Correspondence

Misunderstandings about methyl methacrylate

I read with interest the recent article by Schmid and Isaacson.[1] Unfortunately, the article propagates the myth that methyl methacrylate (MMA) embedded material does not preserve antigens for subsequent immunocytochemistry. The literature is confounded by similar accounts[2,3] which have served to prevent development of the technique and may have been responsible in part for many departments in this country losing interest in the use of resin embedding for diagnostic purposes. Although the use of MMA has become unfashionable for high resolution studies compared with other resins such as those based on glycol methacrylate (JB-4) and polyhydroxy aromatic dimethacrylate resin (LR White), it offers the advantage of access to a wide range of antibodies for immunocytochemistry. Immunocytochemistry can be performed on tissue embedded in resins other than MMA, but the techniques required are often troublesome and idiosyncratic.

In recent years I have been one of the main advocates for the use of MMA as a suitable embedding medium for simultaneous high resolution morphology and immunocytochemistry.[4-6] Though acrylic resins, including MMA, can be polymerised at raised temperatures, perhaps overnight as suggested by Schmid and Isaacson, our studies use polymerisation with a chemical accelerator at room temperature or less, which is quicker and more practical. To date, about 40 monoclonal and polyclonal antibodies have been successfully applied to MMA embedded tissue for the demonstration of hormonal, lymphoid, mesenchymal, neuronal and smooth muscle antigens, but the number is rapidly expanding.

The technique has proved simple, quick, and reliable in a routine laboratory and has been used on a variety of tissues, including lymph node, skin, renal and bone marrow trephine biopsy specimens for diagnostic purposes. Although MMA is being used in some pathology departments, wider recognition of the benefits to be gained will not be helped by perpetuating the fallacy that immunocytochemistry can not be done.

NM HAND
Department of Pathology, Queen's Medical Centre, University Hospital, Nottingham NG7 2UH

1 Schmid C, Isaacson PG. Bone marrow trephine biopsy in lymphoproliferative disease. *J Clin Pathol* 1992;45:745–50.
2 Islam A. A new instrument for longitudinal hemisectioning of undecalcified marrow cores for simultaneous processing and examination with useful and complementary techniques. *J Clin Pathol* 1987;40:112–6.
3 Frisch B, Bartl R. In: Gresham GA, ed. *Atlas of bone marrow pathology*. Dordrecht: Kluwer Academic Publishers, 1990.
4 Hand NM, Morrell KJ, MacLennan KA. Immunocytochemistry on resin embedded tissue for light microscopy: a novel post-embedding procedure. *Proc Roy Microbiol Soc* 1989;24:A54-A5.
5 Hand NM, Morrell KJ. Immunocytochemistry on plastic sections for light microscopy: a new technique. *Proc Roy Microbiol Soc* 1990;25:111.
6 Johns L, Hand NM, Fish DCW, Miller KD. Immunocytochemistry on methyl methacrylate embedded tissue. *J Pathol* 1992; 167:suppl 154.

Processing of trephine biopsy specimens

We read with interest the article by Schmid and Isaacson. The authors discuss methods of processing trephine biopsy specimens and conclude that decalcification remains the method of choice. We strongly disagree with this view for the following reasons.

1 Methyl-methacrylate embedded trephine biopsy specimens provide excellent morphology. This permits detailed, critical assessment of bone marrow architecture and cellular differentiation at a level which we consider impossible in the best prepared decalcified sections.

2 Processing specimens in MMA can be automated and in this laboratory biopsy specimens can be reported within one working day. The method which we have been using for the past two years (about 1 000 biopsies) was developed by Mr N Hand of the Queen's Medical Centre, Nottingham. The method is also used for renal biopsy specimens and is of particular value in the assessment of cutaneous lymphoid infiltrates.

3 Contrary to the statements in this article, immunocytochemistry is not only possible on MMA sections, but can be carried out to a very high standard, which when combined with the degree of morphological detail makes this a very powerful analytical method. A wide range of antibodies can be used permitting the immuncytochemical identification of lymphoid infiltrates, normal haemopoetic cell lines, and metastatic tumours of the types normally seen in marrow. Around 25 antibodies are in routine use in this laboratory: staining techniques are similar to those used in paraffin wax sections.

