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Progress report

The intestinal brush border

The appearance of the striated border of intestinal epithelial cells under the light microscope was described many years ago[1], and the first demonstration that this border consisted of fine projections or microvilli was made by Granger and Baker in 1949[2] using the electron microscope. Since that time the intestines of many species including man have been shown to possess brush (microvillous) borders[3], and there are approximately 1,700 microvilli on each epithelial cell[4]. A single microvillus measures about 1 micron in length and 0·1 micron in diameter, and the total brush borders of the small intestine have been estimated to increase the surface of the absorptive cells thirty- to forty-fold[4]. In 1961, Miller and Crane[5] separated intact brush borders from the epithelial cells of hamster small intestine. Over the last 10 years the microvilli of intestinal epithelial cells have been investigated intensively for digestive, absorptive and binding functions, and they have been the subjects of several reviews[6,7,8,9,10,11] and one symposium[12]. In this report no attempt will be made to review the subject exhaustively but rather to highlight the important functional aspects and also to discuss some more recent developments.

Historical and Technical Aspects

As early as 1880, Brown and Heron[13] showed that enzymes hydrolysing disaccharides were present mainly in the intestinal mucosa rather than in the intestinal juice and this finding has been repeatedly confirmed[14,15,16,17,18]. Nevertheless, for many years it was generally considered[19,20] that the terminal phase of carbohydrate and protein digestion took place by the action of enzymes secreted into the lumen in the intestinal juice or succus entericus. However, the localization of certain enzymes to the brush border by histochemical[21,22,23] and immunofluorescent techniques[24] suggested a digestive function for this part of the intestinal epithelial cell. Examination of the substructure of the intestinal epithelial cell under the electron microscope[3,25] showed the terminal web as a division between the microvilli and the rest of the cell at which under appropriate conditions the microvillous component might be induced to separate. These conditions were provided by Miller and Crane[5] when they homogenized mucosal scrapings from the small intestines of hamsters in hypotonic EDTA solution, during which procedure the epithelial cells were lysed, liberating the brush borders as intact subunits. By filtration and differential centrifugation these brush borders were obtained relatively free from contamination by other cellular particles, and were found to possess most of the alkaline phosphatase and disaccharidase activity of the mucosa[26]. Similar observations have been made by other investigators[27,28,29,30] and confirmed by different methods[24,31] and have provided impressive evidence against the idea that succus entericus played a major role in terminal digestion in the small intestine.

Most workers have gently scraped everted small intestine or simply expressed the mucosa from the cut end in order to obtain material for the preparation of brush borders. More refined techniques have also been used including vibrating everted intestine on a glass spiral[32], or controlled sectioning of frozen intestine[33]. It is difficult to obtain morphologically distinct brush borders in the presence of less than 2·5 mM EDTA solution[32,34]. The EDTA appears to preserve the microvilli and prevent their osmotic disruption possibly by its chelating effect on Ca^{++}[34]. Isolated brush borders so obtained consist of microvilli with the adjacent terminal web plus a rim of apical cytoplasm. Contamination of brush border preparations by other cell particles can be reduced by using buffered EDTA solution (*p*H 7·4) at a total concentration of 35m0s/litre[34,35,36]. Nuclear material can be removed by precipitation[30,37] or by adsorption to glass fibre[32]. At each stage of the preparation it is essential to check for contaminants by means of phase contrast and electron microscopy, supplemented by biochemical tests for the presence of unwanted cell particles[32,28,38,37,39]. In this way, virtually uncontaminated brush border preparations can be obtained[37], but care must be taken to ensure that any purification procedure adopted does not damage the microvilli or drastically reduce the yield.

Development of Brush Borders

The epithelial lining of the small intestine is being constantly renewed by cell division in the crypts, migration of cells along the sides of the villi and extrusion of these cells at the villous tips[3,40,41]. Cell proliferation appears to be confined to the crypts[42] and this process of repeated cell division is accompanied by evidence of rapid protein synthesis[43,44,45]. As undifferentiated cells emerge from the crypts they develop brush borders which rapidly mature morphologically[46], and considerable protein synthesis must be occurring here in the formation of the microvilli. It has been shown[47,48,49] that turnover of protein is occurring in the microvillous membranes and constituent enzymes throughout the life span of the epithelial cells on the villi, indicating that the brush border is a dynamic digestive surface. On the sides of the villi the activity of brush border enzymes is not the same at each level, but rises from low values near the crypts to peak values at or near the villous tips[50,51,52,53] and this pattern may mirror the physiological function of the epithelial cells along the villi. Brush border enzymes develop at different times during foetal life and have varying levels of activity throughout the small intestine in the adult[55,56,57]. Enzyme levels vary with age[58], and can be induced by diet[59,60,61] or by the administration of glucocorticoids[62,63] and vitamin D[64], but it is uncertain whether any of these variations are of physiological importance. Even more intriguing is the problem of how certain brush border functions are located almost exclusively to one part of the small intestine, eg, enterokinase to the duodenum and proximal jejunum[65]; B_{12}-binding function to the ileum[66].

