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SINGLE-VILLUS ANALYSIS OF DISACCHARIDASE EXPRESSION BY DIFFERENT REGIONS OF THE MOUSE INTESTINE

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SUMMARY

1. The present results describe how a new technique of whole-tissue cytochemistry can be combined with automatic scanning of microdissected villi to measure the capacity of individual villi to hydrolyse disaccharides in different parts of the small intestine.

2. Intact villi from the mouse proximal jejunum are found to be eight times more effective than ileal villi in hydrolysing 2-naphthyl-α-D-glucoside, an artificial substrate for enzymes normally hydrolysing sucrose, maltose, isomaltose and trehalose in adult intestine. Homogenates of jejunal scrapings are four times more effective than ileal homogenates in hydrolysing this substrate. This discrepancy arises from relating enzyme activities to homogenate protein in cases where intestinal structure changes.

3. The eightfold difference in villus α-glucosidase activity is associated with a threefold difference in villus surface area. This discrepancy in turn reflects changes in the capacity of individual enterocytes to express α-glucosidase during migration along the crypt–villus axis. These results emphasize the futility of trying to gauge intestinal function from measurement of intestinal structure.

4. Differences between ileal and jejunal villus α-glucosidase activities have been further partitioned into those depending on villus structure and those depending on enterocyte development. Present results are discussed in relation to the ability of luminal nutrition to maintain a proximal–distal gradient of digestive enzyme function in the small intestine. The general applicability of this method of analysis to other studies of adaptive response is also emphasized.

INTRODUCTION

The ability of the jejunum to digest and absorb nutrients has long been known to exceed that of the distal ileum. Part of this difference reflects corresponding changes in intestinal structure brought about as an adaptive response to different levels of luminal nutrition (Grönqvist, Engström & Grimelius, 1975; Rijke, Hanson & Plaisier, 1977; Menge & Robinson, 1978*a*). Another adaptive response not yet studied in relation to luminal nutrition involves an alteration in the way enterocytes express their ability to digest and absorb different nutrients (Smith, 1985). This

response can then be amplified by increasing the size of the villus over which enterocytes migrate. Interaction between structural adaptation and enterocyte development occurs whenever increased villus size becomes associated with crypt cell hyperplasia (Dowling, 1982). How such an interaction affects intestinal function then depends upon whether faster migrating enterocytes still have time to develop fully before being shed from the tips of longer villi.

Some progress in disentangling cellular from structural aspects of adaptation has been achieved recently by applying new techniques of quantitative cytochemistry and autoradiography to pieces of intestine taken from animals adapted to different conditions (Menge, Sepúlveda & Smith, 1983; Dauncey, Ingram, James & Smith, 1983; King, Paterson, Peacock, Smith & Syme, 1983; Hewitt & Smith, 1986; Lund & Smith, 1987). There has, however, been no comparable study of how structural change affects intestinal function.

In order to obtain this information it was first necessary to discover a way to measure surface area and disaccharidase activity in the same villus. This was achieved by developing a new technique of whole-tissue cytochemistry, the appearance of enzyme reaction product then being assessed by applying scanning microdensitometry to microdissected villi. The surface area of these villi was also measured by image analysis. Initial results show that it is possible to use this technique to measure developmental profiles for enzyme expression on individual villi as well as their total capacity to hydrolyse disaccharides. This unexpected finding gives single-villus analysis additional importance as a future way to study fundamental aspects of intestinal function.

METHODS

Animals

Eight-week-old BALB/c male mice kept under controlled conditions of temperature and lighting (daily cycle 12 h light and 12 h dark) were fed a constant diet containing 57% carbohydrate purchased from Labsure, K & K Greef Ltd, Croydon, Surrey, and allowed free access to water until the time they were used for experiment.

Whole-tissue cytochemistry

Mice killed by cervical dislocation had their small intestines removed and rinsed with ice-cold phosphate-buffered saline containing 0·5 mM-dithiothreitol. Tissue samples removed from these intestines, taken immediately distal to the ligament of Treitz and immediately proximal to the ileo-caecal junction (proximal jejunum and distal ileum respectively), were then cut open and pinned out in small Petri dishes containing Silgard 184 silicone elastomer (Dow Corning 6198, Seneffe, Belgium) for preliminary fixation in Formol calcium medium for 10 min at 4 °C. This latter procedure is needed to limit diffusion of naphthol substrate enzyme reaction product from its site of production (Gossrau, 1976). These fixed tissues were then incubated at 37 °C with 6 mM-2-naphthyl-α-D-glucoside as substrate and hexazonium-*p*-rosaniline for colour development of enzyme reaction product. Tissues were shaken vigorously during incubation at maximum setting on a Titertek plate shaker (Flow Labs, Rickmansworth, Herts) and hydrolysis stopped by rinsing in fresh ice-cold Formol calcium medium at pH 6·0. This procedure caused no damage to villus structure viewed under a microscope. A similar method for determining α-glucosidase activities in rat intestine, but applied only to tissue sections and not involving shaking, has been described previously by Gutschmidt, Kaul & Riecken (1979).

