processor, together with the routine tissue blocks, for sectioning the next day. Most of the sections are mounted on 3 × 2 inch slides, though occasionally 4 × 3 inch slides are used. To obtain these sections we use a standard Leitz 1400 base sledge microtome that is very old. We find that the sections produced are of excellent quality and certainly as good as those from standard blocks. No special technical expertise is required. The only additional costs are those of the larger specimen block holders, slides, and cover slips.

The advantages of this method are that it reveals the whole of the lesion and its relation

to the excision margins, and permits an accurate measurement of the lesion. We do not wish to be too dogmatic about this approach and realise that individual pathologists have their own preferences, but there is clearly no reason to abandon this method.

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- 1 Gibbs NM. Large paraffin sections and chemical clearance of axillary tissues as a routine procedure in the pathological examination of the breast. *Histopathology* 1982;6:647-60.

Laboratory handling of impalpable breast lesions

I was surprised to read the authors' comments on the large section method which I developed for the examination of breast specimens in general and which has been in routine use in Guildford since 1979. These specimens include localisation biopsy specimens which only form a small proportion of screening pathology examinations. Most of the blocks we cut measure no more than 7 cm in maximum diameter after fixation and are cut at 4 µm in the line of the duct system on a standard sledge microtome using a large block holder which is readily available (fibre block or standard vice; Anglia Scientific). Each localisation specimen is thus reduced to two or three blocks which are cut and stained to provide a permanent record which can then be re-examined by any pathologist without the need to refer to jig-saw puzzles of numerous small blocks cut at right angles to the duct system. Laboratory x ray machines are not required and there is no need to paint the periphery. This enables clearance margins to be measured accurately as well as the size of the lesions. Discontinuous lesions, such as in situ ductal carcinoma, can be accurately mapped.

The opinion expressed in this review has wider implications because the breast screening programme relies on pathologists to provide not only the diagnosis but full and accurate data concerning microinvasion, multiple malignancy, and multicentric malignancy in all breast resections for cancer. Accurate staging comparisons will be required to assess the progress and comparative efficiency of the screening centres, because mortality statistics will take at least a decade to produce hard facts. It follows that the pathologist who alone makes the diagnosis of cancer is a key figure in the programme and must use techniques suitable for all breast resections where a visible lesion is often only the "tip of the iceberg" of extended field change.

The histological methods outlined in this paper are merely a restatement of conventional time honoured small block histology which was barely adequate when large symptomatic cancers were the rule but this won't do any more.¹ The authors give no data to support their views, presumably because it is too early in the programme to have anything

worth publishing. Why not forget Rokitansky and get into the 20th century while there is still time?

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- 1 Gibbs NM. Topographical and histological presentation of mammographic pathology in breast cancer. *J Clin Pathol* 1988;41:3-11.

Drs Armstrong and Davies comment:

Professor Gibbs reiterates the advantages of large sections which we mention in our paper—namely, the ease of measurement of clearance margins and size of the lesion.

We have contrasting experience in the cutting of large sections, however. The technical staff find the cutting of large blocks on an unfamiliar microtome demanding, and the resulting sections are of significantly poorer quality. This is a particular problem in these days of high staff turnover. The distinction of borderline lesions and microinvasion is rendered difficult by the resultant thicker sections, together with score and chatter marks. Perhaps use of this technique for 12 years may account for the fact that Professor Gibbs finds his sections adequate.

We note with some surprise that Professor Gibbs does not use routine marking of resection margins or specimen slice mammography. The former is necessary to avoid the problem of a false edge caused by buckling of the block during embedding. The latter is invaluable for assessing the extent of tumour in both impalpable and easily visible lesions. Slice mammography is a more sensitive tool than both clinical and whole specimen mammography for visualising the microcalcification and dilated ducts seen in disseminated carcinoma in situ. It thus needs the differentiation of a multicentric carcinoma from a multifocal tumour, as well as identifying associated disseminated carcinoma in situ in palpable tumours.

The method we advocated has been specifically devised to produce conventional small blocks that can be dealt with routinely once they reach the automatic processor. We have now used this method in more than 200 cases, and we have no cases in which we were unable to produce the data Professor Gibbs demands, although we obviously recognise that size would be more accurately measured on large sections.

We like to feel we are building on Rokitansky rather than forgetting him.

Postoperative necrotising granulomas in the ovary

We read with interest the cases presented by LJ McWilliam *et al*¹ and would like to report a further case of postoperative necrotising granulomata in the ovary.

A 41 year old woman had primary infertility. A laparoscopy in 1985 showed noticeable adhesions around both ovaries and tubes. No biopsy specimens were taken at this time. Laparotomy was performed a year later. The right ovary and tube were freed from adhesions and the left ovary was removed. The histology of the left ovary showed no clinically relevant abnormality. Subsequently, several unsuccessful attempts at in vitro fertilisation were made. A total hysterectomy and right salpingo-oophorectomy was done in 1990.

Granulomata showing central necrosis and a giant cell containing intracytoplasmic refractile material (arrow) (haematoxylin and eosin).

The histology of the right ovary showed numerous granulomata confined to the site of tubal adhesions, in which there was central eosinophilic amorphous material, surrounded by a narrow zone of palisaded histiocytes, admixed with small lymphocytes, plasma cells, and occasional eosinophils. A few Langhans' giant cells were also present, one of which contained intracytoplasmic refractile material (figure). Stains for acid and alcohol fast bacilli and fungi were negative. The tube showed no clinically relevant abnormality, and in particular there was no evidence of chronic salpingitis.

