

Technical methods

Staining properties of human intestinal mucosal mast cells after glutaraldehyde fixation

STEPHAN STROBEL, MAHMUD HASAN, ANNE FERGUSON *Gastrointestinal Unit and University Department of Medicine, Western General Hospital, Edinburgh EH4 2XU*

For light microscopy, the demonstration of intestinal mucosal mast cells (MMC) is critically dependent on the fixative used.¹⁻³ In an investigation of lamina propria cell types in endoscopic duodenal biopsies, MMC were identified in only 13 of 78 biopsies, and in these the numbers amounted to only one or two per section (Hasan, Hay, Sircus and Ferguson, in preparation). The specimens had been glutaraldehyde fixed, embedded in Araldite, 1-2 μm semithin sections stained with haematoxylin and eosin and with toluidine blue pH 0.7. It seemed likely that this poor yield of MMC was related to fixation and staining techniques and we have therefore examined in more detail the staining properties of MMC, by comparing glutaraldehyde and Carnoy's fixation, paraffin-embedded and Araldite-embedded tissues, and using, as the stains, Astra blue/safranin pH 0.3^{4,5} and azure II/methylene blue/basic fuchsin,⁶ a method which distinctly stains intestinal MMC in the rat.⁷

Material and methods

FIXATIVE

Operative biopsies of jejunum, and perendoscopic biopsy specimens of duodenum, were fixed in 5% glutaraldehyde (GTA) in 0.1 M sodium cacodylate buffer, pH 7.3 for 24 h, or in Carnoy's solution for 6 h. Where appropriate, specimens were post-fixed with 1% OsO₄ in cacodylate buffer for 1 h.

EMBEDDING

For conventional light microscopy studies, tissue was paraffin-embedded and sections cut at 6 μm thickness. For semithin plastic sections the tissue was Araldite-embedded and sections 1-2 μm thick were cut.

Araldite was removed after sectioning and before staining with Astra blue/safranin. For this, slides were incubated in a solution of 0.5% iodine in 1%

NaOH in ethyl alcohol at 40°C for 30 min, and thereafter rinsed in ethyl alcohol and in distilled water.

STAINS

Astra blue/safranin pH 0.3 (AB/S) This stain was performed as described in detail elsewhere.^{3,4} Paraffin embedded sections were stained in 1% Astra blue solution (BDH Ltd) for 60 min. Plastic sections were stained for 3 days. Counterstaining was achieved by dipping the sections in 0.5% Safranin O (BDH Ltd) in 0.125 N HCl. Specimens were dehydrated, cleared in xylene and mounted in DPX (BDH Ltd).

Azure II/methylene blue/basic fuchsin⁶ (AIIMB)

Solutions were prepared as follows:

Solution A (methylene blue/azure II) methylene blue (Difco, UK) 0.130 g; azure II (Sigma, UK) 0.020 g; glycerol 10 ml; methanol 10 ml; phosphate buffer pH 6.9 30 ml; distilled water 50 ml.

Solution B 3 ml: 3 ml of a stock solution of 1% basic fuchsin (RA Lamb, UK) in 50% ethanol, together with 57 ml distilled water.

Solutions A and B were preheated to 55°C and sections stained in A for 2 h. They were rinsed briefly in tap water and stained in B for 40 min, rinsed and processed as above.

Results

The design of the various experimental comparisons, and the results obtained, are summarised in the Table. Substantial numbers of well-stained MMC were found in specimens which had been Carnoy's fixed and AB/S stained (Fig. 1). In the same tissues, fixed in GTA before staining, MMC could not be identified. Somewhat better demonstration of MMC was obtained in semithin sections post-fixed with OsO₄, however by far the best results were produced by using the stain AIIMB in GTA-fixed biopsies. Distinct staining patterns of all the lamina propria cellular components were obtained with the stain AIIMB, and there was easy differentiation between red eosinophilic granules and dark blue MMC granules (Figs. 2 and 3), for as with toluidine blue, MMC and eosinophils could not readily be distinguished by the colour of their granules.

Discussion

Semithin sections, as used for orientation of tissues

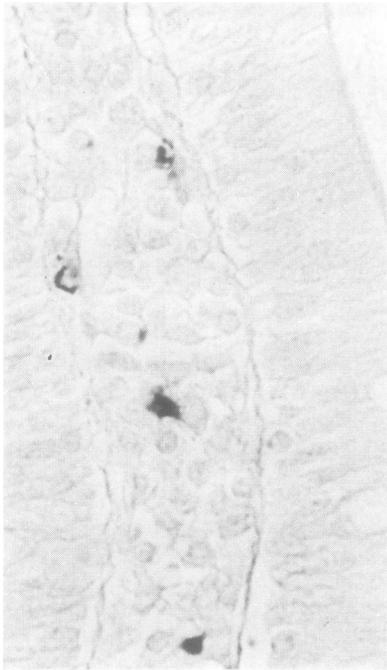


Fig. 1 Operative biopsy of normal human jejunum, fixed in Carnoy's and stained with Astra blue/safranin, pH 0.3. Intensely stained MMC are seen in the lamina propria and the typical granular staining pattern of MMC is well demonstrated $\times 500$.

in transmission electron microscopy work, provide excellent detail of lamina propria cell morphology and we have found that these can be used for counts of lamina propria cells in duodenal mucosa (Hasan, Hay, Sircus and Ferguson, in preparation). However, MMC were rarely recognised in these GTA-fixed, plastic-embedded preparations, when stained with toluidine blue or with Astra blue, but were well demonstrated by using AIIMB.

