Small bowel review: Part I

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ABR THOMSON, JA THOMSON, MJ ROPELESKI, GE WILD. Small bowel review: Part I. *Can J Gastroenterol* 1996;10(3): 179-189. Major scientific advances have been made over the past few years in the areas of small bowel physiology, pathology, microbiology and clinical sciences. Over 1000 papers have been reviewed and a selective number are considered here. Wherever possible, the clinical relevance of these advances have been identified. There have been a number of important and/or interesting developments in the past year that have clinical significance.

Key Words: *Brush border membrane, Diabetes mellitus, Glutamine, Immunology, Lipids, Peptides, Review, Small bowel*

L'intestin grêle en bref : partie I

RÉSUMÉ : De grands progrès scientifiques ont été accomplis au cours des quelques dernières années dans le domaine de la physiologie, de la pathologie, de la microbiologie et des sciences cliniques appliquées à l'intestin grêle. Plus de 1 000 articles ont été passés en revue et certains sont abordés ici. Dans la mesure du possible, la pertinence clinique de ces progrès a été identifiée. Un certain nombre de découvertes importantes ou intéressantes ont eu une portée clinique notable au cours de l'année écoulée.

Major scientific advances have been made over the past few years in the areas of small bowel physiology, pathology, microbiology and clinical sciences. An in-depth review of the small bowel is presented. Topics discussed are the brush border membrane (BBM), resection and adaptation, glutamine, peptides, sugar digestion and absorption, diabetes mellitus (DM), immunology and transplantation, and lipids.

BRUSH BORDER MEMBRANE

The BBM interfaces with a complex external milieu including dietary substances, antigens, microorganisms and digestive enzymes. The cellular and molecular processes associated with maintenance of the structural and functional properties of the BBM remain a major theme in modern cell biology (1). The restriction of hydrolases and specific nutrient transporters to the BBM allows the efficient digestion and absorption of carbohydrates. A summary of some of the

features pertaining to the expression of specific BBM enzymes will be presented.

Sucrase-isomaltase (SI) is an enterocyte-specific BBM hydrolytic enzyme that breaks down dietary sucrose into fructose and glucose. There is little SI activity in crypt cells; peak activity is detected at the base and middle segments of the villus. In addition to the crypt-villus gradient of SI activity, a gradient of diminishing SI activity exists along the longitudinal (ie, duodenal-to-ileal) axis. Recent studies in the rodent as well as in the human small intestine have failed to detect appreciable levels of SI mRNA in crypt cells, whereas there is a prominent appearance of SI mRNA in cells at the crypt-villus junction. SI mRNA is abundant in cells from the base to the mid-villus region, and then SI mRNA decreases as enterocytes move from the mid-villus towards the villus tip. Transcriptional activation likely oc-

An abbreviated list of references appears at the end of this paper. A full list is available from the corresponding author

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curs at the crypt-villus junction, and it appears that the expression of the SI gene as enterocytes emerge from the crypts is regulated primarily at the level of mRNA accumulation (2). Enterocyte-specific transcription of the SI gene is regulated by an evolutionarily conserved promoter that extends approximately 180 base pairs upstream of the transcription start site (3). There are three nuclear protein-binding sites within the SI promoter, each of which acts as a positive regulatory element for transcription in intestinal cell lines. These novel DNA-binding proteins may play an important role in regulating intestine-specific transcription of the SI gene.

Many enzyme proteins that define the differentiated phenotype of enterocytes are expressed primarily in cells located on the villus; these include disaccharidases, fatty acid binding proteins (FABPs) and xenobiotic metabolizing enzymes. Localization of their corresponding mRNAs by *in situ* hybridization suggests that transcriptional activation of genes may be an important mechanism for enterocyte differentiation as cells move from the crypt to the villus compartments. However, there may also be important differences in post-translational processing of these enzyme proteins. For example, variations in the post-transcriptional processing of SI between proximal and distal small intestine have been observed, which may explain the differences in SI activity in these two segments of the small intestine (4-6). Finally SI expression is at least in part developmentally regulated, but dietary factors and conditions such as diabetes or starvation also influence SI activities (4). The conversion of this digestive protein into the alpha and beta units is differentially catalyzed by trypsin, trimming the subunits to unequal amounts and involving a complex series of cleavage steps (7).

Lactase-phlorizin hydrolase (LPH) contains two enzymatic activities, lactase and phlorizin hydrolase, and is the principal carbohydrase of the infant mammalian small intestine. This glycoprotein is restricted to the BBM where it digests lactose to the readily absorbable monosaccharides glucose and galactose. The primary structure of human and rabbit LPH has been deduced from cDNA cloning. Human LPH complex comprises a single polypeptide chain that contains a cleavable signal peptide sequence. In contrast to SI, the catalytic sites for LPH are located on the same polypeptide chain, and this process is catalyzed by an enterocyte-specific mechanism, not by pancreatic protease. LPH processing occurs subsequent to intracellular sorting along the secretory pathway. However, this processing is not an obligate requirement for expression of full LPH activity at the cell surface (8).

LPH is synthesized in the endoplasmic reticulum as a precursor molecule with a relative molecular weight in humans of 205,000 Da. Synthesis is followed by post-translational changes, including proteolytic conversion to the 150,000 Da mature BBM form of the protein, which is bound to the lipid bilayer by a hydrophobic anchor sequence. There are two forms of BBM LPH in human intestine: an N-glycosylated molecule and an N- and

O-glycosylated molecule. The N- and O-glycosylated forms exhibit almost a fourfold maximal activity ($V_{\max}$) rate and are therefore enzymatically more active than the N-glycosylated enzyme (9). Since O-glycosylation is a Golgi event, the generation of the two mezoforms is likely linked to differentiation of intestinal cells.

The pattern of enterocyte differentiation along the villus depends on the age of the cell and of the animal, and varies along the longitudinal axis of the intestine. There is a sharp decline of lactase activity in weaning rats and rabbits, but after weaning there remains residual lactase protein. Low lactase levels in the adult result from alterations in the genetic expression of LPH. Development patterns of LPH expression are most likely regulated at the level of gene transcription (10). However, thyroxine has also been implicated in the regulation of lactase ontogeny by post-transcriptional mechanisms, including altered processing and increased degradation of the protein (11). In the proximal intestine uniform immunohistochemical staining of lactase is seen, but in the distal intestine a patchy pattern of lactase staining is observed (similar to that seen in adult hypolactasic humans), which indicates a heterogeneity of enterocyte differentiation (12).

