Intestinal Mucosal Enzymes in the Diagnosis of Gastrointestinal Metabolic Disease

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ABSTRACT

The small intestinal mucosa is an actively metabolizing, rapidly proliferating, absorptive epithelium with nutritional and homeostatic functions. A metabolic dysfunction of this organ might, therefore, be expected to cause not only gastrointestinal dysfunction, but also systemic symptoms. Several diseases characterized by primary or secondary gastrointestinal metabolic alterations are discussed.

Introduction

The most important functions of the small intestine are unquestionably digestion and absorption. However, a conception of the small intestine simply as an organ in which foods are enzymatically hydrolyzed and from which the end products are transported is an oversimplification. This limited point of view excludes from consideration the uniquely active metabolism of the intestinal mucosa and its relationships with other tissues in the digestive and absorptive processes. Metabolically, the mucosa of the small intestine is the most active tissue in the body. Moreover, the mucosa of the small intestine is the most active tissue in the body. Furthermore, the nutrients consumed in the diet are not usually easily absorbed, nor are they substrates directly usable for normal cellular function. The foods ingested are usually polymers, e.g., starch, neutral fat, and proteins, which require metabolic conversion to the monomers or reconstituted polymers, such as chylomicrons which are transported from the intestine.

The mucosa of the small intestine, moreover, contains distinctive morphological specializations designed to present a maximal digestive and absorptive surface to the intestinal contents. The epithelium is composed primarily of tall columnar cells whose absorptive surface consists of numerous microvilli, which multiply the absorptive surface as much as 40 times over that presented by a flat surface. Furthermore, the mucosal cells are highly differentiated for digestion and absorption. A variety of organelles constitutes the functional specializa-
tion of the absorptive epithelial cells, including terminal web, mitochondria, lysosomes, endoplasmic reticulum, a well-defined supranuclear Golgi complex and, most importantly, the brush border. The brush border, according to Crane, comprises a structurally integrated subcellular organelle specialized for digestion and absorption,—less complex, but clearly of the same functional category as mitochondria. The specific functions of all these organelles in digestion and absorption have not been completely defined, but all have been implicated in gastrointestinal or metabolic disease. Studies of diseases of the human small intestine have been simplified by the development of techniques for obtaining peroral jejunal biopsies. Using this technique, 10 to 15 mg of intestinal mucosa can be obtained in a simple, safe and reproducible manner for direct assay of intestinal mucosal enzyme activity.

The following report reviews a number of conditions in which primary or secondary enzyme abnormalities have been demonstrated in the small intestinal mucosa. Of this group, the most well-characterized are the diseases of the brush border because it is the most thoroughly studied organelle of the epithelial cell. In addition, however, several less well-defined gastrointestinal metabolic disorders will be discussed. Clinical examples from our experience will be used as illustrations when possible.

**Brush Border**

According to Crane and associates, the brush border comprises a structurally integrated subcellular organelle specialized for digestion and absorption. It forms the luminal surface of the epithelial cell and contains the catalysts of digestive and absorptive function organized to provide a kinetic advantage for absorption. It is no longer appropriate to think of digestion and absorption as separate processes because many of the digestive-absorptive functions of the small intestine occur in such close physical proximity and are so cooperatively organized in the brush border, that a distinction between digestion and absorption is artificial.

Biochemical, histochemical and immunological studies have demonstrated high concentrations of many enzymes, including alkaline phosphatase, leucine aminopeptidase, adenosine triphosphatase and disaccharidases in the brush border. Miller and Crane in 1961 first reported the isolation of brush borders from hamster intestine and showed that they contained over 75 percent of the sucrase and maltase activity found in the intestinal homogenates. Following the localization of disaccharidase activity to isolated brush borders, studies were undertaken which also localized peptidase activity to the brush border. Enterokinase (enteropeptidase), an enzyme which converts trypsinogen to active trypsin, was subsequently also demonstrated to be a brush border enzyme.