Pieces of tissue reacted in this way to determine the initial rate of α-glucoside hydrolysis were then either treated for 5 min at room temperature with 1% Alcian Blue dissolved in 3% v/v acetic acid to stain goblet cells or used immediately to provide intact villi for scanning microdensitometry.

Other control microdissected villi were stained with Alcian Blue for goblet cell counting on a TV monitor attached to a Leitz Dialux microscope through a Hitachi CCTV camera. Final goblet cell count was related to different areas of villus surface measured on the same TV monitor.

Scanning microdensitometry

Intact villi microdissected from incubated tissue with the aid of a microscalpel and fine forceps were mounted on microscope slides in a 1:1 mixture of glycerol and phosphate-buffered saline, where they were flattened by applying a 1 kg force to a 2×2 cm cover-slip. The edges of the cover-slip were then sealed with Tipp-Ex (Tipp-Ex Vertrieb GmbH, Frankfurt, F.R.G.) to avoid dehydration and preserve the flattened structure of the individual villus. Applying pressure to villi in this way caused a $19{\cdot}3 \pm 5{\cdot}0$ % increase in *detectable* surface area measured by Magiscan analysis (mean value $\pm$ S.E.M. obtained from six estimates). These villi were then transferred to an MPV3 microdensitometer (Leitz GmbH, Wetzlar, F.R.G.), where they were scanned automatically for colour intensity at a wavelength of 500 nm.

The MPV3 microdensitometer has the ability to record a maximum of 1600 measurements of optical density during a single scan, the width and length of the area covered being controlled through a Hewlett–Packard 85B microcomputer. In practice it was found convenient to use a 10×10 μm light square and scanning matrices of 25×30 and 50×33 to cover the complete surface of ileal and jejunal villi respectively. Polaroid photographs of these villi were also taken routinely to facilitate later analysis of results. Optical densities arising from passing light through villus cores were corrected for by first zeroing the instrument on a suitable crypt containing no enzyme reaction product. Applying this procedure in control scans of unincubated microdissected villi gave negligible readings of optical density. Positive readings of optical density recorded from villi dissected from incubated tissue were both printed and stored on floppy disc. Villus perimeters on paper print-outs were later identified by comparing positive with background readings of optical density. All readings within this perimeter were then summed to calculate the *total* capacity of the villus to hydrolyse α-glucosides. Calculating *mean* values for optical density along the crypt–villus axis also allowed the construction of developmental profiles describing the characteristics of α-glucosidase expression.

Measurement of villus surface area

Surface areas were determined by transferring villus images through a Bosch TYK9A television camera attached to the MPV3 microdensitometer into a Magiscan 2A image analyser (Joyce-Loebl Ltd, Newcastle upon Tyne, Tyne and Wear). Values obtained using the standard Magiscan MENU program were then doubled to give estimates of villus surface area. The assumption made here was that flattened villi could be considered to consist of two flat sheets bent to meet each other only in the region of the villus perimeter.

Control experiments carried out to test the validity of this assumption involved measuring villus thickness directly by focusing first on the upper and then on the lower surface of each villus. Results obtained by carrying out these measurements are summarized in Table 1. Villi from the proximal jejunum are, on average, about 10 μm thicker than ileal villi and there is about a 5–8 μm increase in thickness seen on moving from the villus edge to the centre of the villus core. Further measurements showed villus thickness to remain constant along the long axis of both ileal and jejunal villi ($34{\cdot}3 \pm 1{\cdot}9$ and $40{\cdot}2 \pm 1{\cdot}6$ μm; means $\pm$ S.E.M. of estimates carried out on thirteen and twelve ileal and jejunal villi respectively; $P < 0{\cdot}05$). It is concluded that any curvature which might remain across flattened villi will be too small to materially affect the results obtained.

Biochemical and histological determination of α-glucosidase activity

α-Glucosidase activities were determined in 10 μm sections of frozen tissue taken from the proximal jejunum and distal ileum of the mouse small intestine by incubation with 2-naphthyl-α-D-glucoside for 4 and 8 min respectively at a temperature of 37 °C. Subsequent colour development with hexazonium-*p*-rosaniline was as described previously by Gutschmidt *et al.* (1979). Final estimates of the amount of enzyme reaction product formed were obtained by scanning sections automatically using the MPV3 microdensitometer as described above for whole villi.

The corresponding ability of homogenates of mouse ileal and jejunal scrapings to hydrolyse 2-naphthyl-α-D-glucoside was determined by incubation in 0·1 M-citrate–phosphate buffer, pH 6·0, for 25 min at 37 °C. The amount of azodye formed during incubation was then extracted with

ethylacetate and estimated according to the method of Lojda, Slaby, Kraml & Kolinska (1973). Final α-glucosidase activities in this case refer to the amount of substrate hydrolysed per minute per milligram of protein estimated according to the method of Markwell, Haas, Bieber & Tolbert (1978). All estimates of α-glucosidase activity were carried out using saturating concentrations of substrate under initial rate conditions.