The BBM enzyme dipeptidyl peptidase IV (DPP IV) has also been examined as a marker of enterocyte differentiation in a variety of physiological states. The differentiation-dependent expression of DPP IV in rat jejunum is primarily controlled at the mRNA level (13). Compared with SI and LPH, DPP IV expression does not appear to be influenced by metabolic or dietary factors.

Recent work has focused on the spatial, temporal and cellular patterns of gene expression in the developing intestine. A 'hard-wired' differentiation program is encoded in the intestinal endoderm. Transcriptional activation in fetal gut epithelium is a complex and dynamic process that is spatially regulated along the horizontal axis of the intestine. Initiation of transcription of rat liver FABP (L-FABP) and the cellular expression of apolipoprotein (apo) A-IV mRNA is linked to villus morphogenesis and to histological cytodifferentiation (14).

Mosaicism occurs spontaneously in rats during late gestation, as well as in adult human small intestinal villi as indicated by the pattern of lactase protein expression in some persons with adult-type hypolactasia. There is also variability in the expression of blood group A specificity of the BBM components; this likely reflects subtle differences in the pattern of differentiation between monoclinally derived epithelial cells on the villus (15).

Lactase deficiency is a relatively common clinical condition. In some populations, such as northern Europeans and their descendants, adults commonly retain relatively high lactase levels, but in most of the world's population the LPH level falls with maturation. In adult lactase deficiency there are two phenotypic patterns. Phenotype I deficiency is characterized by a decrease in the rate of synthesis of LPH precursor, and this deficiency appears to result from a decrease in mRNA abundance. Post-translational processing is normal

in these individuals, although in some there may be retardation of intracellular transport and maturation of the LPH precursor. Subjects with phenotype I deficiency show reduced and more patchy LPH reaction over the BBM. In phenotype II lactase deficiency there are both patchy staining by immunocytochemical distribution of LPH and intracellular accumulations of immunoreactivity within the apex of enterocytes (mainly on the upper half of villi) (16). In phenotype II, there is altered post-translational processing of the precursor protein and there may be a partial block in transport from the endoplasmic reticulum to the Golgi apparatus.

It has long been accepted that mucin is an important constituent of the intestinal mucosal barrier. The gene for a rat intestinal mucin-like peptide is expressed by goblet cells of rat intestine and colon. This goblet cell mucin protects and lubricates the intestinal tract. Cholinergic or vagal stimulation increases intestinal vascular and epithelial permeability. This results in the passage of serum proteins into the intestinal lumen, possibly by opening tight junctions and paracellular pathways (17). The extrinsic vagus nerve does not regulate rabbit jejunal mucin secretion, and the cholinergic control of intestinal goblet cells is implemented entirely by the intrinsic enteric nervous system.

The carbohydrate moieties of mucin glycoproteins can bind to bacterial cell surfaces and thereby influence bacterial colonization by interfering with bacteria-epithelial cell receptor interactions. Intestinal hydrophobicity is also determined by mucin, which in turn can be altered by the state of enterocyte maturation, regional differences and mucosal inflammation. These changes in physical properties are likely due to alterations in the mucus layer overlying the intestinal epithelium (18). Mucin secreted in response to an injury insult may contribute to adaptive cytoprotection of the small intestine by delaying attachment of the irritant to the BBM of mucosal epithelial cells (19).

RESECTION AND ADAPTATION

After resection of the small intestine there are a variety of structural and functional changes that occur in the remaining intestine. Within 48 h of resection of a portion of the small intestine there is an increase in the production of crypt cells, villus enlargement and enhanced absorption. The changes in the absorptive capacity of the remaining small intestine after resection may be examined in the context of the adaptive modulation hypothesis put forth recently by Kasper et al (20). According to this hypothesis, a transporter is repressed when its biosynthetic and other costs of maintenance exceed the potential benefits that it provides. Transporters of monosaccharides or amino acids worth calories are upregulated by their substrates, or by dietary carbohydrate or protein, respectively. Water-soluble vitamin transporters are downregulated by their substrate and upmodulated in deficiency. Adaptive modulation by monosaccharides or by amino acids appears to be a robust hypothesis, but while the transport of some minerals and vitamins is in accordance with the adaptive modulation hypothesis, the intestinal transport of choline, pantothenic acid and ascor-

bic acid seems not to be increased in their deficiency states (20). It is possible that vitamin transport may be modulated only if it is by a carrier-mediated pathway.

While the specific signals controlling this intestinal adaptive response are incompletely understood, they are thought to include factors such as luminal nutrients, pancreaticobiliary secretions and humoral factors. The key mediators of adaptive bowel growth include endocrine and paracrine factors, as well as luminal nutrients. Gut glucagon-like immunoreactants may be mediators of this adaptive hyperplasia. Enteroglucagons are composed predominantly of glucocentins and oxyntomodulins, peptides derived from an mRNA encoding proglucagon, which contains additional glucagon-like peptides. The elevation of ileal proglucagon and ornithine decarboxylase (ODC) mRNAs within 12 h after jejunoileal resection in rats and before refeeding shows a nutrient-independent component of the adaptor response (21). Ileal proglucagon mRNA and plasma glucagon-like peptide increase following resection and refeeding, suggesting that in addition to the enteroglucagons other intestinal proglucagon-derived peptides may be mediators of adaptive growth. Other potential candidates for feeding-associated ODC activation include insulin and prostaglandin I₂ (22).

Peptide products of L cell-derived proglucagon, collectively termed enteroglucagon, are thought to be enterotrophic. In addition to proglucagon, the L cells also synthesize peptide tyrosine tyrosine (PYY), which has also been postulated to play a role in mucosal adaptation. Ileal proglucagon mRNA levels increase during ileal adaptation in rats, and four days after resection there is an increase in the intensity of both the proglucagon and the PYY mRNA (23). This parallel increase in PYY mRNA implies an L cell rather than a proglucagon gene-specific response. When ODC activity is blocked by difluoromethylornithine there is a blunted increase in mRNA for enteroglucagon and for PYY (24); this indicates that the response of enteroglucagon and of the PYY genes to massive small bowel resection depends on polyamine biosynthesis.

