SUMMARY  A technique using alkaline phosphatase histochemistry on routine sections of four jejunal biopsy specimens and one necropsy sample was applied to show that alkaline phosphatase activity, normally present in the brush border, occurs in the enterocytes of patients with microvillus inclusion disease. Sections were cut at 5 μm, mounted on to glass slides, and dried overnight at 37°C before staining for alkaline phosphatase activity by the indoxyl phosphate nitro blue tetrazolium method. Incubation periods amounted to 10 minutes for biopsy specimens and 30 minutes to one hour for necropsy samples.

The demonstration of alkaline phosphatase activity in routinely processed biopsy specimens provides an effective, quick, and definitive test in the diagnosis of microvillus inclusion disease without recourse to electron microscopy.

Microvillus inclusion disease, also known as congenital microvillous atrophy, is a disorder which presents from birth with severe intractable secretory diarrhoea. Diagnosis is often delayed because of the difficulty in obtaining a jejunal biopsy specimen in the first weeks of life, and patients are fed intravenously until the diagnosis is made. As there is no effective treatment, accurate diagnosis is important for genetic and prognostic reasons. Histological examination shows a severe hypoplastic partial villous atrophy and an absence of the normal periodic acid Schiff (PAS) positive brush border. The apical portions, both of the surface enterocytes and some of those down the villus towards the crypt, also show PAS positive changes quite distinct from those of the goblet cells. Although this feature may be very suggestive of the diagnosis, electron microscopy is necessary to show the characteristic feature of internalised microvilli and lack or paucity of microvilli on the surface enterocytes. Electron microscopical examination of a suction rectal biopsy specimen may show the diagnostic changes, but the features are not common and may not be evident in all specimens. Suction rectal biopsy, however, is technically simpler in neonates and could provide a diagnosis if microvillus inclusion disease had been considered. In most patients the diagnosis will be made by examination of jejunal morphology in a biopsy specimen or at necropsy. Currently this examination needs to be made by electron microscopy.

This paper describes the results of a histochemical study which shows that the diagnosis can be reached at light microscopy by application of methods for demonstration of alkaline phosphatase activity in sections of routinely fixed and processed tissue.

Material and methods

Jejunal biopsy specimens taken perorally with the Watson paediatric capsule during the investigation of malabsorption, were fixed in 4% formaldehyde in phosphate buffer (buffered formalin) at room temperature and processed routinely into paraffin wax. Necropsy samples of small intestine were fixed in buffered formalin and processed routinely into paraffin wax. Jejunal biopsy specimens from four patients with microvillus inclusion disease were available as well as small intestine taken at post mortem examination in 1976 from one patient in whom the diagnosis was not made until several years later. Jejunal biopsy specimens and necropsy samples from patients without microvillus inclusion disease served as controls. Sections were cut at 5 μm, mounted on to glass slides, and dried overnight at 37°C before staining for alkaline phosphatase activity by the indoxyl phosphate-nitro blue tetrazolium method. The incubation medium contains 2·5 mg p-toluidine salt of 5-bromo-4-chloro-3-indoxyl phosphate (Sigma) dissolved in 0·5 ml dimethylformamide, 10 ml 0·2M veronal acetate buffer (pH 9·5), 5 mg nitro blue.
Microvillus inclusion disease

Results

Brush border alkaline phosphatase activity (fig 1a) was readily demonstrable on villous enterocytes in all of the control biopsy samples which comprised those with normal morphology and those with partial villous atrophy. The activity was not present on crypt cells. A similar well defined staining pattern was found in necropsy samples which had not been autolysed.

In patients with microvillus inclusion disease alkaline phosphatase activity was present in the apical portions of the villous enterocytes. In most enterocytes the activity was present as a diffuse clump (fig 1b), but in some cells well defined ring-like activity could be seen (fig 1c). The brush border activity was severely depleted.