TABLE 1. Positional dependence of villus thickness

	Villus thickness (μm)		
Tissue	S1	S2	S3
Proximal jejunum	56·4±4·0	56·0±3·0	51·6±2·2
Distal ileum	48·0±3·7	38·8±3·7	40·4±3·1

Villus thickness at half villus height was measured by focusing alternately on the top and bottom surface of the villus. Readings starting in the middle of the villus (S1) moved towards the periphery in 30 μm steps (S2 and S3 respectively). Final readings at S3 were about 25 μm from the edge of the villus. Each value gives the mean villus thickness±S.E.M. obtained from estimates carried out on five different villi.

Materials

The α-glucosidase substrate 2-naphthyl-α-D-glucoside was supplied as β-naphthyl-α-D-glucopyranoside by the Sigma Chemical Company, Poole, Dorset. All other reagents were of analytical reagent grade.

RESULTS

Structural properties of mouse intestinal villi

Mouse jejunal villi have a general finger-like appearance with the width at the base being about half the villus height (Plate 1*A*). Incubation of these preparations with 2-naphthyl-α-D-glucoside produces orange-coloured villi with the density of enzyme reaction product decreasing towards the villus tip (Plate 1*B*). A low-power view of these villi reacted with Alcian Blue shows an apparent even distribution of goblet cells along the villus with extracellular mucus staining blue at the base of the crypts (Plate 1*C*). These particular villi have also been flattened in order to measure the total amount of α-glucosidase activity present (Plate 1; compare *B* with *C*). High-power views of similar flattened jejunal villi incubated for 4 and 12 min for α-glucosidase activity show the amount of enzyme reaction product to increase with time of incubation. There is also a marked tendency for some of this enzyme reaction product to accumulate at the surface of goblet cells (Plate 1*D* and *E*). The possible reason for this latter phenomenon and the effect it might have on subsequent scanning patterns for enzyme activity is discussed in a later section.

Ileal villi from mouse intestine are leaf-like in appearance and about half the size of an average jejunal villus. High-power views of these villi, incubated for 7 and 22 min to determine α-glucosidase activity, again show enzyme reaction product increasing during incubation, but with the total activity less than that found in jejunal villi (Plate 1*F* and *G*). Some of this enzyme reaction product again appears to accumulate over the surface of goblet cells. None of these treatments appears to cause any shrinkage or distortion of villus structure. Results showing the average dimensions of flattened villi are summarized in Fig. 1.

The sides of jejunal villi taper gradually to reach a villus tip 460 μm from the villus base. The sides of the ileal villus taper towards a villus tip which is at least twice as wide as that measured in the jejunum. The average ileal villus rises 210 μm from a base which is nearly as wide as that measured in the jejunum. The mean surface areas of these preparations are $0{\cdot}168 \pm 0{\cdot}01$ and $0{\cdot}061 \pm 0{\cdot}004$ mm^2 respectively

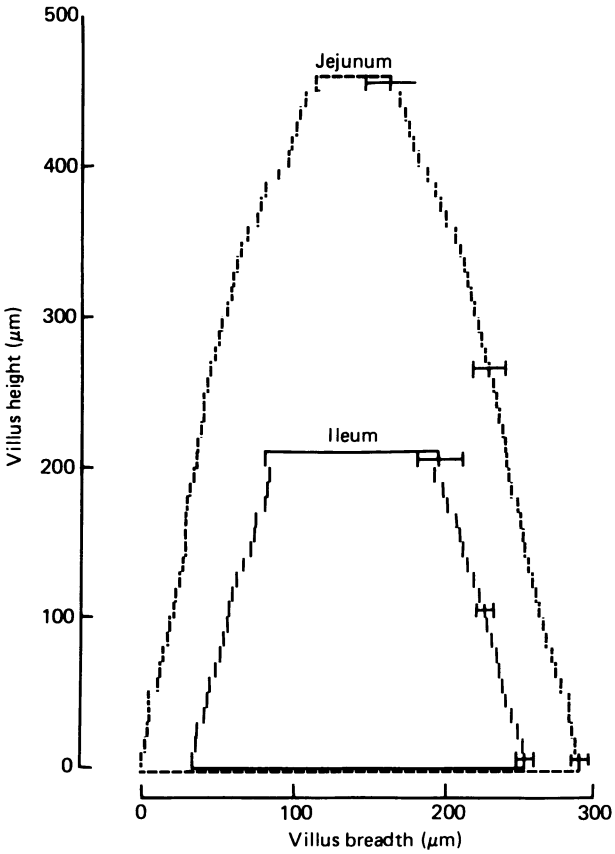

Fig. 1. Scanning reconstruction of villus shapes in mouse small intestine. Villi microdissected from mouse proximal jejunum and distal ileum flattened and scanned for α-glucosidase activity as described in the text had their shapes reconstructed by measuring the number of scanning frames recording positive colour density. Values for villus breadth obtained by this method give means $\pm$ S.E.M. of estimates carried out in thirteen jejunal and twenty-six ileal villi.