Currently there are eleven enzymes for which there is sufficient evidence to establish their loci in the brush border. They are oligopeptidase (enterokinase), gamma glutamyl transferase, enteropeptidase, alkaline phosphatase, glucoamylase, maltase, sucrase, isomaltase, lactase, trehalase and phlorizin hydrolase. Thus, the final stages of hydrolysis of dietary oligosaccharides and polypeptides occur in the brush border, from which the monosaccharide and amino acid products are actively transported into the epithelial mucosal cell. The mechanism of monosaccharide and amino acid transfer across the brush border has not been completely defined.

Under normal conditions, the majority of actively transported monomers are de-
derived from the final stages of carbohydrate and protein hydrolysis on the luminal surface of the brush border. These products of brush border enzyme activity, in comparison to similar monosaccharides and amino acids within the intestinal lumen, have a kinetic advantage for transport. According to Faust, the monomer products move "downhill" through the plasma membrane by facilitated diffusion. He concludes that the intestinal brush border must be considered a multifunctional organelle in any explanation of the molecular basis of small intestinal sugar and amino acid transport.11

**Carbohydrate Digestion and Absorption**

Dietary carbohydrate accounts for about half of the calories ingested by man and consists principally of starch, glycogen, sucrose and lactose. Starch accounts for approximately 60 percent, sucrose for 30 percent and lactose for 10 percent of the ingested carbohydrate. Starch and glycogen are enzymatically hydrolyzed to oligosaccharides in the lumen of the duodenum and upper jejunum. Salivary and pancreatic amylases act on the interior alpha 1,4 bonds of starch, but cannot attack the outer linkages or the alpha 1,6-branch points and have relatively little specificity for the 1,4-links adjacent to the branch points.

Thus, the final products of amylase action are alpha 1,4-linked oligosaccharides (maltose and maltotriose) and large oligosaccharides containing five or more glucose units and one or more 1,6-branching links. Enzymatic hydrolysis to oligosaccharides occurs within minutes in the duodenal lumen.17,24

The final digestion and absorption of glucosyl oligosaccharides produced from starch occurs in the intestinal epithelial cells. Oligosaccharidases are located in the brush border membranes of these cells and are available to substrates at the cell surface. These enzymes are commonly called disaccharidases, but most are actually oligosaccharidases, since they may hydrolyze sugars containing three or more hexose units.17,24

Brush border membranes contain a number of oligosaccharidases: one or two maltase-glucosaminylases, one sucrase, one isomaltase, one trehalase, one lactase and one phlorizin hydrolase.17 The major substrates for these enzymes are the three most common dietary disaccharides,— lactose, sucrose and maltose. As disaccharides are poorly absorbed, they must be hydrolyzed to component monosaccharides, which are readily absorbed.1

The total or partial deficiency of one or more disaccharidases results in a failure of hydrolysis of its specific substrate, which remains in the intestinal lumen and acts to produce abdominal pain, distension, flatulence and diarrhea.1

The disaccharidase deficiencies are the most well-characterized of the brush border membrane diseases, and include congenital lactase deficiency, adult hypolactasia, sucrase-isomaltase deficiency, and trehalase deficiency. The most common pathologic condition among the disaccharidase deficiencies is sucrase-isomaltase deficiency, which has an incidence in the general population of approximately 0.2 percent. Hypolactasia, or lactase deficiency, is actually more common than sucrase-isomaltase deficiency, occurring in 15 to 70 percent of adults. However, hypolactasia is a physiologic condition in adults and cannot be considered pathological. Much less common disorders are congenital hypolactasia and trehalase deficiency.1,17

The first two patients listed in table I are children with disaccharidase deficiency. The first is an infant with lactase deficiency, who presented with malnutrition, diarrhea, and failure to thrive. She may represent a case of congenital hypolactasia. The second is a juvenile with sucrase-isomaltase deficiency. He is
Small Intestinal Mucosal Enzyme Activities in Patients with Gastrointestinal Symptoms