Food in the intestinal lumen is important to maintain normal small bowel structure and function. While total parenteral nutrition (TPN) may be used to maintain total body energy and nitrogen values, intestinal hypoplasia develops. Hexose absorption may be reduced, whereas the absorption of glycine or glycylglycine is not affected. Not all nutrients are malabsorbed with TPN treatment and not all BBM glycoproteins are affected (25). In rats, the TPN-associated gut atrophy can be attenuated by adding to the intravenous infusate gut-specific fuel such as glutamine, ketone bodies and short chain fatty acids. Providing glutamine in the form of glycylglutamine attenuates the histological changes in the intestine seen with systemic sepsis and with TPN, which is associated with increased protein synthesis in the intestinal mucosa.

After an 85% shortening of the rat gut, there are changes in the total nonspecific activities of BBM sucrase and alkaline phosphatase, and the villi become hyperplastic. The enzymatic markers of functional differentiation are not equally

affected by this adaptational hyperplasia (26). This suggests that the functional adaptation is not simply a reflection of increased immature cells lining the villus cell epithelium. However, the relationship between enzyme activity and the cellular events that dictate the overall expression of a particular enzyme (ie, transcription, translation, etc) awaits definition. The availability of appropriate molecular probes for BBM enzymes will assist in elucidating the relative contribution of post-transcriptional events to the expression of enzyme markers associated with villus enterocytes. Three weeks after a 90% small bowel resection in rats, the transepithelial potential differences after glucose or after theophylline administration are lower in resected versus in sham-operated rats. However, by 10 weeks after resection the potential differences between the resected and nonresected animals are lost, even though the length of the villi in the resection group is increased (27). Thus, *in vivo* electrophysiological measurements may be useful to monitor functional adaptation after massive small bowel resection in the rat, but the early phase of adaptation *in vivo* does not correlate with histological changes.

A state of hyperproliferation (not hypoproliferation) occurs in the epithelial cells of the stomach, the small intestine and the large intestine of stable-fed, aged rodents. Compared with young mature rodents, all BBM hydrolase activity may be reduced with age, secondary to bacterial contamination of the small intestine (28).

GLUTAMINE

Glutamine is the primary metabolic fuel of the small intestine, and enteral or parenteral administration of this amino acid enhances intestinal mucosal regeneration after injury. L-glutamine stimulates both electrogenic and electrically silent absorption of sodium, and when given with D-glucose, L-glutamine stimulates metabolism and sodium chloride absorption in piglet jejunum. This occurs possibly by the glutamine stimulating parallel antiports (ie, Na^+/H^+ and $\text{Cl}^-/\text{HCO}_3^-$) in the BBM (29).

Glutamine-enriched TPN attenuates the gut atrophy associated with TPN, and the addition of glutamine to the TPN solution maintains both B and T cell populations at levels similar to those in chow-fed rats (30). Glutamine metabolism by the gut provides a major source of energy for the mucosal cells and ensures adequate amounts of glutamine amide nitrogen to support nucleic acid biosynthesis by the mucosal cells.

BBM transport of glutamine is decreased in septic patients and in endotoxemic rats; soon after endotoxemia BBM glutamine uptake is increased, while consumption of glutamine across the basolateral membrane (BLM) is decreased (31). Patients receiving glutamine-supplemented TPN after bone marrow transplantation have improved nitrogen balance, a diminished incidence of clinical infection, lower rates of microbial colonization and a shortened hospital stay compared with patients receiving standard parenteral nutrition. A potential role for TPN supplementation with glutamine is emerging.

After operative stress there is accelerated release of glutamine from skeletal muscle and a concomitant increase in glutamine utilization by the intestinal tract. Metabolism of glutamine by the small intestinal mucosal cells is highly dependent on the glutaminase enzyme. Dexamethasone increases glutaminase-specific activity as well as glutaminase mRNA, and the increase in message precedes the increase in glutaminase activity, consistent with *de novo* RNA synthesis followed by protein synthesis (32).

Intestinal atrophy is prevented by luminal food proteins, but not by the equivalent amino acids. Epidermal growth factor (EGF) and transforming growth factor- α (TGF- α) are secreted into the intestinal lumen. Fasting human jejunal juice destroys EGF and TGF- α , but EGF (not TGF- α) is preserved when the milk protein casein or an enzyme inhibitor is present in the lumen (33). Elemental diets are ineffective in preserving EGF activity. The addition of enzyme-inhibiting protein such as casein to elemental diets may preserve intestinal integrity and function.

The barrier function of the intestine may be compromised in patients with sepsis, with this increased mucosal permeability associated with the translocation of enteric bacteria and their endotoxins. Septic rats infused with glutamine-supplemented parenteral nutrition with or without EGF treatment survive sepsis better than septic rats given parenteral nutrition (34).

PEPTIDES

The human enteric nervous system develops from the craniocaudal migration of neuronal precursors accompanied by infiltration/colonization of the gut wall by nerves passing through the muscle towards the mucosa. Autonomic nerve fibres are present in different lymphoid organs. Postganglionic sympathetic nonadrenergic nerves are thought to be the functional link between the immune and the nervous systems, and are proposed to act as neuroimmunomodulators. Somatostatin immunoreactive nerve fibres are present in the Peyer's patches of cat intestine, and these terminals are in close contact between leukocytes and plasma cells (35). Receptors for somatostatin, vasoactive intestinal polypeptide (VIP) and substance P have been identified on various types of human and murine lymphoid cells *in vitro*. Human gut-associated lymphoid tissues are positive for somatostatin receptors, and the mucosa, submucosa and myenteric plexus also contain somatostatin receptors (36). This suggests that the germinal centres of the gut-associated lymphoid tissue are a site of action of somatostatin, and that somatostatin and other neuropeptides modulate both cellular and humoral immune responses of gut-associated lymphoid tissue.