(means $\pm$ S.E.M. of fourteen and twenty-nine estimates). These values for villus surface area have been used to normalize all subsequent estimates of α-glucosidase activity carried out on single villi.

Time dependence of substrate hydrolysis by mouse ileal and jejunal villi

Results summarized in Fig. 2 show α-glucosidase activity of ileal and jejunal villi to increase linearly with time. Extrapolation of straight lines drawn through these

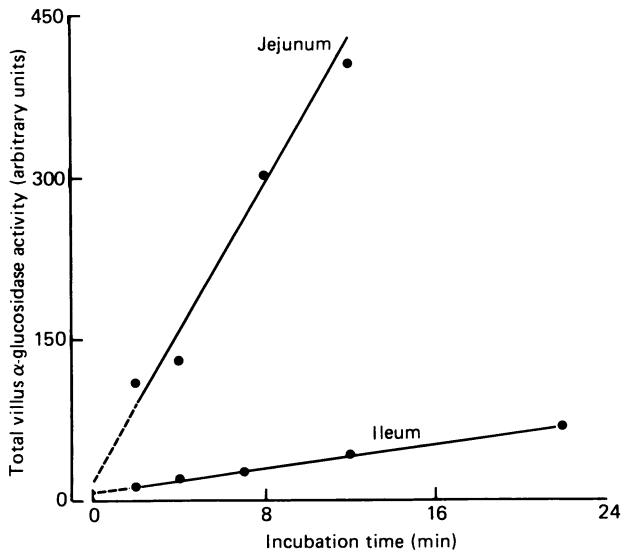

Fig. 2. Time dependence of α-glucoside hydrolysis by different regions of the mouse small intestine. Intact villi prepared from both jejunal and ileal regions of the mouse small intestine incubated previously for different times with 2-naphthyl-α-D-glucoside were flattened and scanned for enzyme reaction product as described in the text. Values give total villus α-glucosidase activity normalized to average jejunal and ileal villus surface areas of 0·17 and 0·06 mm² respectively. Each value gives the mean of determinations carried out on three jejunal and three to six ileal villi. Extrapolation of straight lines through these points are shown as interrupted lines.

TABLE 2. Regional differences in α-glucosidase activity measured in intact villi and mucosal scrapings of mouse small intestine

Preparation	α-Glucosidase activity (arbitrary units)		Activity ratio
	Jejunum	Ileum	
Mucosal scrapings	56·8±9·1 (5)	13·8±1·7 (5)	4·2±0·6 (5)
Intact villi	1773±97 (9)	228±30 (9)	7·8

Homogenates of mucosal scrapings prepared from the proximal jejunum and distal ileum of mice were assayed for their ability to hydrolyse 2-naphthyl-α-D-glucoside as described in the text. Values for α-glucosidase activity here are calculated per milligram of homogenate protein per minute of incubation. Values for α-glucosidase activity determined in intact villi are calculated per minute incubation for ileal and jejunal villus surface areas of 0·06 and 0·17 mm² respectively. All values give means±S.E.M. (number of estimates in parentheses).

time courses also give intercepts approaching zero. The total α-glucosidase activity present on a jejunal villus is about eight times that determined in the ileum. This difference has been investigated further by carrying out similar assays for α-glucosidase activity on homogenates of mucosal scrapings prepared from the same regions of the small intestine. Results obtained in these experiments are compared with those obtained by scanning microdensitometry in Table 2.

The jejunal-to-ileal ratio of α-glucosidase activities measured in homogenates of muscosal scrapings is about four compared with eight measured on whole-villus preparations. It is concluded from these results that estimates of enzyme activities in mucosal homogenates seriously underestimate the relative importance of the jejunum as a site for α-glucoside hydrolysis. This eightfold difference in villus

