Gastrointestinal (GI) system

Hippocrates has been quoted as saying, “death sits in the bowels” and “bad digestion is the root of all evil” in 400 B.C. (Hawrelak and Myers, 2004), showing that the importance of the intestines in human health has been long recognized.

The GI tract runs from the mouth to the anus passing through regions each specialized for a particular stage in the digestive process. The gastrointestinal (GI) tract is an important target organ for toxicity in both pre-clinical and clinical studies but is less well investigated by toxicologists than the liver or kidney (von Richter et al., 2001; Glaeser et al., 2004). A survey of adverse events observed during clinical drug development, and their toxicological correlates have shown GI toxicities were relatively common in clinical trials of novel pharmaceuticals, second only to neurological adverse events (Olson et al., 2000).

Intestine: Structure and Physiology

The intestine, as the main organ of the digestive apparatus, is the primary site of exposure to nutrients/toxicants because of its extensive surface area, and physiological properties (Zucco, 1993). Although the blood flow and tissue volume of the intestine is slightly lower than the liver, the extensive microvilli structure results in a large surface area, ideally suited for absorption. Substances absorbed through the intestine are delivered to the liver via the portal vein, with over 75% of the total blood flow to the liver coming from the intestines (DeSesso and Jacobson, 2001). Since most absorption is thought to occur in the small intestine (Doherty et al., 1997), intestinal first-pass metabolism may determine the balance between bioactivation/ deactivation for orally administered xenobiotics (Wacher et al., 2001).
The intestinal mucosa is now recognized not only as a major determinant of the bioavailability of orally administered drugs regulating absorption, but also for biotransformation into metabolites and possible efflux back to the intestinal lumen (Watkins, 1997; Suzuki and Sugiyama, 2000). Several preclinical and clinical studies have demonstrated the importance of the intestines in first-pass, since both rodent and human intestinal epithelial cells (enterocytes) contain enzymes capable of metabolizing a broad range of drugs and xenobiotics (Obach et al., 2001; Sams et al., 2000). Therefore, there is an increasing interest to assess its role in the fate of other chemicals present in human food and reaching the gastrointestinal tract, including various food contaminants, such as pesticides, heavy metals, polycyclic aromatic hydrocarbons (PAHs), mycotoxins, as well as substances naturally associated with nutrients such as polyphenols.

Following the ingestion of contaminated food, or foods containing natural chemicals such as polyphenols and other secondary plant products, the intestinal epithelial cells may become exposed to significant concentrations of these chemicals. The intestinal mucosa cell monolayer is also the first barrier that either allows or prevents the entry of food antigens including food proteins, commensal gut microorganisms and pathogens, into the underlying tissues (Sergent et al., 2008). The intestine serves principally as the site for digestion of food and absorption of nutrients, water and both beneficial and potentially harmful xenobiotics. The mammalian small intestine is divided into three regions namely a) Duodenum b) Jejunum and c) Ileum (Fig. 1). In rats, the length of the small intestine ranges from 90-110 cm with duodenum comprising of 9.5-10 cm in length (8% of total small intestinal length), while jejunum, the lengthiest portion of the small intestine is of 75-90 cm length (90 % of total intestinal length). Ileum, the shortest of all is of 2.5-3.5 cm (2% of total small intestinal length) (Hebel and Stromberg, 1986).
Anatomy and Histology

Anatomically, the small intestine is divided into duodenum, jejunum and ileum. Each region of the intestinal tract consists of the four cell layers namely, serosa, muscularis, submucosa and mucosa (Fig. 2) (Bowen, 2004). The mucosa is lined with a continuous layer of cells, consisting of enterocytes and goblet cells (Fox, 1999). The enterocytes have a different enzymes and transporters that can metabolize and transport endogenous and exogenous compounds (Kaminsky and Zhang, 2003; Kunta and Sinko, 2004; Chan et al., 2004). Though the blood flow and tissue volume of the intestine is slightly less than the liver, the extensive ‘microvilli’ structure results in a large surface area, ideally suited for absorption (Poet et al., 2003). Substances absorbed through the small intestine are delivered to the liver via the portal vein, with over 75% of the total blood flow to the liver coming from intestine (DeSesso and Jacobson, 2001).
In both humans and rats, numerous microscopic finger-like projections called villi (Fig. 3 and Fig. 4) extend from the intestinal wall into the lumen; these increase the surface area by a factor of 5 in rats and 10 in humans (Shimizu, 2010). Using this vast area, large amounts of nutrients derived from diet are quickly and efficiently absorbed. Intestinal epithelial cells (IECs) covering the internal surface of the intestine are responsible for this absorption (Jenkins and Thompson, 1994).