4 Immunocytochemical studies on trephine biopsy specimens are of great value in identifying minor cell populations such as in early involvement by myeloma, in distinguishing regenerating blasts from large cell lymphoma, and in demonstrating details of the bone marrow microarchitecture. In cases where there is extensive lymphoid infiltration, flow cytometry is the method of choice in establishing the cell phenotype. The need for frozen section immunocytochemistry is minimal.

5 In several recent cases we have extracted DNA from MMA sections and have demonstrated lymphocyte clonality by PCR. In one case the extent of lymphocyte infiltration seen in the biopsy specimen was minimal.

6 In our opinion it is technically more demanding to prepare decalcified sections to a consistently high standard than to use MMA embedding. The standards achieved in many centres are very poor. The equipment costs of MMA embedding are modest in comparison with many standard items of laboratory equipment.

For these reason we regard decalcification as an obsolete method in the preparing trephines. We strongly recommend the use of MMA embedded biopsy specimens, combined with flow cytometric immunophenotyping, in the assessment of all suspected neoplastic disorders of the bone marrow. We agree with the authors that bone marrow biopsy specimens are often regarded as "Cinderella" specimens. Using the methods described above bone marrow, Pathology becomes a demanding and interesting subject, which, if properly applied, has a major role in the management of haematological malignancy.

AS JACK
BE ROBERTS
CS SCOTT
Haematological Malignancy Diagnostic Service, Institute of Pathology, The General Infirmary at Leeds West Yorks LS1 3EX

1 Schmid C, Isaacson Pg. Bone marrow trephine biopsy in lymphoproliferative disease. *J Clin Pathol* 1992;45:745-50.

Drs Schmid and Isaacson comment:

Dr Jack and his colleagues may have read our article "with interest" but clearly, they have not read it with "great care". Nowhere do we state that formalin fixation followed by decalcification and paraffin embedding is the "method of choice". Instead we have given details of several methods, including methyl-methacrylate (MMA) embedding. Indeed, MMA embedding is the method used routinely in the laboratory of one of us (CS) while paraffin wax embedding following formalin fixation with decalcification is the method used in the other author's laboratory (PGI).

We are entirely in agreement with the first two points raised by Jack *et al*, who would, if they had read our paper carefully, have gleaned this from paragraph 5 on page 1 in which we state "the use of methyl-methacrylate (MMA) provides excellent morphology (fig 1B) and sections can be processed and stained within 16–24 hours when rapid fixation is used".

Their third point, relates to performance of immunocytochemistry on MMA sections. We, too, have had some experience with this[1] and have had success with the same range of antibodies that can be applied to paraffin wax sections, with the exception of antiimmunoglobulins where staining of perinuclear space Ig is more difficult. We have encountered some minor problems with the technique relating to the requirement for processing at 4°C and changes in enzyme digestion for certain antibodies. A recent paper by Wolf *et al*[2] reports success with the MMA technique but describes a requirement for specially tailored fixatives and prolonged washes. The range of antibodies listed by Wolf *et al* is also restricted to those that can be applied to paraffin wax embedded material. We doubt, therefore, that Jack *et al* have had success with the wider range of antibodies that can be applied to frozen sections[3] which are sometimes useful in analysing lymphoproliferative and other disorders of the marrow.

We are, of course, in full agreement with point 4, having taken great pains to point out and illustrate the value of immunocytochemistry on bone marrow trephines, and are grateful for the anecdote which forms the fifth point of the letter which, however, has no relevance to our review.

It may be the opinion of Jack *et al* that sections of formalin fixed decalcified bone marrow are morphologically unsatisfactory and technically more demanding to prepare, but others have contested this.[4] We may add that as recipients of numerous consultation cases the standard of MMA embedding of bone marrow trephines is equally poor in many centres.