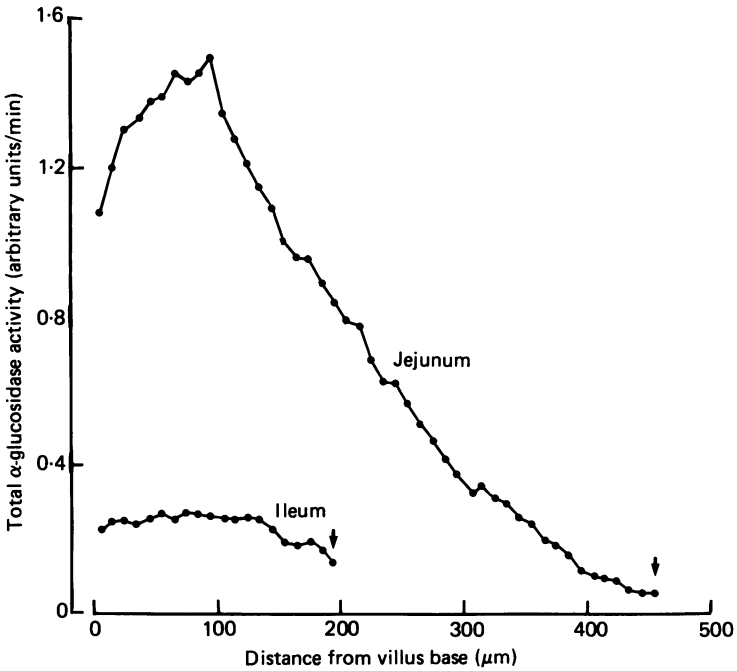

Fig. 3. Villus distribution of α-glucosidase activity. Jejunal and ileal villi dissected from tissue incubated previously with 2-naphthyl-α-D-glucoside (4, 8 and 12 min for the jejunum; 16 and 22 min for the ileum) were flattened and scanned for enzyme reaction product as described in the text. Results give total α-glucosidase activities calculated per minute incubation for successive 10 μm wide perimeter bands of villus surface obtained from scanning nine ileal and nine jejunal villi. Arrows show the positions of the villus tips.

α-glucosidase activity is also three times greater than would be expected from a simple comparison of total surface area (0·17 and 0·06 mm^2 for jejunal and ileal villi respectively). Cellular differences in α-glucosidase expression account for this further discrepancy in enzyme activity.

Positional dependence of total α-glucosidase activity

The ability of jejunal and ileal villi to hydrolyse 2-naphthyl-α-D-glucoside along the crypt–villus axis is given in Fig. 3. Over a fivefold difference in total enzyme activity already exists at the villus base where differences in perimeter are relatively small (see Fig. 1). Cellular changes in enzyme expression must be largely responsible for creating this initial difference. Total α-glucosidase activity then increases on moving up the jejunal villus while that in the ileum remains constant. This secondary

increase in jejunal α-glucosidase must be due to cellular changes in enzyme expression. Total α-glucosidase activity finally falls in the upper half of the jejunal villus to levels below those found in the upper regions of the ileal villus.

Positional dependence of mean α-glucosidase activity

Cellular expression of α-glucosidase activity can be studied in sectioned tissue by measuring enzyme activities sequentially along the crypt–villus axis (Smith, 1985).

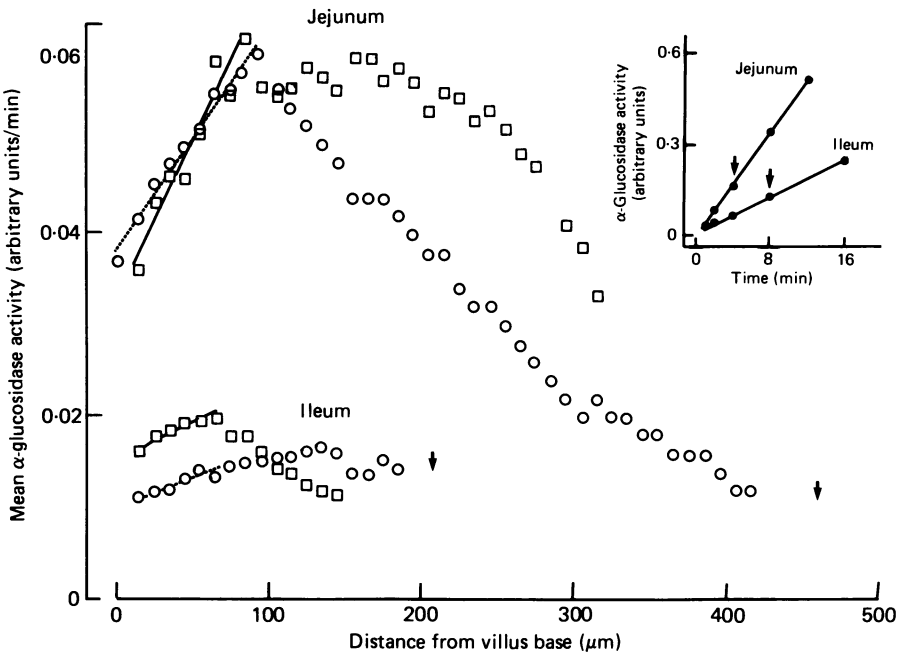

Fig. 4. Positional dependence of α-glucosidase activity in mouse ileal and jejunal enterocytes. Mean α-glucosidase activities were determined at different points on the crypt–villus axis of jejunal and ileal villi by scanning sectioned tissue (□) or intact villi (○) as described in the text. Each value gives the mean of estimates carried out on fifteen villus sections and nine intact villi obtained from two different mice. The inset shows values for α-glucosidase activity plotted in arbitrary units, against the time of incubation in minutes, determined in sections of mouse jejunal and ileal villi. All values for sectioned tissue have been divided by a factor of 2·5 to make them approximately equal to those obtained from whole villi. Arrows in the inset show incubation times chosen to determine developmental profiles for α-glucosidase expression plotted in the main Figure; arrows in the main Figure give the positions of the villus tips.

