the intensity of staining in frozen sections exceeds that in the corresponding fixed and embedded sections. The lectins work satisfactorily on formalin-fixed material but the clarity and intensity of staining is enhanced by trypsinisation. The technique was found to be consistent and reproducible with the exception of SBA. This lectin was unpredictably prone to agglutinate in solution and fail to bind to the tissue. The reasons for this have not been determined.

Discussion

Conjugated lectins provide a simple means of extending the range of histochemical procedures for carbohydrates. Their specificities for saccharides make them useful analytical probes. Reliable preparations and conjugates are now available from a variety of commercial laboratories. Their main disadvantage at present is their high cost, and removal of excess stain for re-use³ is worth doing.

The difference between frozen and fixed material is particularly selective. For example, the comparative intensity of lectin-binding between frozen and fixed glomeruli differs markedly whereas intensity of binding in nearby renal tubules differs only slightly. This is attributed to the extraction of certain carbohydrate containing substances during fixation and processing.⁴

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A method for transmission and scanning electron microscopy of undecalcified human bone marrow biopsy specimens using cryofracture

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The bone marrow biopsy has gradually become a standard diagnostic procedure since its introduction,^{1,2} particularly with the recent improvement of the needles.³ The biopsy permits histological evaluation of marrow specimens of adequate size and is indispensable when aspirated material is unobtainable. Clearly, the biopsy specimen would provide more information if its ultrastructure could be better appreciated. However, electronmicroscopical examination of human marrow biopsy specimens has so far been neglected due to technical difficulties inherent in the preparation of the specimens. Soft marrow tissue proper located in hard bone trabeculae is difficult to cut into thin sections without decalcification, and such a procedure is time-consuming and results in obliteration of considerable detail of these specimens. Consequently, it is necessary to devise a method for thin sectioning of marrow specimens that would separate marrow proper from bone trabeculae without decalcification. Georgii and Thiele⁴ performed this separation manually under a stereomicroscope after 10 to 15 minutes of primary fixation. Unfortunately, the procedure has proved to be tedious and too demanding, as great care is required to avoid crushing the soft marrow tissue located between cancellous bone trabeculae.

We have recently conducted a scanning electron-microscopic study on human bone marrow, observing cryofractured planes of biopsy specimens,⁵ and have found that the bone marrow biopsy specimens could be dissected without decalcification and without deformation. We have used this cryofracture technique for processing undecalcified bone marrow biopsy specimens for transmission (TEM) as well as scanning electron microscopy (SEM).

Materials and methods

Bone marrow specimens were obtained by the method of Jamshidi and Swaim³ using a regular

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adult 11 gauge Jamshidi needle (Kormed). The specimens were fixed in 2% paraformaldehyde, 5.5% glutaraldehyde, and 0.025% calcium chloride in 0.09 M cacodylate buffer, pH 7.4⁶ for 3 to 5 hours at 4°C. Glutaraldehyde fixation was also used, but preliminary work had shown that preservation of structure was better achieved with the combination aldehyde fixative. The fixed specimens were washed in 0.1 M phosphate buffer for 5 to 18 hours at 4°C. The revised tannin-osmium method of Murakami⁷ was used to increase the electroconductivity of the specimens for SEM. The specimens were then dehydrated in a graded series of ethanol. After dehydration, the specimens, well soaked with absolute ethanol were, according to a modification of the method of Humphreys *et al*⁸ immersed directly in liquid nitrogen and fractured with a hammer and chisel into large blocks for SEM and small blocks that could be effectively infiltrated with embedding materials for TEM. The cryofractured blocks were quick-thawed by submersion in absolute ethanol at room temperature. The small blocks were infiltrated with propylene oxide and embedded in Epon 812. Thick survey sections for light microscopy (LM) were cut with glass knives on an ultramicrotome (Porter-Blum MT2-B) and stained convention-

ally with toluidine blue. When regions of calcified bone trabeculae had been located under LM, the specimen blocks were trimmed so that the block faces included only marrow proper (Fig. 1). Thin sections were then cut with diamond knives, stained with uranyl acetate and lead citrate, and examined under a transmission electron microscope (Akashi LEM 2000).