It is difficult to understand the final paragraph in the letter of Jack *et al*. Why, if immunocytochemistry is so easily applied to MMA sections, do they find it necessary to combine this technique with flow cytometric immunophenotyping? We doubt that many routine laboratories have access to such sophisticated technology, desirable though it may be. The aim of our article was to draw attention to the value of the bone marrow biopsy specimen in lymphoproliferative disorders and to provide a balanced view of the different approaches. We trust that we have achieved this.

1 Johns L, Hand NM, Fish DCW, Miller KD. Immunocytochemistry on methyl methacrylate embedded tissue. *J Pathol* 1992; **167**:Suppl 154A.
2 Wolf E, Roser K, Hahn M, Welkerling H, Delling G. Enzyme and immunohistochemistry on undecalcified bone marrow biopsies after embedding in plastic: a new embedding method for routine application. *Virchows Arch (Pathol Anat)* 1992;**420**: 17–24.
3 Frisch B, Bartl R. In: Resham GA, ed. *Atlas of bone marrow pathology*. Dordrecht: Kluwer Academic Publishers, 1990.
4 Gatter KC, Heryet A, Brown DC, Mason DY. Is it necessary to embed bone marrow biopsies in plastic for haematological diagnosis?

Staging and follow up of patients with lymphoproliferative disorder

As a haematologist who both reports marrow biopsy specimens and treats patients based on the results, I wholeheartedly agree with Drs Schmid and Isaacson that trephine biopsies are an integral part of diagnosis, staging and follow up of patients with lymphoproliferative disorders, and that adequate biopsy specimens in terms of size and processing, are essential.[1]

I find it less easy to accept their statement that marrow biopsy specimens are *especially* important (my italics) in the staging of Hodgkin's disease. In their own series of 158 patients only 14 (9%) had marrow disease. Obviously this is of immense importance for those 14 individuals, but one wonders to what extent marrow disease might have been predicted in these patients. Specifically, were the 14 all stage IIIB/IVB, or did they have other features suggestive of marrow disease? I personally do not recall seeing a patient with apparent stage I or IIA Hodgkin's disease who subsequently turned out to be stage IV after marrow biopsy. This is not to suggest that these cases do not ever occur, but I suspect they are extremely rare.

I would be interested to know if the authors feel that marrow biopsy should be performed in *every* patient with Hodgkin's disease (in which case, by their own figures a lot of unnecessary biopsies will be done), or whether a more selected group should be biopsied.

AN STARK
Department of Haematology, Dumfries Infirmary, Dumfries DY 1 4AP

1 Schmid C, Isaacson P.J. Bone marrow trephine biopsy in lymphoproliferative Disease. *J Clin Pathol* 1992;**45**:745–50.

Professor Isaacson comments:

Dr Stark is probably correct in pointing out that by using the term "especially important" with respect to the use of marrow biopsies in the staging of Hodgkin's disease we have slightly overstated our case. Perhaps this reflects our experience as histopathologists since we do in fact receive a staging marrow biopsy from every new case of Hodgkin's disease, a practice which is still strongly recommended.[1]

Our experience is similar to that of Dr Stark and others[2] in that in the series of 158 patients to whom we referred, of the 14 patients (9%) with marrow disease only one was clinical stage 2a.

There is, however, another angle to this argument which we feel is important, if not especially important. We find that in a large percentage of patients without marrow disease as a result of Hodgkin's disease there is a variety of non-specific reactions in the marrow some of which, including leukaemoid hyperplasia and inflammatory changes, have been shown to indicate a poorer prognosis.[3] Moreover, a hypoplastic bone marrow may raise a cautionary warning with respect to chemotherapy and/or radiotherapy effects.

As clinical staging improves less stress will probably be laid on the marrow biopsy but for the reasons outlined above we feel that a trephine biopsy of the marrow should continue to be a part of the staging process.