The results of carrying out similar experiments in the present work are compared with those obtained from whole-villus analysis in Fig. 4.

Mean α-glucosidase activity is much higher at the base of intact jejunal compared with intact ileal villi and the subsequent increase in activity noted on moving up the jejunal villus is now much more obvious than that shown in Fig. 3. A small developmental increase in α-glucosidase activity can now also be detected over the

lower half of the ileal villus. Sections of similar tissues, taken from another animal and incubated at times chosen to produce equal amounts of enzyme reaction product (inset; Fig. 4), have similar profiles for enzyme development to those recorded for intact villi.

In this analysis it has been assumed that goblet cell distribution has no effect upon the determination of developmental profiles in intact villi, even though enzyme reaction product appears to accumulate in these cells (Fig. 1). This assumption should prove valid provided goblet cell distribution remains uniform over the whole surface of the villus. To test this it was decided to measure goblet cell distribution directly in intact villi stained with Alcian Blue. The mean number of goblet cells counted in the basal, mid and top third of jejunal villi, corrected per 10000 μm^2 surface area, were $5{\cdot}4 \pm 0{\cdot}3$, $6{\cdot}1 \pm 0{\cdot}3$ and $4{\cdot}9 \pm 0{\cdot}4$ respectively (means $\pm$ S.E.M. from forty-four villi). The corresponding values for the top and bottom halves of ileal villi were $9{\cdot}0 \pm 0{\cdot}6$ and $9{\cdot}1 \pm 0{\cdot}5$ respectively (means $\pm$ S.E.M. from thirty villi). The proportion of goblet cells on ileal villi is approximately twice that found in the jejunum. Differences in goblet cell distribution along the villus are, however, too small to cause significant distortion of the developmental profiles shown in Fig. 4. A similar even distribution of goblet cells along mouse duodenal villi has previously been reported by Bjerknes & Cheng (1985).

The present results therefore show that it is possible to use whole villi instead of sections to determine the early characteristics of enzyme expression during enterocyte migration along the crypt–villus axis. This then allows a quantitative assessment to be made of the part played by cellular and structural events in creating differences in α-glucosidase activity between ileal and jejunal villi.

Structural and cellular adaptation of ileal villus function

Analysis of how cellular and structural adaptation might account for these net changes in enzyme activity is based on the fact that ileal villi become identical in shape to jejunal villi after transposition to the proximal intestine (Altmann & Leblond, 1970) and that α-glucosidase expression in jejunal villi is similar to that seen in the ileal remnant of proximally resected tissue (Chaves, Smith & Williamson, 1987).

Suppose that the ileal villus grew wider without changing the way each enterocyte expressed α-glucosidase activity. In this case there would be a net increase in enzyme activity due to the extended perimeter of the enlarged villus. The amount of this increase could then be calculated by reference to the villus perimeters in Fig. 1 and the mean ileal villus enzyme activities in Fig. 4. This increase can then be defined as being caused by structural adaptation. The total enzyme activity in the lower half of the jejunal villus is, however, greater than that calculated for the theoretically expanded ileal villus. This difference, which can be calculated by subtracting expanded ileal villus values from those found on the jejunal villus (Fig. 3), is caused by cellular adaptation. How this works in practice is illustrated for different levels of the jejunal villus in Fig. 5.

Results show the major difference between ileal and jejunal function to arise from changes in the way enterocytes express α-glucosidase activity in the basal half of the jejunal villus. Differences in α-glucosidase activity in this region partition 8:1 in

favour of cellular adaptation with the remaining 28% of the total activity being located in the upper half of the villus. The origin of this α-glucosidase activity is classified as being mixed since it depends on the biochemical history of enterocytes being expressed over a villus surface having no counterpart in the ileum.