From our earlier study³ we had concluded that retrospective examination of intestinal MMC in formalin-fixed intestinal biopsies was unlikely to be rewarding. However, it will be feasible to identify and count these cells retrospectively in material which has been GTA/OsO₄ fixed for transmission electron microscopy. The stain AIIMB has the additional advantage of providing a clear and distinct demonstration not only of MMC but also of the other cellular components of the lamina propria and gut epithelium.

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Fig. 2 Endoscopic biopsy of normal duodenal mucosa, fixed in glutaraldehyde, OsO₄ post-fixed, plastic semithin section cut, and stained with azure II/methylene blue. Very distinct staining patterns of all cellular constituents of the lamina propria are demonstrated. Two well preserved MMC (arrowed) can be seen (original magnification $\times 320$).

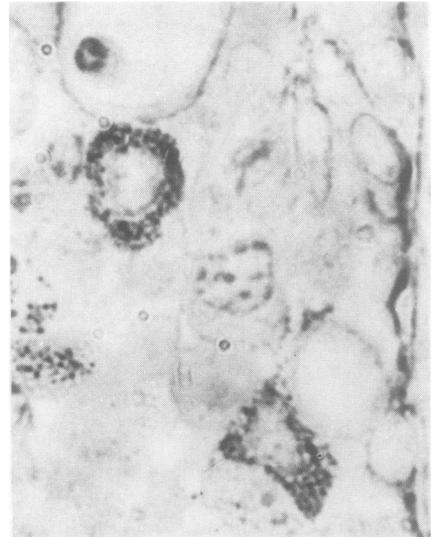


Fig. 3 Higher magnification ($\times 1250$) of the field containing two well preserved MMC, from Fig. 2. These cells exhibit the typical granular staining pattern of MMC.

Summary of experimental designs and results

Tissue	Fixation	Post-fixation	Embedding	Sections (μm)	Stain	MMC	Comments
Operative and biopsy specimens	Carnoy's	—	Paraffin	6.0	AB/S	+++	GTA fixed and paraffin embedded intestinal mucosa is unsatisfactory for light microscopic identification of MMC
	GTA 5%	—	Paraffin	6.0	AB/S	—	
Biopsy specimens	GTA 5%	—	Araldite	1.5	AB/S	—	Routine processing for semithin plastic sections and AB/S gave poor technical and staining results
	GTA 5%	OsO ₄	Araldite	1.5	AB/S	+	
Operative and biopsy specimens	GTA 5%	—	Araldite*	1.5	AB/S	—	Post-fixation with OsO ₄ and staining after Araldite removal resulted in identifiable MMC, very weak staining pattern
	GTA 5%	OsO ₄	Araldite*	1.5	AB/S	+	
Operative and biopsy specimens	Carnoy's	—	Araldite*	1.5	AB/S	+	Staining results after Carnoy's fixation for semithins were poor, marked improvement after OsO ₄ post-fixation
	Carnoy's	OsO ₄	Araldite*	1.5	AB/S	++	
Biopsy specimens	GTA 5%	—	Araldite	1.5	AII/MB	—	Routine processing for plastic sections and AII/MB give excellent and distinct staining pattern of MMC and other cellular components, MMC with blue granules
	GTA 5%	OsO ₄	Araldite	1.5	AII/MB	+++	
Biopsy specimens	Carnoy's	—	Araldite	1.5	AII/MB	(+)	Identification of MMC feasible; non-specific background staining, interfering with MMC identification
	Carnoy's	OsO ₄	Araldite	1.5	AII/MB	(+)	

*Araldite was removed before staining.

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Requests for reprints to: Dr Stephan Strobel, Gastrointestinal Unit, Western General Hospital, Edinburgh EH4 2XU.

Manual screening for immune antitetanus antibodies by means of latex coated with tetanus toxoid

JOHN R BOOTH, PHILIP A NUTTALL *Trent Regional Blood Transfusion Centre, Longley Lane, Sheffield S5 7JN*

Techniques for the selection of donor plasma containing suitably high concentrations of antibody for the production of anti-tetanus immunoglobulin include immunoelectrophoresis (IEOP),¹ haemagglutination,² automated haemagglutination³ and tetanus toxoid coated latex particles⁴ used in both automated⁵ and manual methods. At the Trent Regional Transfusion Centre, Sheffield, IEOP and automated coated latex methods have been used to select blood donors whose serum contains a sufficiently high concentration of tetanus antitoxin for the preparation of antitetanus immunoglobulin. At present this concentration is set at 4 IU/ml.

The manual latex technique has been developed to replace these methods; it is rapid, easy to perform and requires the minimum equipment. Consequently, large numbers of samples can be screened by relatively inexperienced staff, with no outlay for complex automated equipment. The method described can also be used to estimate the response to tetanus toxoid by an individual.

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