Although the epithelium of the villi has different cell types, there is one cell type that is important in the absorption of materials from the lumen and is the predominant cell in all absorbing regions (villi). This cell type is referred to as an enterocyte. Enterocytes are columnar epithelial cells that are bound to their neighboring cells at the luminal surface by tight junctions. Their apical cell membranes possess numerous microvilli (estimated at 3000–7000 per cell in the small intestine), which are visible only with an electron microscope and give the appearance of brush border.
Fig. 3. Histology of the small intestine
(Source: http://williamthecoroner.wordpress.com/2011/10/20/histology-the-small-intestine/)

Fig. 4 Schematic diagram of the small intestinal villi
(Source: Holmes and Lobley, 1989)
Microvilli increase the surface area of rat and human small intestine by a factor of 20 (Snyder et al., 1975; Granger et al., 1985). In both species these anatomical modifications increase surface area to a greater extent in the duodenum and jejunum than in the distal region (Snyder et al., 1975; Hebel and Stromberg, 1986). The epithelium possesses a carbohydrate-rich glycocalyx coat on the surface of the microvilli.

The lamina propria possesses a rich supply of blood and lymphatic capillaries. When villi are present, the lymphatic capillaries are slightly dilated, blind-ending tubes that occupy the centre of the villus; the blood capillaries form a network of vessels beneath the basement membrane. Each villus has a network of ‘capillaries’ and fine lymphatic vessels called ‘lacteals’ close to its surface. The epithelial cells of the villi transport nutrients from the lumen of the intestine into these capillaries (monosaccharides and amino acids) and lacteals (lipids). Underlying the lamina propria is a thin layer of smooth muscle, the muscularis mucosa. The function of this muscular layer appears to be related to the rhythmic movements of the villi that agitate the layer of intestinal secretions and chyme that are in contact with the epithelium and thus promote absorption.

**Cell types in villi**

The small intestinal villi harbor four different cell types: enterocytes, goblet cells, enteroendocrine cells, and Paneth cells (Madara and Trier, 1994). The enterocytes possess an apical plasma membrane which contains closely packed microvilli, designated as ‘brush border.’ Brush border contains glycohydrolases and a large number of other enzymes and transporters (Van Beers et al., 1995).

The goblet cells produce large amounts of secretory mucins which are the major component of mucus gel-layers (Strous and Dekker, 1992). The number and localization of these cells vary along the gastrointestinal tract. Moreover, the quantity of goblet cells can adapt to specific diets or to stress situations. The secretory mucins produced by these cells form a mucus layer
that represents an important protective mechanism. The mucus forms a gel, producing an “unstirred layer,” which functions as a molecular sieve (cut-off at about 10 kDa) (Van Beers et al., 1995). This mucus gel traps defensive molecules such as lysozyme and IgA, but allows access of small molecules to the apical membrane of enterocytes. The mucus gel also offers resistance to physical erosion, and to pathogens.

The enteroendocrine cells constitute a small number of cells, but they produce at least 15 different gastrointestinal hormones (Walsh, 1994). These cells are found all through the gastrointestinal tract and are a very heterogeneous group, i.e. each cell produces more than one hormone but often in different combinations (Van Beers et al., 1995). The Paneth cells are predominantly found in the small intestinal crypts. These cells produce antimicrobial polypeptides such as lysozyme and defensins. Although relatively small in number they apparently are very effective in eliminating bacteria and viruses, and they may be essential to inhibit bacterial overgrowth of the small intestine (Van Beers et al., 1995).

**Functions of intestine**

The first and primary function of the small intestine is digestion and absorption. The apical cell membrane of ‘Intestinal Epithelial Cells’ (IECs) has many transporters which transport nutrients (Shizimu, 2010). paracellular transport is a passive diffusion system using a space or gap at intercellular junctions between IECs (Tsukita et al., 2001). Paracellular transport is regulated by the permeability of the tight junction (TJ), and is thought to be particularly important for mineral absorption (Barthe et al., 1999). Another important function is signal recognition and transduction (Shizimu, 2010) (Fig. 5).
Normal intestinal functions (Schiller, 1979) include:

a) **Digestion**: hydrolysis of food components

Chemical breakdown of food begins in the stomach and continues in the intestine. Small intestine is the place where most chemical digestion takes place. The three major classes of nutrients that undergo digestion are carbohydrates, proteins and lipids. Proteolytic enzymes, including trypsin and chymotrypsin are secreted by the pancreas, cleave proteins into smaller peptides. Aminopeptidase and dipeptidase present as brush border enzymes in small intestine free the end amino acid products. Pancreatic amylase breaks down some carbohydrates (mainly starch) into oligosaccharides. Brush border enzymes takeover from there. The most important brush border enzymes are glycosidases (maltase, sucrase, lactase, trehalase), peptidases (carboxypeptidases, aminopeptidases, dipeptidases) and phosphatases (alkaline phosphatase).
b) **Absorption**: nutrients, ions, water