**TABLE I**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>I.U./Gm Protein</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sucrase</td>
<td>Lactase</td>
</tr>
<tr>
<td>S.J.F.</td>
<td>Lactase deficiency</td>
<td>103.12</td>
<td>0</td>
</tr>
<tr>
<td>E.H.</td>
<td>Sucrase deficiency</td>
<td>0</td>
<td>16.97</td>
</tr>
<tr>
<td>K.M.</td>
<td>Celiac sprue</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>R.M.</td>
<td>Celiac sprue</td>
<td>16.39</td>
<td>0</td>
</tr>
<tr>
<td>W.C.</td>
<td>Fructosemia</td>
<td>14.94</td>
<td>25.19</td>
</tr>
<tr>
<td>D.L.</td>
<td>Galactosemia</td>
<td>81.89</td>
<td>17.04</td>
</tr>
<tr>
<td>Normal</td>
<td>(28-60)</td>
<td>(11-20)</td>
<td>(122-210)</td>
</tr>
</tbody>
</table>

*Method of Dahlquist. µMoles/min/mg protein.

<table>
<thead>
<tr>
<th>Other</th>
<th>*Aldolase B, 0.13 (Normal 1.0)</th>
<th>*Gal-1-P Uridyl Transferase, 0.4 (Normal 8.9-15.8)</th>
</tr>
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<tbody>
<tr>
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a healthy 14 year-old whose only complaint was chronic watery diarrhea.

**Protein Digestion and Absorption**

In addition to dietary protein, the gastrointestinal tract receives daily approximately 60 g of endogenous protein from digestive secretions, desquamated cells, and plasma. Epithelial cells shed from the free ends of villi are cyto-lyzed, their contents released and digested, and their constituents reabsorbed from the lumen. All proteins are hydrolyzed by pancreatic and mucosal proteolytic enzymes to yield amino acids and small peptide end-products for absorption. This suggests that the mucosal cells have the capacity to remove and utilize amino acids for immediate protein synthesis directly from among those passing through the cell during the absorptive process. The mucosa also functions to maintain a constant intraluminal amino acid concentration and pattern, which does not change with time along the length of small intestine. The maintenance of intraluminal amino acid homeostasis prevents wide fluctuations in blood amino acid concentrations, thus preventing the deleterious effects of ingesting imbalanced amino acid preparations.

The digestion of protein within the gastrointestinal tract results in the degradation of high molecular weight, non-diffusible proteins to small diffusible compounds, which are absorbed readily from the intestine. Protein digestion also destroys the biological specificity that makes it antigenic. Under normal conditions, exogenous and endogenous proteins are hydrolyzed in the gastrointestinal tract by enzymes elaborated by the stomach, pancreas, intestinal mucosa and microflora. Among these, the major burden for protein digestion is assumed by pancreatic and intestinal proteases. Hydrolysis by pancreatic enzymes occurs in the small intestinal lumen and
produces neutral and basic amino acids and oligopeptides. The oligopeptides are further hydrolyzed by oligopeptidases localized in the brush border membrane and the cytoplasm of the intestinal cell. Brush border oligopeptidases hydrolyze primarily small peptides composed of neutral amino acids. Hence, only 20 percent of the peptides crossing the brush border are hydrolyzed there. The remaining 80 percent are metabolized by soluble intracellular oligopeptidases, which are specific for peptides containing imino acids (proline, hydroxyproline, glycine) and dicarboxylic amino acids (aspartic and glutamic). A small quantity is transported through the cytoplasm intact, cannot be metabolized by peripheral tissues and is excreted by the kidney.13,16

