Correspondence

Tissue artefacts caused by sponges

We read with interest the short report by Farrell and colleagues on tissue artefacts caused by sponges.1 We note that they have resorted to wrapping some specimens in "permeable paper" prior to processing. Likewise, Rosai suggests the use of tea-bag paper to wrap small fragments of tissue that would otherwise go through the cassette perforations.2 A logical extension of these suggestions is to try empty tea-bags.

We were recently supplied with a quantity of empty, sealed, perforated tea-bags (CWS Ltd, Crewe, England) and investigated whether these would provide a viable alternative to synthetic specimen bags which are already on the market (Shandon UK).

Both were subjected to scanning electron microscopy and neither was found to have sharp spikes, as seen with foam pads, on which tissue might be transfixed (figure). The tea-bag had pores of variable size, but even in the regions of the perforations the largest holes were of the same order of magnitude as the larger holes in the synthetic bags (Brooke Bond tea-bags filter particles of the order of 250 μm).

Both bags can be easily loaded by pouring formalin and biopsy specimens through a funnel into the bags. This is particularly useful for fragmented specimens because there is no need for further manipulation with forceps and the process is less time consuming than picking out tiny bits of tissue from a pot. A bowl left under the bag catches the waste formalin which is discarded. The synthetic bag can be folded in half into the cassette while the tea-bag requires three folds for a near perfect fit.

Both bags survive processing. At the embedding stage the synthetic bag was easily opened and the contents retrieved while the tea-bag was less easily opened and very small pieces of tissue were difficult to separate from the wax impregnated paper. Synthetic bags cost over 9 pence each while an empty tea-bag can be manufactured for about 2 pence.

Small biopsy specimens are likely to remain a major part of the workload in histopathology. They need to be handled efficiently and safely while minimising damage to the tissue. Specimen bags offer one solution but are rather expensive. A standard square tea-bag would require modification of size and its seam before widespread use but could potentially be cheaper.

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Non-nuclear staining of thick tissue sections

Thick sections (1 mm deep or more) of the breast and other organs have been examined histologically since the turn of the century.1 Hitherto, exclusive use has been made of nuclear stains for sections more than 300 μm thick. As a result of our interest in the comparison between radiographs and histological examination of breast tissue we have found, by experimentation, that this restriction is unnecessary. Counterstaining with eosin and direct staining of calcific deposits is possible using such thick sections of tissue, either directly processed from formol-saline,6 or after "back-processing" from archival paraffin wax embedded tissue.7

Pilot studies were performed on mammoplastic resections of excessive breast tissue (macromastia) or breast tissue identified as having mammographically suspicious microcalcification in the United Kingdom national breast screening programme.

In order to apply eosin to thick sections after nuclear staining with Mayer's haematoxylin7 the following schedule proved successful. Once differentiation of the nuclear staining has been performed with acid alcohol and washed in tap water2 the thick section (1 mm or deeper) is placed in 0.1% aqueous eosin Y (Shandon, Runcorn, Cheshire), used diluted 1 part to 9 in 85% methanol for 10 minutes. It is washed again in tap water, dehydrated, and cleared for mounting in methyl salicylate (fig 1).

To show the presence of calcium deposits by cationic complexing with alizarin red S (BDH, Poole) this dye is applied instead of haematoxylin nuclear staining. After the tissue has been partially rehydrated from paraffin wax,1 or dehydrated from formol-saline1 the staining procedure described below is followed.

The tissue is left for 12 to 24 hours in 95% alcohol and transferred to an aqueous 1% solution of KOH for 12 hours, and thereafter into a solution of 1 mg alizarin red S in 100 ml of 1% aqueous KOH for 24 hours. Subsequent dehydration and clearing is as described before,1,4 and the tissue is viewed once mounted in methyl salicylate (fig 2).

One of the reasons for the previous relative neglect of thick section histological examinations has possibly lain in the lack of attempts to stain tissue constituents other than nuclei. The original methods of examining thick sections merit expansion to include study of different tissue components. Such methods are, as we indicate in this letter, both entirely feasible and merely require adjustment to the increased depth of the tissue under study—often achieved by judicial dilution of the reagents used for 5 μm sections and by increasing the time of staining. The new-found ability to select specific lesions from conventional 5 μm sections for examination in thick sections should motivate the development of further methods.