The somatostatin analogue octreotide is effective in the treatment of chronic refractory diarrhea caused by the short bowel syndrome and idiopathic secretory diarrhea. Octreotide is also useful to treat diabetic diarrhea, and its therapeutic benefit may be related to a marked increase in the mouth-to-cecum transit time (37). Octreotide reduces growth hormone hypersecretion in patients with acromegaly, and reduces the symptoms of malignant carcinoid

syndrome and VIPoma. Low dose octreotide frequently causes adverse gastrointestinal symptoms in normal individuals. High dose octreotide (used for the normalization of growth hormone hypersecretion in some patients with acromegaly) slightly increases D-xylose absorption and fecal fat excretion, and may precipitate biliary colic in patients with preexisting cholelithiasis (38).

Patients with postoperative gastrointestinal fistulas close these tracts after a short time when treated with TPN plus somatostatin versus TPN alone; this treatment is associated with a significantly lower morbidity (39).

VIP functions as a neuroendocrine regulatory peptide, and VIP mRNA levels in the ileum increase after estrogen treatment (40). In the developing human gastrointestinal tract, the VIP gene is expressed first in the upper gut (41).

SUGAR DIGESTION AND ABSORPTION

The physiology and pathophysiology of intestinal absorption have been reviewed (42). Major advances in our understanding of the molecular structure and function of the BBM sodium/glucose cotransporter (sodium-dependent glucose transporter [SGLT1]) have come from the work of Wright and colleagues (28,43). Immunocytochemical and *in situ* hybridization techniques suggest that the transcription of SGLT1 is initiated as the enterocytes emerge from the crypt and increases as the cells migrate up the villus. The mature enterocytes on the tip of the villus have the highest levels of SGLT1 activity, of protein and of mRNA; mRNA abundance increases sixfold from the base to the tip of the villus of rabbit small intestine. This suggests that the high rate of sugar transport across the tips of the villi is due to cellular events that regulate the transcription of the SGLT1 gene in mature enterocytes, the subsequent translation of SGLT1 mRNA and the insertion of the functional SGLT1 cotransporter into the BBM of the cells lining the villus tip. There is evidence for N-glycosylation of SGLT1 at a single site; however, the importance of this post-transcriptional event is unknown (44).

Lysine residues are believed to be involved in glucose binding to SGLT1, whereas tyrosine residues are thought to be involved in sodium binding. Amino acids with sulfhydryl and carboxylic acid groups are involved in substrate transport, although not at cotransporter sodium or glucose binding sites. There appears to be a transport-essential sulfhydryl group located within the BBM or at an intracellular site. The first class of carboxylic acid sites is located at or near the cotransporter glucose site, and the second class of carboxylic acid sites possibly acts as a sodium barrier within the sodium substrate channel. The carboxylic acid residue(s) involved in sodium-glucose cotransport has been interpreted to be near an endogenous nucleophile; the phlorizin binding domain and the glucose binding domain may overlap, but are not identical (45).

Kinetic evidence accumulated over the past decade is compatible with the existence of both high and low affinity pathways for this cotransporter. However, *in situ* hybridization and immunocytochemical techniques examining the

tissue distribution of SGLT1 have failed to confirm this suggestion. A careful examination of the kinetic data obtained with a fast-sampling rapid filtration apparatus suggests the presence of only one carrier in rabbit jejunal BBM vesicles (46). The resolution of this conflict remains to be achieved.

The addition of glucose to fluid bathing the mucosal surface of intestinal epithelia activates cytoskeletal contractile proteins in the mucosa, thereby widening intercellular junctions and permitting permeation of nutrients through the paracellular channels. A nonspecific increase of intracellular osmotic pressure during active transport may lead to contraction of the perijunctional actomyosin, formation of junctional dilation and exposure of the lateral membranes. Widening of these junctions can be detected by changes in the permeability to solutes, alterations of transepithelial electrical impedance or electron microscopy. In the jejunum of mice and hamsters, the transmucosal impedance correlates with the transport of sugars and amino acids (47). Luminal but not vascular glutamine enhances the intestinal absorption of glucose by unknown mechanisms and generates one or more humoral or nervous 'hepatotropic' signals in the small intestine, which enhance the hepatic uptake of absorbed glucose (48).

Animals control the uptake of monosaccharides and amino acids at three levels: mucosal hyperplasia increases uptake nonselectively; individual enterocytes increase the transport capacity of specific transporter systems; and the transporters are modulated by solute and ion electrochemical gradients (49). Most nutrient uptake occurs in the upper portion of the villus, and the substrate-dependent upregulation of intestinal glucose transport involves increased numbers of transporters along the crypt-villus axis (50). A change in the carbohydrate content of the diet alters glucose uptake, but there is a time lag of approximately one day between switching the diet and the first appearance of enhanced BBM glucose transport. The signal for glucose transporter regulation is probably perceived in the crypts, and this observed lag in uptake is likely due largely to cell migration times (50).

DIABETES MELLITUS

DM has numerous effects on the motility, digestive and transport functions of the intestines. Symptoms such as constipation or diarrhea are common in diabetic patients. Gastrointestinal motility is altered in DM, and there is a close association with both peripheral and autonomic neuritis and the duration of either type I or II diabetes. Only the symptom of constipation correlates with the appropriate regional delayed transit (ie, delayed colonic transit), but diabetics with delayed transit in any intestinal region have more overall gastrointestinal symptoms than those without (51). Decreased intestinal beta-adrenergic responses have been demonstrated in DM, resulting from a decrease in the number of beta-adrenoceptors, due to impaired receptor turnover (52). Insulin has a beneficial effect on this impaired receptor turnover.

In diabetic patients autonomic neuropathy affects motor

functions throughout the gastrointestinal tract, and the prevalence of symptoms caused by this dysfunction may affect three out of four diabetics. Delayed gastric emptying as a result of gastroparesis is probably the best known and most extensively evaluated motor disturbance of the gastrointestinal tract in diabetic patients, but there may also be delayed emptying of the gallbladder and slowed colonic transit (43).

Bacterial overgrowth in DM may be due to autonomic neuropathy or altered substrate availability. Untreated diabetes results in an overgrowth of mucosal-associated small bowel aerobic and anaerobic microbial populations, compared with populations in normal nondiabetic age-matched control rats. Insulin treatment and pancreatic transplantation prevent this microbial overgrowth (53).