Enzymatic and Binding Functions of Brush Borders

A list of the enzymatic and binding functions demonstrated in brush border preparations up to the present time is given in the Appendix. Disaccharidases and alkaline phosphatase have been found predominantly in the brush border,

and significant amounts of ATPase have also been located at this site[67,68]. Recently, hamster brush borders have been shown to possess a β-glucosidase which hydrolyses phlorhizin to phloretin and glucose[69]. The significance of this finding is not clear but data concerning the mechanism of sugar transport derived from experiments utilizing phlorhizin should be interpreted with caution.

Most of the leucyl naphthylamidase activity of the small intestine has been found in isolated brush borders[70]. In contrast, only 5-10% of dipeptidase activity has been found in the microvilli[70,71], the majority being present within the epithelial cell (cytosol). However, certain tripeptidases and oligopeptidase activity[35,71] have been found in the brush border in amounts which suggest that they have digestive functions comparable to that of sucrase[72]. Enterokinase, which hydrolyses trypsinogen to trypsin and thus initiates protein digestion, has also been located to the microvilli[65]. Folate deconjugase (pteroyl polyglutamate hydrolase) was originally thought to be present in the microvilli[73] but subcellular fractionation studies suggest a lysomal site[74].

Significant enzymatic activities for cholesteryl esters and retinyl (vitamin A) esters have been detected in isolated microvilli[75,76], suggesting that the release of cholesterol and retinol occurs at this site, as well as in the intestinal lumen, by the action of similar pancreatic enzymes. Glyceride synthesis has been reported to occur in isolated brush borders[77] but this activity may have been due to contamination by microsomal membranes[78]. Sphingomyelinase[79] and phospholipase A[80] appear to be concentrated at the brush border.

The ability of brush borders to bind certain amino acids, eg, L-alanine[81], L-histidine[82], has been demonstrated. Ferrous iron is bound preferentially by brush borders from the proximal intestine[83]. In the case of calcium, it has been suggested from autoradiographic evidence[84] that the brush border is the site of localization of a specific binding protein. In the presence of intrinsic factor, vitamin B_{12} binds to brush borders from distal but not from proximal intestine[66], and this uptake of vitamin B_{12} can be inhibited by antibodies to distal microvilli[85]. Hamster brush borders have also been shown to bind D-glucose[86,87]. Whether these binding functions of isolated brush borders are of physiological significance and concerned in transport in the small intestine is not clear, but it is likely that the binding of iron by proximal, and of vitamin B_{12} by distal, brush borders is related to their absorption at these sites.

The functions of brush borders shown in the Appendix have been demonstrated using the hamster, guinea pig, and rat as experimental animals. There has been little information available concerning human brush borders due to the difficulty of obtaining suitable fresh material at necropsy or at operation, and to the difficulty in preparing brush borders from the small amount of mucosa obtained by peroral intestinal biopsy. Recently[36], some of these difficulties have been overcome and brush borders prepared from the duodenum and from the ileum removed at operation have been shown to possess alkaline phosphatase and disaccharidase activities located to the microvilli.

Transport Functions of the Brush Border

Sugars and amino acids are absorbed from the lumen of the intestine by

active transport processes, which have been located to the brush border of the epithelial cell[31,88,89,90] and which appear to be dependent on the presence of Na^+ ions[91]. Crane[91,92] has indicated a way by which transport of water-soluble substances across the lipoprotein microvillous membrane could occur. The substrate and Na^+ are reversibly attached to a specific membrane receptor or 'carrier' which effects translocation through the membrane. Sugar (and amino acid) can be transported against its concentration gradient, the energy for the transport process being provided, at least in part, by a concentration gradient of Na^+ across the membrane which is maintained by the Na^+ pump effectively removing Na^+ from the epithelial cell. Morphological identification of a 'mobile' carrier in the brush border membrane has not yet been made, though the isolated sucrase-isomaltase enzyme complex appears to possess carrier-like functions as well as hydrolytic properties[93]. The suggestion[94] that trehalase acts as a membrane carrier has been challenged[95].