Small intestine is the place where most of the nutrients from ingested food are absorbed. Villi and microvilli increase the amount of surface area (approximately 600-fold) available for the absorption of nutrients (Van Beers et al., 1995). Absorption of the greater part of nutrients takes place in the jejunum, with few exceptions (Sherwood, 2006).

- Iron is absorbed in duodenum
- Bile salts and vitamin B12 are absorbed in the terminal ileum,
- Water and lipids are absorbed throughout the small intestine.

Different modes of absorption like is active transport (Sodium bicarbonate), co-transport (glucose and amino acids) and facilitated diffusion (Fructose) are seen.

c) **Secretion**: mucopolysaccharides, hormones, water, electrolytes,

The enzymes come into the small intestine in response to the hormone 'cholecystokinin,' which is formed in the small intestine in return to the presence of nutrients in the ingested food. The hormone 'secretin' induces the release of bicarbonate into the small intestine from the pancreas to facilitate the neutralization of the potentially harmful acid coming from the stomach (Schneeman, 2002)

d) **Metabolism**: synthesis, degradation

Though the liver has long been thought to play a major role in metabolism in the body, the capacity of the intestine in this respect is now recognized, as an array of a metabolic machinery is also observed in this organ (Kaminsky and Fasco, 1991; Lin et al., 1999; Doherty and Charman, 2002; Ding and Kaminsky, 2003). This is supported by the manifestation of high expression of drug metabolizing enzymes (DMEs) (Glaeser et al., 2004). Several anatomic and physiologic features play a major role in organ’s metabolic competency. Among these are: the significant length of the small intestine; the presence of the
metabolically competent epithelium as a single layer of enterocytes; and the magnification of the luminal surface of the small intestine by numerous finger-like projections of enterocytes-lined villi and, at their bases, buried crypts. Together these features provide an extensive surface for xenobiotic absorption, with a subsequent significant potential for first-pass metabolism. *In vivo* studies proved the importance of first-pass metabolism by the intestine for the bioavailability of verapamil, cyclosporine and midazolam (Glaeser *et al.*, 2004).

e) **Detoxification**: conjugation, hydroxylation, hydrolysis

Both phase I and phase II metabolic enzymes are expressed in the intestine together with associated transporters so that the amount of drug reaching the systemic circulation-referred to as bioavailability can be substantially reduced by both intestinal and hepatic metabolism through a process called first pass metabolism. Since most absorption is thought to occur in the small intestine (Doherty and Pang, 1997), intestinal first-pass metabolism may determine the balance between bioactivation/deactivation for orally administered xenobiotics (Wacher *et al.*, 2001). In addition, P-glycoprotein (multidrug resistance protein) on the apical borders of enterocytes is energy-dependent drug efflux pump capable of lowering intracellular drug/chemical concentrations (Zhang and Benet, 2001). The role of intestinal metabolism and active extrusion of absorbed drugs are of interest since collectively they may be a major determinant of oral drug bioavailability (Zhang and Benet, 2001).

Expression of intestinal CYPs have been analysed under pathological conditions and have been related to some disease. Dysregulation of CYP3A4, CYP2C9 and CYP3A7 activity in the colon was suggested to contribute to the pathophysiology of ulcerative colitis (Langmann *et al.*, 2004). While decreased intestinal immune-reactive CYP3A has been also reported in celiac disease (Lang *et al.*, 1996), CYP3A4, CYP3A5 and P-glycoprotein levels were reported to be significantly elevated in children with Crohn’s disease (Fakhoury *et al.*, 2006).
f) **Immune response**: local antibodies, IgA

The intestine is continuously exposed to microbial and dietary antigens. The host has to maintain the intestinal homeostasis to keep the commensal and pathogenic bacteria in hand. Some of the mechanisms that the intestine does are, the expression of innate immune receptors (e.g. Toll-like receptors-TLRs), secretion of IgA, production of antimicrobial peptides, or autophagy of intracellular bacteria (Santaolalla *et al.*, 2011). Unluckily, in some cases the innate immune response fails to protect the host and chronic inflammation and may result in clinical disease with the most relevant being Crohn’s disease (Rebeca and Maria, 2012).