Lithium, like insulin, stimulates both glucose utilization and glycogen synthesis. Treatment of diabetic rats with lithium chloride normalizes the decreased intestinal responses of the alloxan-diabetic rat, suggesting a direct action on the diabetic smooth muscles (54).

Diabetic rats have hypercholesterolemia and a higher sensitivity to dietary cholesterol, and insulin treatment reduces the percentage of cholesterol that is absorbed. In DM, cholesterol production is unchanged, so that cholesterol absorbed in the diet may be the major contributing factor for the development of hypercholesterolemia. Also, the activity of intestinal acyl-CoA:cholesterol acyltransferase (ACAT), the rate-limiting enzyme for cholesterol absorption, is increased in diabetic rats, and ACAT likely plays a major role in the initiation of diabetes-associated hypercholesterolemia (55).

A high fat diet may worsen glucose control, and in mildly streptozotocin diabetic rats a high fat diet causes a marked increase in fasting glycemia and a deterioration in the intravenous glucose tolerance. This exacerbation occurs primarily by an increase in hepatic glucose production (56).

The concordance of diabetes in identical twins is less than 50%, suggesting the presence of environmental factors that nurture the disease. Diabetes does not occur in diabetes-prone rodents reared for the first two to three months of life on a diet free from cows' milk. Exclusive breast-feeding with delayed exposure to infant formula based on cows' milk reduced the risk of diabetes in a study of Finnish children, and whey protein bovine serum albumin has been suggested to be the trigger molecule in this diet-associated development of diabetes (57). Children with insulin-dependent DM had elevated serum concentrations of immunoglobulin G anti-bovine serum albumin antibody but not of antibodies to other milk proteins. This suggests that patients with insulin-dependent DM have immunity to cows' milk, with antibodies to albumin peptide that are capable of reacting with a beta cell-specific surface protein. Such antibodies could participate in the development of islet cell dysfunction in DM.

As part of the diabetic intestinal adaptation process, marked increases in total and specific activities of mucosal SI have been observed. The specific activity of BBM SI is increased two- to threefold in diabetic animals compared with

controls. Changes in SI activities are commensurate with increases in SI immunoreactivity, and are not a result of altered functional activity (6). SI expression along the crypt-villus axis of nondiabetic rats corresponds to gradients of the SI mRNA level, whereas steady state SI mRNA levels are similar in the jejunum and ileum despite differences in SI activity at these two sites. This suggests that regional differences in translational or post-translational events may determine SI expression along the longitudinal axis of the intestine. In diabetic rat intestine, increases in SI activity are paralleled by increases in SI mRNA. However, analogous to nondiabetic rat intestine, no difference in SI mRNA abundance is observed between corresponding enterocyte fractions from ileum and jejunum of diabetic rat intestine (4). Diabetes may induce increased total and specific activities and mRNA abundance of intestinal SI, largely through the stabilization of SI mRNA. It is also suggested that differences in proximal to distal SI expression are determined at the translational or post-translational level. Transport of glucose and fructose across the BBM and of glucose across the BLM is increased in DM. The levels of the SGLT1 mRNA are increased in 30-day and 60-day streptozotocin-treated rats, but not in acutely diabetic animals (58).

The intestinal hyperplasia that occurs in diabetic rats is associated with increased villus and crypt enterocyte activity of ODC, the enzyme that catalyzes the conversion of ornithine to putrescine, and DM increases the intestinal content of putrescine and spermidine but not of spermine. Spermine may be responsible for the intestinal epithelial hyperplasia in diabetic rats and for the normal growth of intestinal epithelium in nondiabetic animals (59).

Insulin modulates the permeability of the occluding junction of T84 cell monolayers through a receptor-mediated process; this probably involves a change in protein synthesis and cytoskeletal structure (60). Insulin may be absorbed from the gastrointestinal tract, and insulin permeability differs among the various intestinal regions. In the rat ileum, insulin absorption is linearly related to the logarithm of the co-administered dose of aprotinin; the hypoglycemic effect of insulin is promoted with sodium caprate and Na₂EDTA (61), and sodium glycocholate improves colonic insulin efficiency. It is hoped that means to administer insulin by mouth will become possible.

IMMUNOLOGY AND TRANSPLANTATION

Transplantation of gastrointestinal organs has been reviewed (62). Despite recent advances in immune suppression, the results of intestinal transplantation remain relatively disappointing. Small bowel transplantation procedures include transplantation of the small intestine only, combined transplantation of the small intestine and liver, and multivisceral transplantation of the small intestine with other organs such as the liver. Selective cell transplantation is being investigated as an alternative to whole organ transplantation (63). For the intestine, selective cell transplantation potentially avoids the transplantation of large amounts of lymphoid tissue and passenger leukocytes. Basement

membrane component enhances isolated enterocyte growth in rabbits (64), and in the future this may prove to be a useful approach to allow selective enterocyte transplantation. Computed tomographic findings following small intestine transplantation have been described (65).

The absorption of water, nutrients and electrolytes requires a highly coordinated integration of extrinsic and intrinsic neuronal functions. With vascularized intestinal transplantation there is transection of all extrinsic nerves. Loss of innervation is associated with abnormal motility, absorption and secretion. Water flow is initially secretory, but becomes absorptive eight days after small intestinal transplantation in rats (66).

Intestinal intraepithelial lymphocytes (IEL) and lymphoepithelial interactions in the human gastrointestinal mucosa have been reviewed (67). The majority of IEL are T cells, predominantly of the cytotoxic-suppressive phenotype (CD8+). Methods have been described to isolate functionally active IEL and enterocytes from human small and large intestine (68). IEL show poor *in vitro* proliferative responses to phytohemagglutinin, anti-CD3 and phorbol ester-calcium ionophore.

There are two main populations of IEL in the small intestine of mice. First, there is the thymus-dependent CD3+, Thy-1+ population, most of which express $\alpha\beta$ T cell receptor. Second, there is the thymus-independent CD3+, Thy-1- population, most of which express $\delta\tau$ T cell receptor (69). Thy-1-enriched IEL are capable of proliferation and lymphokine secretion after stimulation with concanavalin A, phorbol myristate acetate and anti-CD3 monoclonal antibody. Lamina propria lymphocytes have toxic activity induced by anti-CD3 and phytohemagglutinin; IEL from inflamed mucosa mediate reduced cytotoxicity *in vitro*, compared with normal mucosa (70). IEL provide neither helper nor suppressor functions for immunoglobulin synthesis by B cells, and do not mediate spontaneous cytotoxicity. IEL may perform immune surveillance of the epithelial layer and may regulate mucosal or humeral responses to exogenous antigen. IEL can be distinguished by several phenotypic characteristics, and the functional activities of these cells may influence both local immune cell populations and epithelial differentiation (71).

