Technical method

Orcein as a mucin stain for gastrointestinal tissue sections

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Although orcein has been in use for a long time as a stain for elastic fibres, it is only recently that it has been rediscovered for the demonstration of a variety of other substances. These include hepatitis B surface antigen (HBsAg),^{1,2} copper-associated protein,^{3,4} and more recently, intestinal sulphated mucins.^{3,5} Sipponen⁵ studied the normal distribution of orcein-positive mucin in the gastrointestinal tract and compared the results with those obtained by other mucin stains. We here report our findings using a slightly modified orcein staining method after oxidation, on a variety of gastrointestinal lesions as well as normal gastrointestinal tissues.

Material and methods

Sections, 4 μ m thick, were cut from routinely processed, formalin-fixed paraffin-embedded, surgically removed tissues. These were derived from stomach (3 cases), small intestine (3 cases), appendix (one case) and large intestine (18 cases). Consecutive sections were stained with haematoxylin and eosin, and with orcein (see below). Some large intestinal sections were also stained with the high iron diamine/alcan blue method (HID).⁶

ORCEIN STAIN

The method used was that of Shikata¹ with some modifications. After deparaffinisation, sections were oxidised in a solution of 0.25% potassium permanganate and 0.25% concentrated sulphuric acid in distilled water. Sections were then washed and decolorised in 2% oxalic acid. After rewashing, the sections were stained for one and a half hours in the orcein solution. This was prepared by dissolving 1.0 g natural orcein (BDH) in 100 ml of 70% alcohol and then adding 1.0 ml concentrated hydrochloric acid. Sections were then rinsed in tap water and differentiated for a few seconds in 1% acid-alcohol. This was followed quickly by dehydration, clearing and mounting.

Results and discussion

The goblet cell was the only normal positively stained cell throughout the whole length of the gastrointestinal tract (Fig. 1). All other cells and

Fig. 1 *Jejunum, normal. Positively stained goblet cells along villi and crypts. Orcein $\times$ 120.*

tissue, except for elastic fibres, showed faint brown staining which was considered negative in this study. Sipponen⁵ described two types of normal positively stained cells: goblet cells, in the small and large intestine, and surface foveolar epithelial cells of the stomach. We could not confirm the presence of orcein-positive mucin in the latter cell in the few cases examined. However, goblet cells seen in gastric intestinal metaplasia were positively stained (Fig. 2).

In the normal large intestine, the distribution of orcein-positive cells was similar to that of HID stained cells;⁷ namely the cells lining almost the whole length of the glands, together with a few scattered surface epithelial cells. The distribution was basically the same in all parts of the large bowel.

In large bowel carcinomas, goblet cells when present in the tumours were equally stained with orcein and HID (Fig. 3). Some well differentiated

Fig. 2 Stomach, intestinal metaplasia. Positively stained goblet cells. Orcein $\times 300$.

Fig. 3 Large intestine, papillary adenocarcinoma. The normal mucosal glands (left) and a few scattered neoplastic goblet cells (right) are positively stained. Orcein $\times 50$.

neoplastic acini showed positive staining with HID but not with orcein.

HID is known to be specific for staining acidic sulphated mucin.^{8,9} The almost identical results obtained with orcein when compared with HID support Sipponen's suggestion that orcein, with prior oxidation, also stains sulphated mucins.⁵ The minor differences in staining patterns noticed in our study probably indicate that HID is slightly more sensitive, being capable of staining the small traces of sulphated mucin that might be present. However, as HID needs overnight staining, orcein has the advantage of being quicker and cheaper.

The technique described is simple, can be completed in two hours and can be equally used for the staining of elastic fibres, HBsAg and copper-associated protein as well as acidic sulphated mucin.

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