g) **Structural barrier**: selective penetration

The barrier function of the intestine is maintained by the epithelial cells that line the luminal surface of the intestine and the specific tight junctions between them (Montrose *et al.*, 1999).

h) **Elimination**: mobility

Co-ordinated contractions of smooth muscle of the small intestine participate in a number of ways to facilitate digestion and absorption in the small intestine. Two types of motility predominate, a) segmentation contractions chop, mix and roll the chyme and b) peristalsis slowly drives it toward the large intestine. In a broad sense, any variation in the transit of foods and secretions into the digestive tract may be considered as intestinal motility disorder. Irritable bowel syndrome (IBS) is considered as one of the intestinal motility disorders (Ouyang and Locke, 2007).

i) **Colonization**: enteric metabolism

The normal gastrointestinal (GI) tract contains a huge number of aerobic and anaerobic bacteria, which in general enjoy a symbiotic relationship with the host but can have unfavorable effects with local and systemic outcomes. The small intestine comprises a zone of transition between the sparingly populated
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stomach and the lavish bacterial flora of the colon. Regulation of the intestinal flora depends on intricate interactions between various factors including secretion of gastric acid, biliary and pancreatic secretions, intestinal motility, the surface glycocalyx and mucus layer, local immunity and diet. Microbial interactions are also important, and involve alterations in substrate depletion, redox status, and production of substances such as bacteriocins that restrain bacterial growth (Batt et al., 1996). The useful effects of the normal enteric flora include the competitive elimination of potentially pathogenic microorganisms and the production of nutrients like short-chain fatty acids (an important energy source for the colonic mucosa) and vitamins. Disadvantageous effects of the enteric flora include competition for essential nutrients and calories, particularly by bacteria located in the small bowel, and the ability to damage the mucosa, in some circumstances inducing or contributing to inflammatory bowel disease (Batt et al., 1996).

**Brush border membrane: Morphology**

Brush border membrane (BBM) constitutes one of the most important cellular membranes in the intestine because of its role in digestive and absorptive functions (Dudeja and Mehmood, 1982). The intestinal brush border is present at the luminal pole of the enterocytes and constitutes a functional organelle serving in terminal digestion and absorption of the end products of ingested food (Holmes and Lobley, 1989). Brush border membrane is complementary to and integrated with the basolateral membrane of the enterocyte, ensuring effective transfer of food components across the enterocytes from the intestinal lumen to the interstitial fluid and blood.

From a morphological viewpoint, the brush border is composed of numerous finger like apical microvilli overlying a transverse fibrillar meshwork, the terminal web (Holmes and Lobley, 1989). Ultrastructurally and functionally, the brush border is considered as containing two different structures, the surface (microvillus) membrane and the underlying brush border cytoskeleton,
each with its own characteristic molecular composition. The microvillus membrane consists of a lipid bilayer, the external (luminal) surface of which is lined with a carbohydrate-rich 'hairy coat' or glycocalyx. The membrane possesses a large number of integral membrane proteins, most of which are glycoproteins and have been identified by specific brush border enzymes (Holmes and Lobley, 1989).

**Brush border membrane: Composition**

Brush border membrane contains cholesterol, phospholipids and various neutral lipids, together with a variety of glycolipids (Holmes and Lobley, 1989). The molar ratio of cholesterol to phospholipid is higher in the membranes and closely resembled that reported for myelin (Forstner et al., 1968). Unesterified cholesterol is the major neutral lipid. On the other hand, 30% of the neutral lipid fraction was accounted for by glycerides and fatty acids. Five phospholipid components have been identified and measured in the brush border membrane, including phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, lysophosphatidylcholine and sphingomyelin. Among these, phosphatidylethanolamine is the chief phospholipid (Forstner et al., 1968). In contrast with other plasma membranes in the rat, the polar lipids of the microvillus membrane were rich in glycolipid. The cholesterol: polar lipid (phospholipid + glycolipid) ratio is roughly around 1:3 for the microvillus membrane. There are Differences in the proportion of some fatty acids in membrane and cellular glycerides. These differences may reflect the presence of specific membrane glycerides in the brush border membrane (Forstner et al., 1968).

**Functions of the brush border**

The primary functions of the brush border undoubtedly relate to the terminal digestion and absorption of nutrients. However, in recent years, newly discovered enzymes and transport functions have to be integrated into the overall picture. In addition, there are several newly recognized functions, mainly of a regulatory nature, such as the possible modulation of paracellular
permeability through the brush border cytoskeleton, and of ion transport by receptors, and other regulatory proteins located in the brush border (Holmes and Lobley, 1989).

