Technical methods

Tissue carbohydrate identification by the use of lectins

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The long-established histochemical methods such as periodic acid-Schiff and alcian blue, though invaluable for general identification and localisation of carbohydrate-containing structures, yield limited analytical information. This limitation can be substantially overcome by the use of lectins, a group of substances characterised by their ability to bind specifically to certain saccharide residues.^{1 2} These are briefly summarised in the Table. We have used FITC-conjugated lectins regularly for over three years and have found them to be simple and effective on both frozen and fixed tissues.

Summary of lectin specificities

Con A	mannose/glucose
WGA	glucosamine
SBA	galactosamine
RCA(60/II)	galactose
UEA	fucose
PSA	mannose/glucose
LCH	mannose/glucose
BSA	{ galactosamine (terminal)* galactose (terminal)*

* depends on isolectin content of preparation.

Material and methods

Tissues were obtained from routine surgical histopathology specimens and from a variety of experimental animals. Both neutral phosphate-buffered, formalin-fixed, paraffin-embedded sections and frozen sections were used.

The FITC-conjugated lectins concanavalin A (Con A), wheat germ (WGA), *Lens culinaris* (LCH) and soya bean (SBA) were obtained from Miles Laboratories (Stoke Poges, Berks, UK); Sigma Ltd (Poole, Dorset, UK); and E-Y Laboratories (San Mateo, California, USA). *Ricinus communis* (RCA 60/RCA II) was obtained from Miles and E-Y. *Ulex europaeus* (UEA), *Pisum sativum* (PSA) and *Bandeirea simplicifolia* (BSA) were obtained from Sigma and E-Y. At least two different batch

numbers of each lectin from each source have been used.

The conjugated lectins were dissolved in phosphate-buffered saline (pH 7.2, 0.15 M). The optimum concentrations in established use were 200 µg/ml for WGA and UEA, 500 µg/ml for BSA, and 400 µg/ml for the others.

The procedure for fixed and embedded sections is:

- 1 Take slides to water,
- 2 Wash in PBS for 10 min,
- 3 Trypsinise in 0.1% solution of the enzyme in PBS at room temperature for 30 min,
- 4 Wash in PBS for 10 min,
- 5 Cover section with FITC-lectin and leave in a moist environment at room temperature for 30 min,
- 6 Wash in PBS for 20 min,
- 7 Mount in 90% glycerol, 10% PBS.

For frozen sections only steps 5, 6 and 7 are used. Control sections to determine the presence of autofluorescence are prepared by using PBS by itself in step 5.

Results

Typical results are shown in the Figure. As a rule

Formalin-fixed, paraffin-embedded, trypsinised section of breast carcinoma with a cribriform pattern demonstrating cell surfaces and contents of spaces, FITC-WGA X 256.

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

The technical and clerical assistance of Mr MJL Rae, Mr C Shields and Mrs GL Holmes is gratefully acknowledged.

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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

Accepted for publication 5 August 1981