Discussion

The alkaline phosphatase activity of the brush border of small intestinal enterocytes is well recognised in cryostat sections of frozen tissue. Its distribution, with activity on the villous enterocyte brush border and absence within the crypts, serves as a useful marker in the assessment of crypt:villus ratios. Although the early studies on alkaline phosphatase activity were on tissues fixed in cold reagents for short periods of time followed by careful processing, it has not been widely recognised that human intestinal alkaline phosphatase is sufficiently robust to withstand routine fixation and processing. Once the tissue has been fixed and embedded in paraffin wax, alkaline phosphatase activity remains stable and can be shown in biopsy and necropsy tissue for at least 11 years. Under the same conditions the activity of other alkaline phosphatases is only poorly preserved (kidney proximal tubular enzyme) or may not be demonstrable (vascular, endothelial enzyme).

Almost any method for demonstration of enzyme

tetrazolium (Sigma grade III) and 0.08 ml 1M MgCl₂. Incubation at 37°C for 10 minutes is required for biopsy specimens and for post mortem samples 30 minutes to one hour. Kernechtrot is a suitable nuclear counterstain.

Fig 1  Jejunal biopsy specimens stained to show alkaline phosphatase activity by indoxyl phosphate-tetrazolium method. The nuclei have been counterstained with Kernechtrot. (a) normal jejunum showing discrete brush border activity; (b) microvillus inclusion disease showing clumps of activity in apical portions of enterocytes. Brush border activity is very weak. (c) Microvillus inclusion disease with more prominent clumps of activity. Well defined ring of activity is also shown (arrow).
activity can be used. For practical purposes the indoxyl phosphate-tetrazolium method is preferred, although Gomori’s calcium-cobalt method is also suitable. These two methods give high contrast with a black reaction product on an unstained background, making interpretation of the slides straightforward. The simultaneous coupling method, with Naphthol AS-TR phosphate as substrate and hexazotised new fuchsin coupler, is insensitive and fails to highlight the pathology in microvillus inclusion disease.

The presence of alkaline phosphatase activity within the apical part of the villous enterocytes, when it should be in the brush border, suggests that insertion of the enzyme into the brush border membrane material is not defective. That the general synthesis of membranes forming the brush border and insertion of brush border enzymes into the membranes is normal in microvillus inclusion disease, is supported by the histochemical demonstration of several other brush border enzymes (lactase, aminopeptidase M, dipeptidyl peptidase IV) in the apical portion of villous enterocytes. Although disaccharidase and peptidase activity can be shown in microvillus inclusion disease, their intracellular distribution renders them inaccessible for the normal digestive process, a factor in the malabsorption which affects the patients.

It has been reported that alkaline phosphatase activity is not found on the internalised microvilli, but the study was at ultrastructural level and details of the methodology were not given in the summary. In our study the morphological appearances suggest that internalised microvilli (ring-like structures, fig 1c) as well as the secretory granular component described at electron microscopy (diffuse clumps) both expressed alkaline phosphatase activity. Further studies on the ultrastructural localisation of alkaline phosphatase activity are planned when fresh material becomes available from this rare condition.

The pathology is consistent with the suggestion that microvillus inclusion disease is a disorder of the cytoskeleton. Depleted amounts or disordered distribution of cytoskeletal proteins, or both, have been reported for actin and cytokeratin, myosin, and vinculin. Similar ultrastructural changes can be produced experimentally in human fetal intestinal organ culture by treatment with cytochalasins B and D, although there are some differences in the detailed ultrastructure. Colchicine also disturbs the cytoskeleton with formation of lateral membrane microvilli and displacement of the Golgi complex in enterocytes of the treated rat. Surface microvilli, however, were unaffected.

The early diagnosis of microvillus inclusion disease is particularly important as there is no effective treatment and any child supported on total parenteral nutrition with a diagnosis of microvillus inclusion disease would pose an ethical problem.

The demonstration of alkaline phosphatase activity in routinely processed biopsy specimens provides an effective, quick, and definitive test in the diagnosis of microvillus inclusion disease.

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References