The absorptive epithelium of mammalian small intestine constitutively expresses low levels of glycoprotein products of the major histocompatibility complex (MHC). MHC molecules are expressed on macrophages and on B lymphocytes. MHC class I products present antigens to cytotoxic/suppressor T lymphocytes, and class II molecules present antigens to helper T lymphocytes. These class II gene products support the *in vitro* presentation of soluble protein antigens and alloantigens to mucosal T cells (72). Class II molecules on small intestinal epithelial cells activate T lymphocytes in an antigen-specific manner, and the expression of most MHC molecules depends on the presence of intestinal microorganisms (73). In pathological conditions involving activation of mucosal lymphocytes, concomitant increases in epithelial class II expression are seen. For example, there is

increased class II expression in the villus epithelium of a number of small intestinal disorders, including experimental and clinical graft-versus-host disease (GVHD), intestinal nematode infection, whole body infusion of interferon-alpha, idiopathic inflammatory bowel disease, gluten-sensitive enteropathy, radiation colitis, acute infectious colitis and enteric inflammation associated with arthropies. Furthermore, class II expression is extended to the immature small intestinal crypt epithelium and to the epithelium of the large intestine, which do not normally express class II glycoprotein products.

The histological changes of cell necrosis at the base of the crypts, mucosal sloughing, hemorrhage and diarrhea may occur with cytomegalovirus infection following the administration of cytotoxic drugs and irradiation, and may occur in patients with acute GVHD. Human leukocyte antigen DR expression is increased on enterocytes in GVHD. Cellular adhesion on molecules may also improve the diagnostic specificity between GVHD and the histological changes produced by irradiation and cytotoxic drug used before transplantation (74).

There are wide interpatient differences in the systemic bioavailability of orally administered cyclosporin A (CyA). Although over 17 different cyclosporin metabolites have been identified in human blood and bile, the major pathways of cyclosporin catabolism involve monohydroxylation and N-D methylation. The product of each of these CyA metabolites is catalyzed by a single subfamily of phase I enzymes, termed P450III_A, which are present in rat jejunal enterocytes. These enzymes likely contribute to the 'first-pass' metabolism of orally administered CyA (75).

Nitric oxide (NO) is an endogenous mediator previously termed endothelium-derived relaxing factor (76). NO is thought to have an important physiological role in the regulation of bloodflow. This mediator is synthesized from the amino acid L-arginine by the enzyme NO synthetase, of which two forms have been characterized. NO is produced during immune reactions and has an important effector function. Vascular endothelial cells have been shown to have both the constitutively expressed and inducible forms of NO synthetase. NO also plays an important role in the tumoricidal and antimicrobial activities of macrophages both *in vitro* and *in vivo*. NO is involved in the killing of transplanted syngeneic pancreatic islet cells *in vitro* and in the induction of diabetes by streptozotocin. Treatment of mice with a specific inhibitor of NO synthesis ablates the pathology associated with a proliferative form of intestinal graft-versus-host reaction (77). NO is responsible for this activity, and a microsensor has been developed and applied to monitoring NO release (78).

LIPIDS

Intraluminal and intracellular phases of intestinal fat absorption have been reviewed (79,80). Long chain free fatty acids (FFA) taken up across the BBM are incorporated into triacylglycerol and phospholipid, are packaged into lipoprotein particles and are secreted into the lymph via the BLM

(81). FFA from the plasma are also taken up into the enterocyte via the BLM and, being primarily oxidized or incorporated into phospholipid, are metabolized differently from those absorbed from the lumen.

Luminal fatty acids are absorbed as FFA and are esterified to 2-monoacylglycerol, via either the monoacylglycerol pathway or the phosphatidic acid pathway. Fatty acids absorbed from the intestinal lumen are preferentially incorporated into triglycerides, whereas those absorbed simultaneously from the blood stream are likely to undergo beta-oxidation or incorporation into phospholipids.

Dietary intake of polyunsaturated fats is a major contributor to the body's source of lipid hydroperoxides. With lipid peroxidation, oxygen-derived free radicals attack lipids in the membrane and cause its destruction. The mucosal glutathione peroxidase/oxidized glutathione reductase system may play an important role in the intestinal handling of luminal lipid hydroperoxides in rat small intestine (82). This peroxidation involves an oxidative degradation process of polyunsaturated fatty acids in the phospholipids of biological membranes, resulting in a change in the chemical composition of membrane phospholipids. Peroxidation causes a decrease in the lipid fluidity of the membrane and an increase in the positive charge on the membrane surface (83).

The intestinal mucosa is constantly in contact with the luminal contents, which include dietary materials such as transition metals, ascorbic acid, rancid fat and bacterial metabolites; some of these are potential pro-oxidants. Pro-oxidants, along with iron in the lumen, do not have a damaging effect on the intestinal mucosa of rats, whereas free radicals, which do not require iron, result in lipid peroxidation and increased water and electrolyte secretion (84).

The kinetics of alpha-linolenic acid uptake by isolated hamster intestinal epithelial cells suggest an active, carrier-mediated mechanism common to long chain fatty acids (85). BBM fatty acid uptake into Caco-2 cells is sixfold higher than BLM uptake when expressed per unit surface area of the membrane (86). Unsaturated long chain fatty acids have an absorption-enhancing efficiency on various poorly absorbable drugs such as heparin and interferon. This effect is achieved by sulfhydryl modification of membrane proteins (87).

Lipid absorption is inhibited by drugs that alter cytoskeletal structure and function. Cytochalasin decreases the permeability of the mucosal membrane to linoleic acid, and the administration of colchicine, EDTA, or cytochalasin plus colchicine increases the permeability of the rat jejunal serosal membrane to linoleic acid (88).