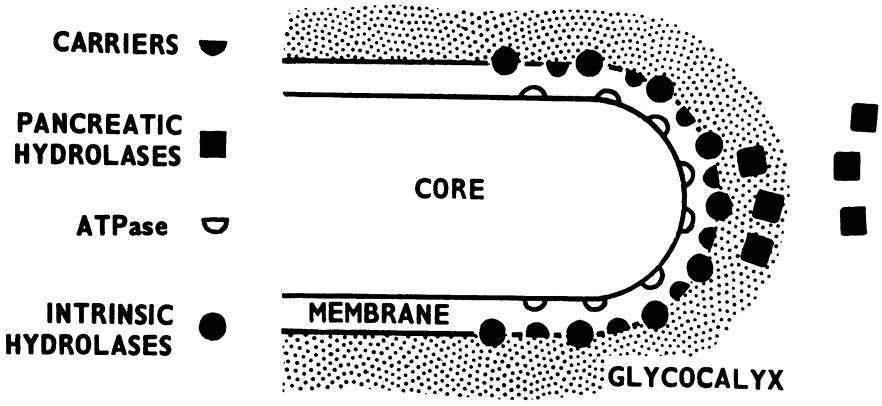

Fig. 1 *Diagrammatic representation of the organization of components of the microvillus (after Crane[9]).*

Isolated brush borders are not suitable for use in direct studies of transport phenomena due to the destruction of their physiological integrity with the rest of the mucosal cell during preparation. However, isolated 'curled' microvillous membranes apparently show transport functions comparable to those found in intact intestine[96].

Structural and Functional Relationships of Brush Border Components

Virtually all the enzymatic activities of isolated brush borders have been found in the microvillous membrane[30,38,97], which constitutes the luminal surface of the intestinal epithelial cell. Histochemically, alkaline phosphatase has been localized to the outer surface[97], and an ATPase to the inner surface[98,99] of the brush border membrane. Much remains to be learned of the orientation of the enzyme components in the microvilli, but a regular, orderly arrangement has been inferred[9] from the results of papain digestion

of the membrane[100], in which the enzymes were removed in a sequential manner, the disaccharidases first, then leucine aminopeptidase, the trehalase and alkaline phosphatase remaining behind.

The transport processes or mobile carriers of the membrane have been located at a site internal to the disaccharidase activity[31,89]. Thus, the membrane components for digestion and absorption are envisaged as forming two layers[7], the outer layer possessing hydrolytic functions and the immediately subjacent inner layer possessing transport or carrier properties. An intimate structural relationship between the components of these two layers has been inferred, especially for sucrase which appears to be activated by Na^+ in a similar way to the Na^+-activation of glucose transport[101]. Recent observations[102] suggest that the hamster possesses two closely related monosaccharide transport systems: one accepting free monosaccharide, and another accepting glucose only from disaccharide hydrolysis, the disaccharidase involved itself contributing transport function.

The close association of the membrane components subserving hydrolytic and transport functions confers a 'kinetic advantage' for the absorption of disaccharides as compared with monosaccharides[7], and thus, glucose is better absorbed when given as sucrose than when given as free glucose[89,103]. Similarly, some amino acids can be absorbed faster when given as peptides than as free amino acids[104,105]. It would appear that substrate hydrolysis by the enzymatic subunit of the membrane releases products which come into such a functionally intimate relationship with the carrier that conditions for optimum transport are attained.

The brush border possesses an enteric surface coat, the 'fuzzy coat' or glycocalyx, which is not adsorbed mucus but is firmly attached to the outer membrane[97]. It appears to be synthesized continuously by the epithelial cell[106,107] and to be an integral part of its structure, but its function has not been clearly defined. By negative staining, knobs or particles about 60 Angstrom units in diameter have been demonstrated on the outer surface of the microvillous membrane[99,108,109]. These particles possess disaccharidase activity[99,109], and it has been suggested that they constitute the glycocalyx[109,110]. However, other experiments localizing sucrase to the membrane after removal of the glycocalyx[111] are against this idea. It is possible that these enzymatic subunits do not reside solely in either the microvillous membrane or the glycocalyx but possess a structure which is common to both components of the brush border.

