Technical methods

Enzyme histochemistry on jejunal tissue embedded in resin

NM HAND *From the Department of Pathology, University Hospital, Queen's Medical Centre, Nottingham*

To obtain a definitive diagnosis from small intestinal biopsy specimens where malabsorption is suspected, enzyme histochemical staining for alkaline phosphatase (ALP), leucine aminopeptidase (LAP), acid phosphatase (AcP), and specific disaccharidases on frozen sections is often required.

During the past 20 years, there have been several studies reporting the demonstration of enzyme histochemistry on glycol methacrylate (GMA) embedded tissue, including diagnostic applications such as on bone marrow trephines and lymph nodes. In addition to permitting some enzyme histochemistry, semithin resin sections provide high quality morphology, especially nuclear cytology.

This paper describes the diagnostic application of enzyme histochemistry on jejunal tissue embedded in the commercial GMA resin mix JB-4 (Polysciences Ltd), including showing the presence of the disaccharidases lactase and sucrase, neither of which has previously been reported on resin sections.

Material and methods

Jejunal tissue is received fresh and examined under a stereo microscope to observe morphology and villous shape. A flat longitudinal edge is required, which, if absent, is created by bisecting the biopsy with a scalpel. Tissue larger than 2 mm wide should also be bisected before fixing in cold (4°C) 4% neutral formal calcium for four hours, followed by washing in cold (4°C) 3% sucrose in 0.2M cacodylate buffer pH 7.4 for 16 hours. The tissue is then processed as follows:

- 1 Process with three changes of JB-4 monomer at -20°C each for 30 minutes.
- 2 Infiltrate with monomer and catalyst (0.4%) at -20°C for 30 minutes.

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3 Embed in catalysed monomer (0.4%) + accelerator (40:1) at 4°C.

All the processing and embedding solutions are prechilled.

After infiltration with catalysed monomer the tissue is removed from the filter paper with prechilled forceps and orientated on the flat surface in the open tray plastic embedding moulds (Polaron Equipment Ltd) so that the villi will be sectioned longitudinally. Polymerisation of the resin is achieved at 4°C in a dessicator containing cold water, in which the moulds are partially immersed to minimise and dissipate any heat produced. The dessicator is filled with nitrogen, as oxygen inhibits polymerisation. After polymerisation the blocks are allowed to reach room temperature and sections are cut at 3 µm using a glass knife on a Reichert-Jung Autocut. Sections are floated out on a room temperature water bath and mounted on cover glasses coated with 0.1% poly L lysine (molecular weight 90 000). They are allowed to air dry. Excess water on the cover glass is removed by careful blotting around the section to avoid a long delay before staining. Drying for one hour has proved satisfactory.

STAINING

One section is stained with haematoxylin and eosin for microanatomical structure and the other sections for certain enzymes (table).

Staining of lactase and sucrase is accomplished using the indigogenic and azocoupling (6-Br-2 naphthyl-α-D glucoside) procedures, respectively. Sections stained for ALP and lactase are counterstained with 1% nuclear fast red in 5% aluminium sulphate; for AcP, LAP, and sucrose 0.5% methyl green (chloroform washed) is used. The sections are rinsed in water, blotted, air dried, and rinsed in xylene before mounting in Permount.

Results

Figs 1 and 2 show lactase and sucrase activity on the villi. It has been shown that only jejunal biopsy specimens are suitable for assessing of malabsorption, with staining for disaccharidases being essential. Lactase and sucrase are the most popular and represent a range of sensitivity to injury of enterocytes.

Table *Staining procedures for microanatomical structure and for certain enzymes*

Enzyme	Source	Staining conditions		Location
Alkaline phosphatase	Rutenberg <i>et al</i> ¹	15 minutes	37°C	Brush border
Acid phosphatase	Barka, modified ²	30 minutes	37°C	Lysosomes and macrophages
Leucine aminopeptidase	Nachlas <i>et al</i> ³	30 minutes	37°C	Brush border
Lactase	Lojda and Kraml ⁴	2 hours	37°C	Brush border
Sucrase	Lojda ⁵	1 hour	37°C	Brush border

Fig 1 JB4 section of jejunum showing lactase activity on brush border of villi. Original magnification $\times 276$.

Discussion

The use of formalin as a fixative for enzyme histochemical studies using frozen sections is well documented, and the variant formol calcium is recommended. Subsequent enhancement of enzyme activity by washing in 3% sucrose in 0.2M cacodylate buffer has been recommended for tissue required for resin embedding. Although a similar resin processing schedule has been previously described for enzyme histochemistry,⁶ further (unpublished) studies indicate that disaccharidases are more sensitive to processing than most other hydrolytic enzymes and require special attention. Processing tissue via JB-4 monomer produces satisfactory preservation of disaccharidase activity.

Although other procedures for preparing tissue on resin sections have been reported,⁷ the procedure described in this paper is easy and more convenient in a diagnostic laboratory.

In conclusion, the results of this study indicate that malabsorption may be investigated and assessed using enzyme histochemistry on resin embedded jejunal biopsy specimens. Recent studies in which the biopsy specimen has been bisected for subsequent frozen and resin sections have shown comparable enzyme histochemistry.

Fig 2 JB4 section of jejunum showing sucrase activity on brush border of villi. Original magnification $\times 276$.

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Requests for reprints to: Mr NM Hand, Department of Pathology, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH, England.