Once the amino acids have accumulated in the intestinal epithelial cells, they may follow one of two major pathways. The majority diffuse outside the cell to the intestinal capillaries, which deliver them to the peripheral sites of utilization via the hepatic portal system. A significant portion is used by the epithelial cell for protein synthesis, while a lesser fraction is transaminated and enters the tricarboxylic acid cycle of the mucosal cell.12,13,14,16,27

The relative significance of intestinal versus pancreatic protein digestion is difficult to assess. Exclusion of pancreatic secretions by duct ligation or pancreatectomy appears to be detrimental but not fatal.38 There is evidence, however, that deficiencies of intestinal peptidases may also have a deleterious effect. Patients with celiac sprue, for example, have reduced levels of intestinal peptidases but normal pancreatic function, and demonstrate delayed protein digestion and absorption.14,38

The only primary peptidase deficiency of clinical significance in humans is enteropeptidase or enterokinase deficiency. Enterokinase, like the oligosaccharidases and peptidases, is localized to the brush border of the intestinal epithelial cell.7,25 It initiates the pancreatic digestion of protein by the proteolytic conversion of trypsinogen to trypsin on the surface of the epithelial cells. Trypsin, in turn, activates the other pancreatic proenzymes, chymotrypsinogen and procarboxypeptidase. Enterokinase deficiency, therefore, results in functional deficiencies of trypsin, chymotrypsin and carboxypeptidase. Thus, although enterokinase deficiency is a primary intestinal brush border disease, its clinical presentation may be indistinguishable from pancreatic insufficiency. Patients present with diarrhea, failure to thrive, anemia, hypoproteinemia and steatorrhea in early infancy.41 Treatment consists of pancreatic enzyme replacement.

Small Intestinal Adaptation

Adaptability is a fundamental property of any biologic system. The environment of the intestine is composed, in large part, of the food one ingests, and dietary composition has the capacity to influence intestinal enzyme activities. For example, enzymes of carbohydrate digestion and metabolism can be regulated by dietary sugars.34 Furthermore, rats depleted of protein for three weeks were reported to absorb amino acids from hydrolyzed beef blood serum more rapidly than rats fed a 19 percent protein diet and rats adapted to an 88 percent casein diet absorbed amino acids more rapidly than rats adapted to a 13 percent diet.38,39,45 Moreover, investigations in rats indicate that intestinal brush border enzymes and transport systems demonstrate diurnal rhythms, and that the synchronizer for these fluctuations in activity is the time of feeding. The activities of these systems are highest around feeding time, but there is significant variability with relation to feeding.38,40
The activities of sucrase and maltase in humans vary with dietary carbohydrate. Sucrose feeding increases mucosal sucrase and maltase activities significantly within two to five days but does not change lactase activity. The removal of sucrose results in a decrease in activity within two to five days.\textsuperscript{34,40} Fructose has been shown to produce the same effect in normal volunteers and in a patient with sucrase-isomaltase deficiency.\textsuperscript{34} It has been postulated, therefore, that the fructose is the active component of the sucrose molecule. Its effect occurs at the crypt cell level and becomes apparent once the cell matures. Oral fructose administration to a child with sucrase-isomaltase deficiency produced a fourfold increase in sucrase activity and an improvement in sucrase tolerance.\textsuperscript{34} The presence or absence of lactose in the diet, however, has no effect on lactase activity.\textsuperscript{34}

Studies in rats and humans indicate that glucose, fructose or galactose feedings cause an increase in the glycolytic enzyme activities of the jejunum mucosa, which is a function of the sugar ingested.\textsuperscript{34} The enzymes responding are determined by the specific dietary carbohydrates: Hexokinase was highest on a glucose diet; fructokinase, fructose-1-phosphate aldolase and pyruvate kinase activities were highest on fructose; and galactose metabolizing enzymes were highest on galactose. These enzyme responses occurred within hours, implying that their adaptation occurred at the villus epithelial cell rather than at the crypt level.\textsuperscript{32,34}

