Correspondence

Misunderstandings about methyl methacrylate

I read with interest the recent article by Schmid and Isaacs. 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 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 the topic has been one of the main advocates for the use of MMA as a suitable embedding medium for simultaneous high resolution morphology and immunocytochemistry. Though acrylic resins, including MMA, can be polymerised at raised temperatures, perhaps overnight as suggested by Schmid and Isaacs, 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, neural 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.

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Dr Schmid and Isaacs comment:

Dr Jack and his colleagues may have read our article "with interest" but 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 one's own, 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 MMA and have had difficulty with the same range of antibodies that can be applied to paraffin wax sections, with the exception of anti-immunoglobulins 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 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 not 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 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.
Staging and follow up of patients with lymphoproliferative disease

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 preparation are essential.

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 II A 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.

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 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 Bactec/Alert system currently being used in the United Kingdom. Bactec/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 no information from the suppliers of Bactec/Alert that such bottles will be available in 1993.

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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.

Our experience is similar to that of Dr Stark and others 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 leukae-moid hyperplasia and inflammatory changes, have been shown to indicate a poorer prognosis. 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.


Both the Sentinel and BacT/Alert trials were based on the inoculation of 5 ml of blood per bottle. As there is a direct correlation between volume of blood cultured and microbial yield, Becton Dickinson has recently developed a high volume (10 ml) resin based blood culture system—NR BacT Plus (BP). In a multicentre study, excluding paediatric patients, the BP system showed a significantly increased isolation rate over its 5 ml non-resin BacTec counterpart. In our own limited prospective study of BP and Sentinel systems, BP showed similar advantages. Of 148 four-bottle blood culture sets evaluated, 146 clinically significant micro-organisms were cultured: 96 isolates were detected by both systems, (90%) 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.

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Dr. J. Stevens comments:

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

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