Figure 1 Thick section (1500 μm deep) of a reductio mammoplasty stained by Mayer's haematoxylin and counterstained with 0.01% eosin Y. The collagenous swatches of connective tissue encircle the adipose tissue islands. The focal lobular units stained by haematoxylin are visible in the grey eosinophilic collagen and in the paler islands of adipose tissue.
for staining other components of thick tissue sections.14

Lateral in-depth assessment of microcalcification and the changes in macromastia1 may also facilitate appropriate analysis of mammographic lesions and the diagnostic histological dilemmas resulting from reduction mammoplasty4 for macromastia.

Prospective comparative study of computer programs used for management of warfarin

Poller et al refer to their experience with, among others, the Hillingdon system for computer-assisted warfarin maintenance.1 Their numbers are small, and we should like to record our current experience with a larger series. Nearly all of our patients are given a target INR of 2-8. To compare these as nearly as possible with those of the above authors, who used a range of 2-0 to 3-0, we have used 2-3 to 3-3. The ranges used by these authors are narrow, and divergence from them does not necessarily mean that anticoagulation is at a level which is either ineffective or dangerous. We followed their division into the first 26 weeks of treatment and the period, if any, after that point. The results are shown in the table.

With the important exception of the interval between visits, our experience is not significantly different from that of Poller et al as shown in their table 4. Our mean intervals, both for early and later periods of treatment, are longer than theirs: this may be partly attributable to the maximum permissible interval having been increased from eight to 10 weeks during the period under consideration. We can add that the average interval at the latest visit was 7-04 weeks.

We have only six patients (187 visits) to compare with those who had a higher target INR. Having so few, we would only say tentatively that the average intervals before and after 26 weeks were 2-24 and 3-36 weeks, respectively, and that the interval at the latest visit averaged 3-83 weeks.

The mean interval is important, both for the convenience of patients and economy in the use of hospital resources. Our data support the inherently probable propositions that intervals become longer as treatment proceeds, and are much shorter with higher INR targets. Because they require more frequent attendances, and also because they are more difficult to achieve, high targets require much justification on grounds of clinical necessity.

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Vasculopathy and antiphospholipid antibodies in systemic lupus

We read with great interest the article by Ellison and colleagues showing intramural deposition of platelet derived material in small cerebral vessels in four patients with systemic lupus erythematosus (SLE).1 The authors recall the strong association between the presence of antiphospholipid antibodies (aPL) and the occurrence of ischaemic cerebrovascular events in SLE. The results of tests for aPL (lupus anticoagulant or anticardiolipin antibodies), however, are not mentioned in their patients with SLE. Such information could probably be obtained from the patients’ charts or from stored serum or plasma. At least the full description of extra- and intracerebral events—arterial or venous thrombosis, recurrent fetal loss, or thrombocytopenia—could suggest the presence of this peculiar family of antibodies. Such data could allow the hypothesis that intramural deposition of platelet derived material is a feature of a aPL-associated non-inflammatory “vasculopathy” to be tested. The absence of such material in the two patients with SLE and active vasculitis6 is consistent with this hypothesis, due to the lack of an association between aPL and vasculitis in SLE.3

Furthermore, the search for intramural deposition of platelet derived material should be performed in other forms of vascular lesions encountered in patients with antiphospholipid syndrome, either “primary” or secondary to SLE, such as non-inflammatory non-atheromatous large artery lesions2 3 and heart valve thickening,4 the latter being mainly present in patients with SLE with long-lasting disease.4 The pathogenesis of these lesions remains unknown: it could involve a complex aPL mediated interaction between platelets and endothelial cells, resulting in platelet derived material incorporation into vessel or heart valve wall, which would explain the “mysterious” thickening frequently observed. Similar remarks could also apply to Sneddon’s syndrome, a condition closely related to aPL,5 the pathological basis of which has been recently detailed,6 but the pathophysiology remains obscure.

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