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New instrument for longitudinal hemisectioning of undecalcified marrow cores, for simultaneous processing and examination with useful and complimentary techniques

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In addition to morphological studies, cytochemical and immunological studies are now performed routinely on bone marrow aspirate samples obtained from patients with leukaemia or lymphoma. Similar studies, if performed on bone marrow biopsy specimens, could be far more informative because tissue organisation would be maintained in histological preparations, and sampling errors inherent in the bone marrow aspiration technique avoided. Unfortunately, however, the combination of an improved histological embedding technique for semithin sectioning of undecalcified bone in methyl-methacrylate (MMA) with special enzyme or immunohistochemistry methods, has been unsuccessful to date. The shortcomings have been attributed to the MMA embedding medium.¹ They can, however, be overcome by dividing the cylindrical marrow core in half, longitudinally, and then processing one half into methyl-methacrylate for optimal morphology^{2,3} and the other half into glycolmethacrylate (GMA) for enzyme or immunohistology.^{4,5} Water miscibility and low temperature processing make GMA a suitable embedding medium, and in this way the adjacent areas of optimally processed marrow cores can be examined by useful and complimentary techniques.

This paper describes the invention of a simple instrument (fig 1) that longitudinally hemisections cylindrical biopsy specimens of cancellous bone and reconciles the recent technological improvements that are necessary for optimal morphological, histochemical, and immunohistological analysis of bone marrow biopsy specimens.

Material and methods

INSTRUMENT

The instrument has four basic parts: (i) the circular saw (0.1 mm thick and 80 mm in diameter), mounted on (ii) an electrically operated drill; (iii) a brass recep-

tacle with a small brass top to hold the biopsy specimen in place while it is being split; and (iv) an on/off switch. The brass receptacle has a centre groove with a 0.3 mm slit for the saw to pass through and has been specially designed for bone marrow samples obtained with the standard Islam bone marrow biopsy, or similar needles⁶ that provide a long uniform core of marrow tissue with little or no distortion of marrow architecture. A straight uniform core of marrow tissue of adequate length and diameter is essential. Marrow cores which are broken, fragmented, or too narrow in diameter are unsuitable.

HEMISECTIONING PROCEDURE

The brass receptacle has been designed so that immediately after the biopsy needle is withdrawn from the patient, the biopsy sample can be pushed directly into the receptacle's biopsy receiving groove. When the biopsy is in place, the small brass top is placed over the biopsy specimen to hold it in place. The marrow biopsy is then divided in half longitudinally by the electrically operated circular saw, as the brass receptacle glides forward over an aluminium platform. The speed of the saw is regulated to prevent frictional damage or burning of the tissue. When the biopsy is divided, the portions are removed, appropriately fixed and dehydrated, and processed separately into MMA and GMA. The cut surfaces of the biopsy sample are placed downward during embedding so that the maximum width of the specimen is available for sectioning and examination.

Results

The instrument has been tested on a large number of bone marrow biopsy samples obtained from patients with various haematological malignancies. In each case the specimens were adequately divided longitudinally into two equal halves, with no damage or distortion to the marrow tissue. Morphological and structural preservation of the marrow was good (fig 2b), and the results for immunostaining methods were excellent (fig 3).

Discussion

Bone marrow biopsy specimens are now used routinely to investigate and diagnose various haematological and non-haematological malignant conditions.⁷⁻⁹ The conventional method of decalcification and paraffin embedding, however, produces shrinkage, considerable loss of cellular details, and reduces the quality of staining of haemopoietic tissue. Thus cells from the haematopoietic system are more difficult to identify in the sections of paraffin embedded tissue. To overcome these disadvantages various plastic embedding techniques have been intro-

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Fig 1 Schematic representation of biopsy splitting and embedding procedures, showing (a) circular saw, (b) electrically operated drill, (c) brass receptacle with small brass top, and (d) aluminium platform and (e) on/off switch.

Fig 2 Details of bone marrow biopsy preparation from patient with non-Hodgkin's lymphoma. Histopathology of bone marrow biopsy section processed in conventional way suggested diagnosis of diffuse well differentiated lymphocytic lymphoma, but plastic embedded bone marrow biopsy section clearly showed presence of plasma cells and large number of well differentiated mature looking lymphocytes; (a) after decalcification and paraffin embedding (Haematoxylin and eosin) $\times 600$; (b) one half of divided biopsy after embedding in methyl-methacrylate without decalcification. (May-Grünwald-Giemsa stain.) $\times 600$.

Fig 3 Bone marrow biopsy section from same case as in fig 2, showing large number of plasma cells that are strongly positive for immunoglobulin IgA. This is other half of divided biopsy, which was processed glycolmethacrylate without decalcification for immunohistology. Semithin section was stained for immunoglobulin IgA by indirect immunoperoxidase method and counterstained with Mayer's haematoxylin. $\times 600$.

duced,¹⁰⁻¹³ which obviate the need for decalcification, thus avoiding a major source of artefactual change. With plastic embedding techniques, distortions and tissue shrinkage are minimal and thinner sections of 1–3 μm can be cut. This improved resolution enhances identification of cells, interpretation, and diagnosis.² Most plastic embedding procedures for bone marrow biopsy specimens offer a definite advantage over the conventional paraffin embedding techniques, but to date, most have been unsuccessful for enzyme or immunohistochemistry, with the possible exception of glycolmethacrylate. Immunological studies have been done on Epon and Araldite embedded tissues,^{14,15} but as with the paraffin embedding technique, a wider application of different immunological methods using various monoclonal and polyclonal antibodies has been unsuccessful.