The efficacy of cholesterol absorption is about 50% in humans, and restriction of cholesterol consumption and/or absorption results in a reduction of the plasma cholesterol concentration in some persons. Cholesterol absorption is a multistep process that includes hydrolysis of cholesterol esters in the gut lumen, formation of mixed micelles, transport of cholesterol into enterocytes and its re-esterification, and assembly and secretion of chylomicrons as well as nascent

high density lipoproteins (HDLs). The human intestine in organ culture has been used to develop a new model of cholesterol uptake which involves rapid and slow phases of inhibition of cholesterol acyl transferase. Treatment with monoensin reduces cholesterol uptake, but treatment of cultures with lovastatin (an inhibitor of 3-hydroxy-3-methyl-glutaryl coenzyme reductase) stimulates cholesterol uptake, and mevalonic acid reverses this stimulatory effect of lovastatin (89). In contrast, reduction in 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase activity by lovastatin reduces the absorption of dietary cholesterol (90).

Patients without intestinal malfunction can be divided into three distinct groups based on low, medium or high rates of cholesterol uptake into small bowel biopsies. In subjects with medium or high rates of cholesterol uptake, there is a positive correlation between cholesterol uptake and synthesis (91). Cholesterol synthesis in the intestine is not the subject of a simple feedback regulation by dietary cholesterol. The membrane-bound microsomal enzyme ACAT plays an integral role in cholesterol homeostasis. Rat liver and intestine possess significant amounts of ACAT activity throughout development in the rat from day 21 of gestation to postnatal day 60 (92). ACAT activity can be inhibited in the intestine and liver, and this lowers cholesterol absorption and plasma lipid concentrations (93). Thus, hypo- and hyperresponsivity can be ascribed at least partly to the genetically regulated peculiarities of microsomal ACAT activity.

FABP in the cytosol of intestinal mucosa has been reviewed (64). The rat intestinal epithelium is known to express three distinct cytosolic FABPs: the L-FABP, the intestinal FABP (I-FABP) and a 15 kDa cytosolic protein (I-15P). This latter protein is a potent lipid-binding protein detected in the distal small intestine as well as in the ovary and adrenal gland, suggesting that I-15P may play a role in the cellular metabolism of steroids (94). A cDNA encoding I-15P has been isolated and sequenced from a rat ileum-specific cDNA library (95).

The L-FABP but not the I-FABP gene is expressed in Caco-2 cells (96). FABPs in the cytosol and membrane of the enterocyte play a role in lipid absorption. Rat I-FABP may be labelled fluorescently (97).

Enterocytes are a major site of production of extracellular lipid transport proteins, particularly apo A-I, apo A-IV and apo B-48. Apo B-48 is an apolipoprotein found in intestinal chylomicrons, and its synthesis is increased by EGF and hydrocortisone (98). The EGF-induced increase in apo A-I synthesis is blunted by concomitant treatment with hydrocortisone, whereas the combination of insulin and hydrocortisone induces an increase in apo A-I synthesis. Apo A-IV secretion into mesenteric lymph in rats is increased when fat emulsion is infused into the intestinal lumen, and dietary fat-dependent and -independent factors are involved in the elevation of intestinal apo A-IV mRNA (99). Apo A-IV is a protein associated with lipoprotein that may act as a physiological signal for satiation in rats (100).

Oxygenated derivatives of cholesterol are potent stimulators of intracellular cholesterol metabolism. They suppress HMG-CoA reductase activity and reduce low density lipoprotein (LDL) receptor activity; this is achieved mainly by repression of transcriptional expression of the genes through sterol regulatory elements. Oxygenated sterols stimulate the formation of cholesterol esters by increasing the activity of microsomal ACAT. ACAT catalyses the formation of long chain fatty acyl esters of cholesterol and plays an important role in cholesterol absorption (101). This ester formation is suppressed by staurosporine, a kinase inhibitor.

The sphingomyelin content of intestinal cell membranes also influences cholesterol absorption (102). Sphingomyelin, a phospholipid found in the plasma membrane of mammalian cells, has a high affinity for cholesterol and is strongly correlated with the amount of cholesterol present in membranes. Depletion of plasma membrane sphingomyelin causes a rapid flux of plasma membrane cholesterol into the cell. Human pancreatic juice contains neutral sphingomyelinase activity; hydrolysis of apical sphingomyelin inhibits the cellular uptake of micellar cholesterol and decreases the secretion of unesterified cholesterol (102).

The absorption of a physiological load of lipid into lymph does not affect apo B synthesis in the intestinal mucosa or apo B secretion into lymph. During active lipid absorption the number of chylomicrons remains the same, the size of these particles increases, and the number and triglyceride content of very low density lipoprotein (VLDL) particles synthesized and secreted by the small intestine remain relatively constant (103).

The mechanisms by which the type of dietary fat alters plasma lipoprotein concentrations are poorly understood. Diets containing fish oils reduce the circulating concentration of triacylglycerols by reducing the synthesis and secretion of hepatic VLDL, and not by a decrease in the intestinal absorption of lipids (104). Dietary saturated fatty acids raise, *n*-6 polyunsaturated fatty acids lower and monounsaturated fatty acids have no effect on plasma cholesterol concentrations. African green monkeys fed an oleinate and fish oil diet absorb a lower percentage of dietary cholesterol compared with animals fed lard (105); this effect is seen only with a high level of cholesterol uptake. Indeed, at a lower level of dietary cholesterol, the percentage of cholesterol absorption is not affected by the type of dietary fat. Substituting long chain triglycerides for glucose in a mixed diet lowers the trend for an increased mucosal mass in the proximal segment, whereas there is a rise in that trend in the middle segment of the intestine (106). Increasing cell phospholipids in Caco-2 cells by enriching the culture medium with fatty acid increases leukotriene B₄ synthesis (107).

Olestra, a sucrose polyester, is the generic name of nonabsorbable, synthetic hexa-, hepta- and octaesters of sucrose and fatty acids. These have the physical properties of conventional dietary fats. Olestras are not hydrolyzed by pancreatic lipase or colonic bacteria, and therefore cannot be absorbed. Olestras are potential acaloric substitutes for dietary fat. Substitution of up to 30 g of olestra in a 45 g fat meal

does not alter gastrointestinal transit in healthy subjects (108).