Other cryofractured marrow blocks were observed in a scanning electron microscope (Hitachi S-700) after the blocks had been "critical point" dried and sputter-coated as previously described.⁵

Results

Human marrow biopsy specimens prepared according to the above methods are illustrated in Figs. 2 and 3. The preparations consistently demonstrated excellent structural preservation and offered wide fields for observation by both TEM and SEM. Occasional tears in specimens themselves, not in sections, prepared for TEM were encountered (see also Fig. 1). These tears probably occurred during cryofracturing of the specimens and represent cracks caused by mechanical impact. However, the cytoplasm of the cracked cells showed no other discern-

Fig. 1 Light micrograph of a thick survey section of a marrow specimen from a patient with chronic myelogenous leukaemia, prepared as described. The face of this specimen block contains unsectionable bone trabecula (B) in addition to the marrow proper. There are several tears in the specimen (arrowheads). The lines indicate how this block should be trimmed in order to cut thin sections. Scale marker = 500 μ m.

Fig. 2 A transmission electron micrograph of a marrow specimen from a patient with chronic myelogenous leukaemia has a large area of observation and a well preserved morphology except for a few tears (arrowheads) in the specimen. Scale marker = 5 μ m.

ible morphological changes in detail. In the SEM specimens, some cells, particularly free blood cells in the lumen of venous sinuses, were ready to fall off the fractured plane during the preparing process and preparation of the specimen resulted in exposure of the lumina of the vasculature.

Discussion

The ultrastructural study of human bone marrow biopsy materials has long been hampered by the problem of decalcification. In animals, tubular bones are commonly used and decalcification step is thus by-passed. In man, however, tubular bones are not generally haemopoietically active and haemopoiesis is limited to cancellous bone requiring decalcification before thin sectioning; otherwise the path of the knife, alternating between the hard (bone) and soft (marrow) media would shatter the tissue, making it impossible to draw any meaningful interpretation, not to mention that the knife itself would be ruined. Most decalcifying solutions are harsh on the rather fragile marrow and tend to alter the structure, making it unsuitable for interpretation.

To overcome this most stubborn problem, we used a cryofracture technique which enabled us to dissect the fixed, but not decalcified, specimens without deformation into pieces that were small

Fig. 3 A scanning electron micrograph of a cryofractured plane of a marrow specimen from a patient with chronic myelogenous leukaemia. Venous sinus (S) exposes its lumen. Scale marker = 5 μ m.

Technical methods

enough to be well embedded with epoxy resin. After embedding the pieces of specimen, it was not difficult to trim off the calcified bone trabeculae and cut thin sections for TEM. Moreover, three-dimensional structure of the marrow specimen could also be studied by observing the cryofractured planes with the scanning electron microscope.

The role of electron microscopy in the field of human marrow aspiration cytology has been well established. Using the technique described in this report, electron microscopy can now be extended to histology of human marrow biopsy specimens.

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Letters to the Editor

Photographic recording of the ESR

In the article "An automated method for recording the Westergren erythrocyte sedimentation rate,"¹ King, *et al* describe a method of recording the ESR photographically. The principle is not new and photography was used for this purpose many years ago. In the collection of old and obsolete medical laboratory instruments housed at Colchester and under the aegis of the Joint Working Party of the R C Path and the IMLS is an instrument (photograph) on which is stamped: "The Lee Sedimentometer, 1760/15, AL Hawkins Ltd, London W1." It consists of a holder for a sedimentation tube, to one side of which is a frosted glass window and a source of light. On the other side is a long, narrow, rectangular tube, completely enclosed except for a long slit in the far wall. Beyond this slit and the wall is a clockwork movement, wound by a key in a light-proof container. This carries an old-type photographic plate slowly across the slit presumably in an hour, thus recording the ESR as the cells fall and

The Lee Sedimentometer