Although we knew necrotising granulomata could occur in the prostate and other organs postoperatively, at the time of reporting we were unaware of their existence in the ovary. Consequently, the sections were reported as containing necrotising granulomas of uncertain aetiology and that tuberculosis could not be confidently excluded. Therefore, antituberculous treatment was recommended.

We believe a wider recognition of this condition could save patients from unnecessary treatment.

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I Wilson GE, Haboubi NY, McWilliam LJ, Hirsch PJ. Postoperative necrotising granulomata in the cervix and ovary. *J Clin Pathol* 1990;43:1037-8.

Duration of toxoplasma infection

Joynson *et al* report the potential role of IgG avidity in the estimation of the duration of toxoplasma infection and its application to the detection of infection acquired during pregnancy.¹ From the data presented by these workers it is apparent that quantitative IgM

measurement represents an alternative to avidity assays in this context. Avidity estimation performed at the two study centres correctly identified seven out of seven patients with onset of lymphadenopathy within three months. Of 11 patients with lymphadenopathy for more than six months, however, one was incorrectly classified by the Swansea avidity findings and two were misclassified by results of testing at Oxford. Of 19 samples tested at both centres, four discordant results were also recorded.

In contrast, all seven acutely infected patients had "strongly positive" IgM findings, and all 11 with chronic lymphadenopathy had lower IgM titres ("positive", "equivocal", or "negative"). The IgM ELISA method used has been shown to be highly consistent. Of 30 sera tested at three separate centres, 29 sets of results were consistent and only one discrepant result was reported.²

In our laboratory we undertake semiquantitative specific IgM measurement to estimate the duration of toxoplasma infection in a number of contexts including pregnancy. It has been shown that the immunosorbent agglutination assay (ISAGA) is significantly more sensitive than ELISA for the detection of toxoplasma specific IgM.³ In our experience ISAGA reactivity persists for an average of 18 months after acute infection; ELISA reactivity is usually lost within six months. Furthermore, IgG antibody measured by the dye test rises during the initial two months of infection to reach a plateau, with a slow decline subsequently.⁴ Consequently our approach to the serotesting of pregnant women is to perform the dye test as well as estimating IgM using both ELISA and ISAGA.

If specific IgM is detected a repeat sample of serum drawn at least 10 days after the first is tested in parallel with the initial sample by all three assays. Patients with rising dye test titres and detectable IgM are said to be infected within the preceding two months;

those with stable dye test findings and positive ELISA and ISAGA results can be classified as having been infected for between two and six months; patients with stable dye test titres, negative ELISA, and positive ISAGA findings are classified as having been infected for six to 18 months. Other combinations of findings are rarely seen.³

The disadvantage of our approach is its requirement for a second sample with a consequent delay in diagnosis. In practice, rising dye test values are rarely seen. Of 200 pregnant women with detectable toxoplasma specific IgM tested in our laboratory over the preceding two years, none has shown rising dye test findings when the repeat assay was performed.

The experience of Joynson *et al* and our own group illustrates the need for a large prospective study comparing different methods for dating the duration of toxoplasma infection. Potential methods include quantitative IgM assessment, IgG avidity, IgA estimation, differential agglutination reactions and immunoblotting.⁴ Until such a study is complete any estimation of the duration of infection should be treated with caution, in view of individual variation within the immune response.

Finally, it should be remembered that the duration of infection is only an indirect measure of the crucial factor in toxoplasmosis associated with pregnancy—the presence of parasitaemia itself. Direct testing for the presence of the parasite using novel methods such as the polymerase chain reaction can be used.⁵

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- 1 Joynson DHM, Payne RA, Rawal BK. Potential role of IgG avidity for diagnosing toxoplasmosis. *J Clin Pathol* 1990;43:1032-3.
- 2 Joynson DHM, Payne RA, Balfour AH, Prestage ES, Fleck DG, Chessum BS. Five commercial enzyme linked immunosorbent assay kits for toxoplasma specific IgM antibody. *J Clin Pathol* 1989;42:653-7.
- 3 Duffy KT, Wharton PJ, Johnson JD, New L, Holliman RE. Assessment of an immunoglobulin-M immunosorbent agglutination assay (ISAGA) for detection of toxoplasma specific IgM. *J Clin Pathol* 1989;42:1291-5.
- 4 Holliman RE. The diagnosis of toxoplasmosis. *Serodiag Immunother Infect Dis* 1990;4: 83-93.
- 5 Savva D, Morris JC, Johnson JD, Holliman RE. Polymerase chain reaction for detection of *Toxoplasma gondii*. *J Med Microbiol* 1990;32:25-31.

Drs Joynson and Payne comment:

We agree with the author that the IgM ELISA method which we use is highly consistent in identifying acute infection. The difficulty in determining the duration of infection, however, occurs when relatively low, stable dye test titres are found and IgM ELISA and ISAGA results are positive or equivocal. We have obtained positive IgM ELISA results in sera taken more than six months apart, in some instances more than two years apart. In most of these sera high IgG avidity titres can be shown: we believe that in these circumstances this additional test does help to interpret the serological result.

A prospective study of different methods for dating the duration of toxoplasma infection, including IgG avidity testing, is currently in hand.