The nutritional and medical importance of gamma-linolenic acid has been reviewed (109). The hepatic and intestinal activities of HMG-CoA reductase and ACAT are modified after interruption of the enterohepatic circulation. The relative availability of saturated and unsaturated fatty acids for phospholipid synthesis is determined by the diet, and by the activity of elongase and desaturase enzymes. The activities of $\Delta 9$ and $\Delta 5$ desaturases are increased in the jejunal mucosa of rats undergoing intestinal resection (110). The Caco-2 cell line may also prove to be useful for the study of fatty acid interconversion catalyzed by $\Delta 6$ and $\Delta 5$ desaturases (111).

Apo B-100, B-43, E, A-I, A-IV and C-III have been identified in Caco-2 cells by immunoprecipitation (112). Differentiation of Caco-2 cells causes changes in apo gene expression (113). In Caco-2 cells, eicosapentaenoic acid (EPA) impairs triglyceride transport by inhibiting apo B synthesis and secretion, compared with the effects of oleic acid. This inhibition of apo B synthesis by EPA acid may be related to a decrease in gene transcription or in mRNA stability (114). Apo B secretion by Caco-2 cells incubated with oleic acid is increased compared with cells incubated with EPA (115).

Normally, dietary triglycerides are absorbed and packaged into chylomicrons. Even in the absence of dietary fat, intestinal epithelial cells are an important site of VLDL production. The intestine also secretes intact HDL. Chylomicron synthesis is modulated by insulin, but neither the lipid composition of chylomicron nor that of VLDL, LDL or HDL is affected by insulin (116).

The enterohepatic cycling of bile salts is a major factor in the maintenance of the bile salt pool. Bile salts are passively absorbed in the jejunum and are actively absorbed in the ileum. They are then carried back to the liver by the portal blood. Passive jejunal uptake of taurocholate decreases between 14 and 40 days of age in rats, whereas hepatic taurocholate clearance increases (117). The active transport of bile acids in rabbit ileum is mediated by a saturable, high efficiency, low affinity carrier with $V_{\max}$ values in this order: taurocholic acid then tauroursodeoxycholic acid then taurochenodeoxycholic acid; passive transport is highly efficient for unconjugated bile acid, with values in the following order: deoxycholic acid then chenodeoxycholic acid then ursodeoxycholic acid then cholic acid (118). Thiol and amino groups are involved in active ileal bile acid uptake, and a membrane polypeptide of apparent relative molecular weight of 90,000 Da is a component of the active sodium-dependent bile-acid reabsorption system in the BBM of the terminal ileum from rabbits (119). The mRNA coding for the sodium-dependent bile salt cotransporter is present in the enterocytes lining the whole length of the small intestine, but transporter function is only expressed in the BBM of the distal small intestine (120). The reason for this site difference in activity is unknown. The transporter appears

around the time of weaning, and there may be a modulatory influence of diet on the ontogenic expression of this process.

Bile acid biosynthesis from cholesterol is regulated by the flux of bile acids through the hepatocyte. This biosynthesis is mediated by changes in the activity of the rate-limiting enzyme cholesterol 7- α -hydroxylase. In the guinea pig, ingestion of a taurocholate-enriched diet results in a 75% decrease in the absorption rate of ursodeoxycholytaurine, whereas cholysarcosine ingestion causes an increase in absorption. This suggests that bile acid metabolism is also regulated by feedback inhibition of active ileal transport in addition to feedback on hepatic cholesterol 7- α -hydroxylase (121).

Selenium-75 homocholic acid taurine ($[^{75}\text{Se}]\text{HCAAT}$) is a synthetic analogue of the natural conjugated bile acid taurocholic acid, with ^{75}Se in the side chain. It may be used to assess bile acid malabsorption. Current methods for measuring $[^{75}\text{Se}]\text{HCAAT}$ absorption in humans by whole body or total abdominal retention or by fecal excretion provide only semiquantitative data. Measurement of the isotope in the enterohepatic circulation by daily gallbladder scintigraphy or by total abdominal retention four and seven days after isotope administration are useful measurements for the detection of bile acid absorption (122). Since there is a close relationship between the intestinal loss and hepatic synthesis of bile acids, the measurement of 7- α -hydroxy-4-cholesten-3-one in serum may be useful to assess the presence of bile acid malabsorption indirectly in patients with diarrhea (123).

Cholylsarcosine is a synthetic deconjugation-resistant and nonsecretory conjugated bile acid analogue that improves dietary fat absorption in a canine model of severe bile acid malabsorption (124). In humans, cholylsarcosine is not metabolized, is nontoxic and has similar effects on biliary secretion as cholyltaurine (125). Thus, cholylsarcosine pos-

sesses the physical, chemical and physiological properties required for a suitable bile acid replacement in deficiency states (126).

Bile salts, at levels as low as 5 and 50 $\mu\text{mol/L}$, depress cholecystokinin-induced contractions of guinea pig terminal ileum. The bile salt-associated inhibition of excitatory, cholinergic, enteric neurons may slow transit through the ileum, thereby enhancing the time for absorption of bile acids and conserving the bile salt pool. Diarrhea may induce mild secondary steatorrhea; the excretion of up to 14 g/day of fat is not specific for a primary defect in fat digestion or absorption and therefore may represent a false positive result (127). While the measurement of a three-day stool fat collection is a reliable alternative to the detection of more severe malabsorption, this is technically and esthetically a difficult test. A simpler semiquantitative method to determine fecal fat concentration is the 'steatocrit test' performed after a fatty meal. In children with known celiac disease, the steatocrit test does not yield any false positive or false negative results, while the D-xylose test shows both results (128). The reference values of the steatocrit test have been identified for children of different ages: the maximal steatocrit value is observed in neonates and the value progressively declines to an undetectable level in children older than two years (129).

Fat malabsorption is common in persons with progressive systemic sclerosis. This fat malabsorption may be caused by hypomotility, by diverticula of the small bowel causing bacterial overgrowth and by sclerosis of intestinal lymphatic channels. A patient with progressive systemic sclerosis and malabsorption has been described who developed a peripheral sensory and motor polyneuropathy and marked depression of T lymphocyte function, in conjunction with a severe vitamin E deficiency arising from malabsorption of fat (130).

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