Pancreatic enzymes can bind loosely to the glycocalyx and their hydrolytic activity can be demonstrated there[112,113,114]. Hydrolysis of substrates by adsorbed pancreatic enzymes at or in the glycocalyx has the potential advantage of releasing products in a situation immediately adjacent to the enzymes and transport processes of the microvilli, but it is unlikely that such attached enzymes play more than a minor role physiologically, in view of the high concentration of pancreatic enzymes found in the intestinal lumen during digestion[115,116]. The activity of digestive enzymes of intestinal origin have been studied when adsorbed onto the mucosal surface[112,117], and it has been postulated that both pancreatic and intestinal enzymes normally act in this way, the overall function being called 'membrane (contact) digestion'. However, the hydrolytic enzymes of the small intestine appear to be integral parts of the brush border structure[9,100,111] and not present merely by adsorption to the mucosal surface.

Brush Borders and Digestion

The mechanisms involved in the integration of the digestive processes are illustrated in Figure 1. Thus, pancreatic α-amylase hydrolyses polysaccharides in the intestinal lumen, and the products are hydrolysed to their constituent monosaccharides by the brush border enzymes before absorption[9,116,118]. Similarly, the digestion of proteins by pancreatic enzymes releases oligopeptides which undergo further hydrolysis to amino acids at the brush border. The membrane peptidases may also liberate dipeptides[72] which can be absorbed directly[104,105] and hydrolysed in the epithelial cell[35,119].

One may ask whether the epithelial cell brush border is the sole location of membrane digestion or whether some could occur in the intestinal lumen? Effete epithelial cells are being constantly desquamated from the villous tips, and intact brush borders derived from them have been recognized in intestinal perfusates[36,120]. It is doubtful whether these brush borders make any significant contribution to luminal digestion, but some hydrolysis of folate polyglutamates could occur in the small intestinal lumen[72] by the action of pteroyl polyglutamate hydrolase released from disintegrated villous tip cells. The brush border enzymes enterokinase[121] and alkaline phosphatase[122] appear in duodenal fluid following secretin-pancreozymin stimulation in humans, and this has been attributed to the solubilizing effect of bile salts on the microvillous membrane[123], and not to direct hormonal stimulation[124,125]. Bile salts can effect the release of enterokinase[123] and alkaline phosphatase[126] from isolated brush borders *in vitro*, but it is not known whether this is a physiological mechanism occurring in the intestine during digestion.

Brush Borders and Disease

The brush border membrane forms a digestive-absorptive surface and alterations in its functional organization provide a rational explanation of certain conditions of impaired digestion and absorption found clinically[6]. Thus, primary malabsorption is due to the congenital or acquired absence or inactivity of a specific functional component of the brush border membrane. Examples of this type are sucrose-isomaltose malabsorption of children, and lactose malabsorption of children and adults in whom the intestinal cell structure appears normal but the specific enzyme is virtually absent[127]. The rare disease of children glucose-galactose malabsorption may represent the specific absence or inactivity of the transport system for glucose[128,129]. Intestinal enterokinase deficiency[121,123,130] can be explained on the basis of a primary deletion of the hydrolytic enzyme at the membrane level. In Hartnup disease[131] and cystinuria[132], certain dipeptides can be absorbed but the absorptive process for the amino acids appears to be lacking. Patients with secondary malabsorption can be considered as suffering a reduction in the total available digestive-absorptive surface as a consequence of other diseases, for example coeliac disease[133]. Here effective treatment of the underlying condition can be expected to bring about restitution of the digestive-absorptive surface leading to recovery of intestinal function.

The development of techniques to isolate and characterize the microvillous membranes from mucosal biopsy specimens obtained perorally can

be expected to yield much information concerning brush border function in man, in health and disease.

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Appendix

Enzymes and Binding Functions of Intestinal Brush Borders

ENZYMES

Maltase
Isomaltase
Sucrase
Lactase
Trehalase
Phlorhizin hydrolase
Alkaline phosphatase
ATPase
Leucyl naphthylamidase
Dipeptidase
Tripeptidase
Oligopeptidase
Enterokinase

Cholesteryl ester hydrolase
Retinyl ester hydrolase

BINDING FUNCTIONS

L-alanine
L-histidine
Iron
Calcium
Vitamin B_{12}
Glucose