The observation of mucosal glycolytic enzyme response to folate therapy in a patient with tropical sprue prompted a study of the effect of folate in normal adults, in whom significant increases in jejunal glycolytic and gluconeogenic enzymes were also demonstrated.\textsuperscript{35,34} The increase in activity occurs within hours after physiologic or pharmacologic doses of oral folate and returns to normal once folate is discontinued. Oligosaccharidase activities are not affected. The mechanism of the folate effect is not known. However, in a patient with congenital formiminotransferase deficiency in whom folate metabolism is blocked, the normal glycolytic enzyme adaptive response to folate was not observed, which implicates folic acid in the regulation of carbohydrate metabolism.\textsuperscript{33,34}

There are chronic gastrointestinal complaints for which no enzyme deficiency state or other pathologic lesion can be demonstrated. Selected patients from this group, therefore, were studied to determine glycolytic enzyme response to carbohydrate feeding. They were patients with chronic, non-specific, gastrointestinal complaints, usually including diarrhea, whose symptoms worsened on high carbohydrate diets and who had normal gastrointestinal function by conventional evaluation. High carbohydrate diets in some of these patients failed to induce increases in jejunal glycolytic enzyme activities, and they were characterized as having a “maladaptation syndrome”.\textsuperscript{34} They improved with low carbohydrate diets. Similar symptoms were produced in normal subjects by the feeding of carbohydrate-free diets for three days followed acutely by oral glucose loading.\textsuperscript{35,33,34}

Celiac Sprue
Gluten-sensitive Enteropathy

Celiac sprue is a disease characterized by multiple biochemical degradations and abnormalities of brush border and lysosomal enzymes.\textsuperscript{28,31} The pathologic lesion is characterized by atrophy of the intestinal villi and malabsorption resulting from toxic effect of gluten, a wheat protein, or gliadin, one of its fractions. The mucosal abnormality results in a loss of absorptive surface and the malabsorp-
tion of dietary components. Villus atrophy is induced by the ingestion of the gliadin; the removal of gliadin results in a return of normal mucosal structure and function. The re-introduction of gliadin is followed within hours by epithelial cell damage, thickening of the basement membrane and the appearance of inflammatory cells in the submucosa. This epithelial reaction is so rapid and consistent that it has become a diagnostic feature.

Two primary hypotheses are generally accepted to explain the pathogenesis of celiac sprue. The first postulates the deficiency of a hypothetical mucosal peptidase, which is essential for the breakdown of gluten. Such a defect would result in incomplete digestion of gliadin and the accumulation of fractions directly toxic to the epithelial cells. The second is based on the observation that the reintroduction of gluten causes an acute local inflammatory reaction and an increase in synthesis of IgA and IgM in the mucosa, which is at least partly attributable to increased antigliadin antibody production and the formation of immune complexes in the intestinal mucosa. Gliadin, or one of its metabolites, is thought to act as an immunogen, which causes a local cytotoxic reaction mediated by immunologic processes. Patients with celiac sprue are thought to have an abnormal susceptibility to react immunologically to gliadin or its metabolites in intestinal mucosal sites.

Small intestinal biopsies from untreated patients with gluten-sensitive enteropathy demonstrate decreased brush border enzyme activities, which return to normal after gluten withdrawal. Comparison of lysosomal enzyme activities of normal human intestinal mucosa and mucosa from patients with celiac sprue demonstrates increased activities of acid hydrolases in the patients, which returned to normal after treatment. Townley emphasized similarities between celiac sprue and sucrase-isomaltase deficiency, the latter a well-defined, autosomal recessive inborn error of metabolism associated with sucrase malabsorption and diarrhea.