1 Schmoll HJ, Peters HD, Fink U. In: Kompendium Internistische Onkologie Teil II. Springer Verlag. Berlin, 1986:113.
2 Bartl R, Frisch B, Burkhardt R, Huhn D, Pappenberger R. Assessment of bone marrow histology in Hodgkin's disease: correlation with clinical factors. *Br J Haematol* 1982;**51**:345–60.
3 Frisch B, Lewis SM, Burkhardt R, Bartl R. In: Beckenkammbiopsien, Klinisch interpretiert. Springer Verlag. Berlin. 1987;215–216.

Sentinel and Bactec blood culture systems

The report by Stevens and colleagues on the Sentinel (Difco) and Bactec (Becton Dickinson) blood culture systems is likely to be welcomed by many diagnostic laboratories.[1] In a recent review of blood culture methods in the United Kingdom, the Bactec system was used by all but one of the 124 machine based laboratories.[2] The introduction of the fully automated Sentinel system should therefore generate greater competition within the commercial market. BacT/Alert (Organon Teknica), another automated blood culture system based on colorimetric microbial detection, also seems to compare favorably with the Bactec system.[3] In addition, other fully automated systems are either available (Bactec 9240) or being evaluated (Vitek, Biomerieux).

Both the Sentinel and BacT/Alert trials were based on the inoculation of 5 ml of blood per bottle.[2 3] As there is a direct correlation between volume of blood cultured and microbial yield,[4] Becton Dickinson has recently developed a high volume (10 ml) resin based blood culture system—NR Bactec Plus (BP). In a large multicentre study, excluding paediatric patients, the BP system showed a significantly increased isolation rate over its 5 ml non-resin Bactec counterpart.[5] In our own limited prospective study of BP and Sentinel systems, BP showed similar advantages. Of 1 148 four-bottle blood culture sets evaluated, 146 clinically significant micro-organisms were cultured: 96 isolates were detected by both systems, 44 (30%) by BP alone, and six (4%) by Sentinel alone.

In the United States clinical trials are planned to compare a high volume resin based BacT/Alert system with its Bactec counterpart (JA Washington, personal communication). We hope that the Department of Health will support similar trials in this country when new systems are available to compare with BP.

RPD COOKE
CT JENKINS
Department of Medical Microbiology, District General Hospital Eastbourne, East Sussex BN21 2UD

1 Stevens M, Patel H, Wellers A, *et al*. Comparison of Sentinel and Bactec blood culture systems. *J Clin Pathol* 1992;**45**: 815–18.
2 Brown DFJ, Perry SF. Methods used in the United Kingdom for the culture of microorganisms from blood. *J Clin Pathol* 1992; **45**:468–74.
3 Thorpe CT, Wilson ML, Turner JE, *et al*. Bac T/Alert: an automated colorimetric microbial detection system. *J Clin Microbiol* 1990; **28**:1608–12.
4 Washington JA, Ilstrup DM Blood cultures: Issues and Controversies. *Rev Infect Dis* 1986;**8**:792–802.
5 Kountz FP, Flint KK, Reynolds JK, Allen SD. Multicenter comparison of the High Volume (10 ml) NR Bactec Plus system and the Standard (5 ml) NR Bactec system. *Diagn Microbiol Infect Dis* 1991;**14**:111–18.

Dr Stevens comments:

As Messrs Cooke and Jenkins point out, and as is well documented, the amount of blood cultured and the presence of resin have been shown to increase the isolation rate of blood culture systems.

The Department of Health (Medical Devices Directorate) is intending to conduct a trial of the Bactec 9240 blood culture instrument using high volume resin-based media in comparison with the BacT/Alert system currently being used in the United Kingdom. BacT/Alert bottles designed for the inoculation of up to 10 ml of blood which contain resin are not at present obtainable in the United Kingdom. We have been informed by the suppliers of BacT/Alert that such bottles will be available in 1993.

PS. I have taken the liberty of correcting a few spelling and nomenclatural errors in the letter from Drs Cooke and Jenkins.

In the United States, clinical trials are planned to compare a high volume resin-based BacT/Alert system with its Bactec counterpart (JA Washington, personal communication). We hope that the Department of Health will support similar trials in this country when new systems are available to compare with BP.