In a recent study¹⁶ we found that embedding bone marrow biopsy specimens in MMA offers considerable advantage over various other plastic embedding techniques for light microscopic examination. Unfortunately, however, we were unable to combine this improved histological embedding technique with special methods of enzyme or immunohistochemistry. The shortcomings were attributed to the MMA embedding medium.

The adaptation of plastic embedding to undecalcified marrow cores provides excellent preservation of the marrow architecture and cellular detail, but, even in such an improved histological section preparation, it is sometimes difficult to identify precisely the

nature of disease in the marrow, and enzyme or immunohistological studies become essential to characterise the type of cells or sanctuary of cells participating in the malignant process. These can be done by processing one half of the biopsy specimen in MMA and by snap freezing the other half, and then sectioning it in a cryostat, as has been advocated by Bartle *et al.*¹ This technique, however, has its own inherent disadvantages,¹⁷ all of which can now be overcome by embedding tissues in glycolmethacrylate (detailed methods of GMA embedding technique for enzyme and immunohistochemistry will be published elsewhere).

The instrument described here offers a possibility of combining optimal morphological studies with enzyme and immunohistological studies to be done simultaneously on one biopsy specimen. In this way valuable information can be obtained which would otherwise not be noticed—for example, in a recent case of non-Hodgkin's lymphoma a bone marrow biopsy specimen processed in paraffin showed dense marrow infiltration by mononuclear lymphoid cells (fig 2a). Diffuse well differentiated lymphocytic lymphoma was diagnosed. Plasma cells were reported to be present, but not increased noticeably. The one half of the bone marrow biopsy specimen processed in MMA, however, clearly showed marrow infiltration by a large number (30%–50%) of plasma cells (fig 2b), in addition to the mature looking mononuclear lymphocytes. Immunological studies on the other half of the same bone marrow biopsy specimen processed in GMA clearly showed the presence of IgA and κ immunoglobulin (fig 3) in those plasma cells, indicating the clonal nature of plasma cell proliferation.

Bone marrow disease with lymphoma and other malignancies may be focal, and the presence of lymphoid nodules in otherwise normal marrow may make it difficult to identify it as normal. In such cases longitudinal hemisectioning of long core biopsy specimens and then processing the bone marrow biopsy halves in MMA and GMA for optimal morphology and immunohistology would have an important role in identifying and characterising a malignant process entailing the bone marrow.

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Modified latex agglutination test for anticytomegalovirus, suitable for pretransfusion screening

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The increasing requirements of immunosuppressed patients for blood and blood components from donors uninfected with cytomegalovirus (CMV) led us to search for a sensitive, rapid, convenient and economical test for screening blood donations for antibodies against this virus.

Initially, we used a modification of a commercial haemagglutination (HA) assay (Cetus Corporation, Berkeley, California); this entailed 10-fold dilution of the red cells provided in the kit and assessment of agglutination in a microplate inclined at 70° after centrifugation at 387 g for one minute.¹ Although this test proved suitable for the selection of plasma with high titre anti-CMV for the manufacture of specific immunoglobulin, its sensitivity and specificity was unacceptably variable for selecting CMV free donations. This became apparent when 23% (28 of 124) donations found negative for anti-CMV by modified HA were found to be positive for CMV antibodies by radioimmunoassay. We therefore decided to use a modified latex agglutination as our routine screening test (CMV Scan, Becton and Dickinson, Baltimore, Maryland). We tested this assay in parallel with the modified HA test (MHA) using a sensitive radioimmunoassay described by Berry *et al*² as a standard for comparison.

Methods

For the latex agglutination test 25 µl of test serum was pipetted on to black reaction cards and 15 µl of latex coated with CMV antigen was added and mixed, according to the manufacturer's recommendations. Positive and negative control sera were also tested. The card was placed on a humidified rotator for eight minutes and then read. Samples positive for anti-CMV showed clear agglutination, whereas negative ones remained cloudy. The test was made more economical by diluting two volumes of the antigen coated latex with one volume of the titration buffer provided with the kit. Furthermore, the volumes of reagents were reduced to 15 µl of test serum and 5 µl of diluted latex. Even with these reduced volumes, the reactions could be read easily.

Results

Before using the modified latex test for screening donor sera, we confirmed that the reduced volume modifications, with or without dilution of the latex, provided the same end point titres as the standard method when either sera or plasma with high titre or low titre anti-CMV were assessed. When 252 donor serum samples found to give negative CMV antibody results by modified haemagglutination screen tests were rescreened under routine testing conditions by the reduced volume latex agglutination test (method A), 58 (23%) were found to be positive by the second test. These 58 samples were then retested by various methods (table 1). A small number scored differently on this repeat testing.

Table 2 gives detailed results for nine negative or "discrepant" sera at repeat testing. Furthermore, when 50 serum samples from donors found to be negative for anti-CMV by modified haemagglutination and by the two latex test modifications, were tested by monoclonal radioimmunoassay, 48 were clearly negative, and two samples showed only traces of antibody to CMV. The figure shows the