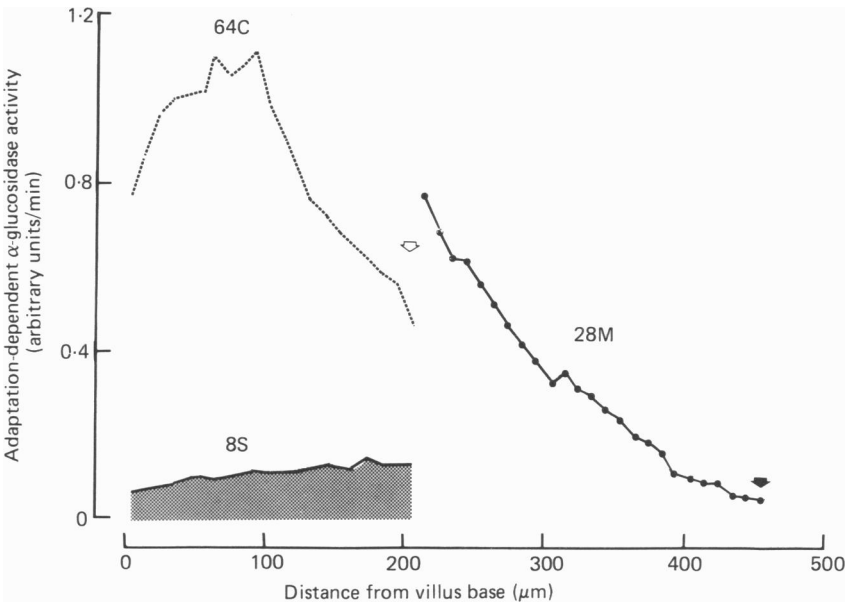

Fig. 5. Structural and cellular origins of differences noted between mouse ileal and jejunal α-glucosidase activities. The amount of α-glucosidase activity needed to convert an ileal into jejunal villus has been divided into that accounted for by changes in structure (structural adaptation S; shaded area; 8% of the total difference) and that caused by changing the profile of enterocyte development (cellular adaptation C; dotted line; 64% of the total difference; values obtained by reference to data summarized in Figs 1 and 4). The remaining 28% of α-glucosidase activity (continuous line corresponding to that drawn for the upper half of the jejunal villus in Fig. 3) is caused by a mixture (M) of both changes in structure and cellular expression of enzyme activity. Open and closed arrows: tip positions for ileal and jejunal villi respectively.

DISCUSSION

The present method of analysis allows one to estimate both cellular and structural aspects of adaptation in intact villi and to relate these findings to what is happening in the whole intestine. The only problem encountered so far in using preparations in this way involves uneven distribution of enzyme reaction product on the surface of the villus. This distribution seems to coincide with that of goblet cells which, it is suggested, act as traps for diffusible enzyme reaction product rather than sites for substrate hydrolysis. The amount of product detected under these circumstances will depend on the number and arrangement of goblet cells on the villus. Present results show no obvious correlation, however, between goblet cell distribution and developmental profiles for α-glucosidase activity. The strongest evidence that goblet

cell trapping of hydrolysed substrate does not affect these developmental profiles is, however, provided by comparing results obtained using sectioned and microdissected tissue (Fig. 4). The close correspondence found between these two sets of data, in spite of the fact that sections show no goblet cell accumulation of hydrolysed substrate, indicates that any effect on villi is too small to influence the final shape of the developmental profile.

The corresponding measurement of surface area depends upon the villus surface being flat. Measurements designed to check the flatness of compressed villi suggest that this assumption is operationally acceptable. Comparison of normal with flattened villi (Plate 1 *B* and *C*) further suggests a proportional spreading of the villus rather than stretching during compression. The best analogy here is probably with a paper cylinder which, which compressed, retains its length-to-breadth characteristics while at the same time exposing the maximal area for measurement.

The search for factors responsible for maintaining the villus size gradient along the small intestine has long proved of interest to those studying cell proliferation and the regulation of tissue growth. From this work it is concluded that luminal nutrition is the main factor affecting villus structure. One of the strongest lines of evidence supporting this conclusion comes from intestinal resection experiments (see Dowling, 1982). Further attempts to establish how these changes in structure affect intestinal function have, however, led to the production of conflicting results. Proximal resection appears, for instance, to stimulate nutrient absorption when related to intestinal length (Dowling & Booth, 1967; Garrido, Freeman, Chung & Kim, 1979) but not when related to intestinal weight or mucosal surface area (Weser & Hernandez, 1971; Menge & Robinson, 1978*a*). Disaccharidase activities are also said to be reduced when measured in intestinal homogenates (Weser & Hernandez, 1971; Bury, 1972; Menge & Robinson, 1978*b*) but not when measured cytochemically in tissue sections (Chaves *et al.* 1987). The present results directly demonstrate the errors involved in relating disaccharidase activities to homogenate protein and villus surface area (Table 2). Differences between normal jejunal and ileal villus size account for a surprisingly small amount of the discrepancy noted between their corresponding disaccharidase activities measured cytochemically. Most of this difference arises instead from the way enterocytes carry out their programme of development at these two sites within the intestine.

Differences occurring between the way ileal and jejunal enterocytes express α-glucosidase activity in the mouse are very similar to those seen to take place in the ileum following proximal resection in the rat (Chaves *et al.* 1987). Similar but less pronounced changes also take place in the jejunum of rats fed a carbohydrate-rich diet (King *et al.* 1983). Cellular adaptation in all cases involves a net increase in α-glucosidase activity over the lower half of the villus. Enterocytes are also known to migrate twice as fast over ileal remnant compared with normal ileal villi (Chaves *et al.* 1987) and similar differences exist between mouse jejunum and ileum (Hagemann, Sigdestad & Lesher, 1970). From this it can be seen that enterocytes substantially increase the rate at which enzyme activity appears in their brush-border membranes in response to both naturally occurring and artificially induced changes in luminal nutrition.