Cornell and Townley separated a peptic-tryptic digest of gliadin into ten fractions by column chromatography and incubated each fraction with a homogenerate of duodenal mucosa. Mucosal homogenates from patients with celiac sprue failed to digest one fraction, while the control homogenates digested all ten completely. Additional studies using cultured fibroblasts and intestinal mucosal cells obtained from children with celiac sprue demonstrated severe degenerative changes after incubation with gluten. In contrast, fibroblasts and mucosa showed no changes when incubated in a gluten-free medium; fibroblasts from normal controls showed no degeneration when incubated with gluten. These observations were thought to be consistent with the absence of specific peptidase in both skin and intestine. Rogentine and associates, however, using an organ culture model for celiac sprue (gluten-sensitive enteropathy), concluded that the pathologic injury is mediated by an endogenous mechanism of tissue toxicity and that there is little evidence in support of an enzyme deficiency and much against it.

The third and fourth patients listed in table I are brothers with celiac sprue prior to treatment. Both presented with significant growth retardation, but only K.M. had diarrhea, despite marked decreases in all disaccharidase activities measured in both children.

Acrodermatitis Enteropathica

Acrodermatitis enteropathica is a rare, inherited, autosomal recessive disorder,
which appears with weaning. Symptoms are invariably precipitated by the ingestion of cow's milk or other foreign protein, while breast milk results in prompt remission. Diagnosis is based on the observation of alopecia, erythematous and vesicular-pustular dermatitis in an infant with diarrhea. Biochemically, hypolipoproteinemia and tryptophanuria can be demonstrated.\textsuperscript{18,22} Zinc deficiency is a consistent finding, and the clinical abnormalities may be attributed to zinc deficiency.\textsuperscript{18,22,23} The three most common features,—acrodermatitis, diarrhea, and alopecia in animals. Anorexia and failure to thrive are prominent features of both zinc deficiency and acrodermatitis enteropathica.\textsuperscript{18}

Moynahan has demonstrated reduced succinic acid dehydrogenase and leucine amino-peptidase activity in jejunal biopsies from patients with this disease.\textsuperscript{21} Ultrastructural and histochemical studies of duodenal and jejunal biopsy material and the favorable response when breast milk is made the sole dietary protein source prompted Moynahan and co-workers to postulate the deficiency of an enzyme,—an oligopeptidase, produced by the intestinal epithelial cell, which hydrolyzes a small peptide produced during the hydrolysis of all proteins except human milk protein. The absence of the oligopeptidase permits the accumulation of a toxic peptide, which interferes with zinc absorption, and inhibits the activity of mitochondrial enzymes in the intestine. Its absorption into the blood causes psychic changes, eczema and abnormal keratinization.\textsuperscript{22}

Until recently, the accepted treatment for acrodermatitis was oral diiodohydroxyquin. Other therapies reported to be effective were dietary restriction of tryptophan to breast milk as the sole protein source.\textsuperscript{22} The primary defect is not known. Any hypothesis, however, must explain all biochemical and enzymologic studies, and the therapeutic response to oral zinc, diiodohydroxyquin, and breast milk. Hambidge suggests that the primary abnormality is a congenital defect of the gastrointestinal tract, which causes zinc malabsorption and zinc deficiency.\textsuperscript{18} Moynahan postulates the absence of an intestinal oligopeptidase.\textsuperscript{22}

Conclusion

It is apparent that metabolic dysfunction at multiple levels in the intestinal epithelial cell may cause gastrointestinal symptoms. Obviously, diseases of the brush border membrane cause diarrhea. Second, diseases of unknown etiology,—such as celiac sprue and acrodermatitis enteropathica, may be characterized by alterations in epithelial cellular metabolism, morphologic changes, and malabsorption.\textsuperscript{22,28} Finally, the maladaptation syndromes provide evidence that alterations in intestinal metabolism not directly related to absorption may cause gastrointestinal symptoms.\textsuperscript{34} The latter observation is supported by our findings in the last two patients in table I. These infants, one with fructosemia and the other with galactosemia, both demonstrated severe diarrhea as a prominent component of their presenting symptomatology.\textsuperscript{15,30}

References