Some attempt has already been made to define ways in which enterocytes adapt

to changes in their immediate environment (Smith, 1986). Use of the isolated villus now allows the effects of structural adaptation to be incorporated into this general scheme of adaptive response. Seven hydrolytic enzymes in the brush-border membrane are already amenable to this form of cytochemical analysis (Gossrau, 1980) and there is no shortage of adaptive stimuli able to change intestinal function. Choice of one particular model to study the mechanisms controlling adaptation in these circumstances has proved difficult in the past. The ease with which single-villus analysis can be performed is, however, encouraging as is the total information obtained from single scans of villi. There seems therefore no reason to doubt that further application of this method to different adaptational situations will prove scientifically rewarding in the future.

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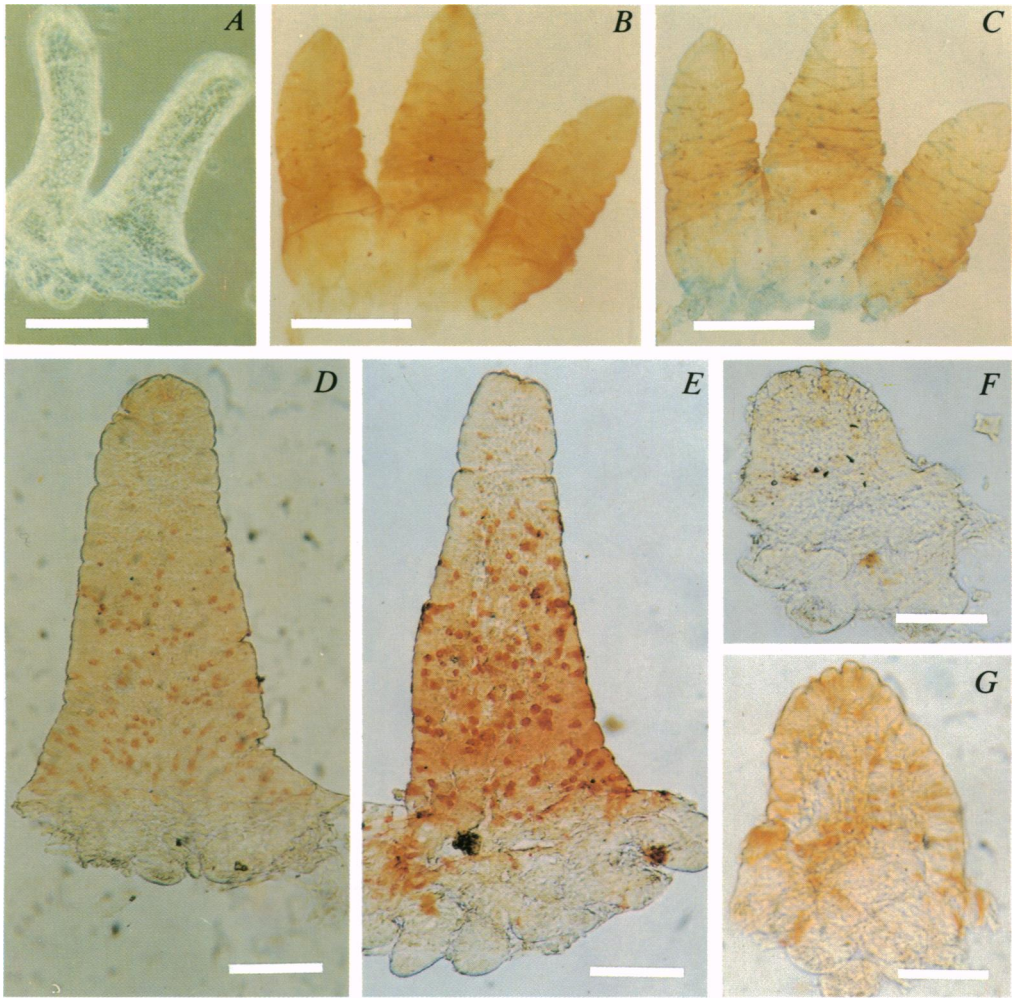
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EXPLANATION OF PLATE

Mouse jejunal (*A–E*) and ileal tissue (*F* and *G*) was microdissected to provide individual villi before (*A*) or after (*B–G*) incubation with 2-naphthyl-α-D-glucoside at 37 °C as described in the text. All tissues had been fixed previously in Formol calcium medium for 10 min at 4 °C. *A*, control jejunal villi; phase-contrast microscopy; 300 μm scale bar. *B*, test jejunal villi; 9 min incubation with α-glucosidase substrate; 300 μm scale bar. *C*, identical villi to those shown in *B* after 5 min reaction with 1% Alcian Blue at room temperature; villi compressed as described in the text; 300 μm scale bar. *D* and *E*, jejunal villi incubated with α-glucosidase substrate for 4 and 12 min respectively; 100 μm scale bars. *F* and *G*, ileal villi incubated with α-glucosidase substrate for 7 and 22 min respectively; 100 μm scale bars. Photography was either on Ektachrome (*A–C*) or Vericolor II film (*D–G*).