I. Digestion

The brush border enzymes which have been investigated in detail share a number of common features. All are relatively large (70-320 kDa) glycoproteins and most are apparently composed of two or more subunits (Holmes and Lobley, 1989). They appear to be essentially globular structures that are attached to the external face of the membrane by a small (2-5 kDa) anchoring segment embedded in the lipid bilayer (Semenza, 1986). Glycosidase (sucrase, maltase, lactase, trehalase), peptidases (aminopeptidases, carboxypeptidase, endopeptidase, $\gamma$-Glutamyl transpeptidase, leucine aminopeptidase) and phosphatases (alkaline phosphatase) are the most common brush border enzymes.

i) Glycosidases: Glycosidases cleave disaccharides present in food and release single sugar units. The intestinal brush border glycohydrolases work in concert with salivary and pancreatic enzymes, and are essential to the digestion of complex dietary carbohydrates to absorbable monosaccharides (Van Beers et al., 1995). This hydrolysis is crucial, as di, oligo and polysaccharides cannot be transported across the plasma membrane of cells. Monosaccharides are transported across the intestinal epithelium via specific transporters and are released into the circulation to be used in numerous metabolic pathways. The small intestine harbours four membrane bound glycohydrolases: maltase-glucoamylase, sucrase-isomaltase, lactase, and trehalase. All small intestinal glycohydrolases have disaccharidase activity, which is their main function (Van Beers et al., 1995) (Fig. 6).
Fig. 6 Digestion of dietary carbohydrates in small intestine

Very important for the survival of young mammals is the hydrolysis of lactose that is present in milk. Lactase is the only enzyme in the small intestine possessing the necessary β (1-4) galactosidase activity and is thus a crucial enzyme during early post-natal mammalian development (Van Beers et al., 1995). In contrast, sucrose is part of the human diet after weaning and during the remainder of adult life. Sucrose is exclusively hydrolyzed by the sucrase subunit of the sucrase-isomaltase heterodimer. Small intestinal trehalase is the sole enzyme responsible for hydrolysis of trehalose. The significance of trehalose for human nutrition, however, is rather obscure, as this disaccharide is confined to yeast, mushrooms and insects (Van Beers et al., 1995).
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a) **Trehalase**: Trehalase is present in mammalian small intestinal and renal tubular epithelium (Galand, 1984). Its natural substrate specificity seems limited to trehalose, which serves as a storage disaccharide in many fungi (especially young mushrooms) including yeast. In the small intestinal brush border, trehalase is a relatively less abundant enzyme, constituting less than 0.1% of total brush border protein (Galand, 1984, 1989). Molecular masses, estimated by SDS-PAGE, gel filtration, or density gradient centrifugation, ranged from 65 to 75 kDa (Morin and Potier, 1987). The active site of the 67 kDa rat intestinal trehalase was investigated in detail with kinetic studies, which showed that the active site of this enzyme can be functionally divided into a binding site and the catalytic site (Chen et al., 1987). One site most likely contains a thiol-group, which can be non-competitively inhibited by N-ethylmaleimide, Hg$^{2+}$ and iodoacetate, while the other site involves a carboxyl-group which can be competitively inhibited by tris.

b) **Lactase**: In the mammalian intestine, lactase is the most important glycohydrolase during early post-natal life. The precursor of lactase is proteolytically cleaved into its mature form of 130 to 160 kDa and a pro-peptide. Apart from its enzymatic specificity toward lactose, lactase also has catalytic activities towards hydrophobic substrates, such as glycosylceramides and phlorizin. It has been demonstrated that there is most likely one major active site, which is capable of hydrolyzing lactose as well as hydrophobic substrates, and a minor active site with residual activity towards hydrophobic substrates (Van Beers et al., 1995).

c) **Sucrase-isomaltase**: This enzyme complex represents quantitatively the most important maltase activity in humans, comprises about 10% of brush border membrane proteins. It further contains all intestinal sucrase activity, which is essential for the digestion of sucrose (Van Beers et al., 1995). The enzyme complex is most specifically detected by its sucrase activity, which is distinctive to this complex, and distinguishes it from the maltase-glucoamylase complex.
Preparations of the mammalian enzyme complex from human, rat, and pig consist of two noncovalently associated subunits of which the smaller one (120 to 140 kDa) is sucrase, while the larger one (140 to 151 kDa) represents isomaltase (Hoffman and Chang, 1991). Only the isomaltase subunit is directly associated with the membrane, while the sucrase subunit interacts noncovalently with the isomaltase subunit, but not with the membrane (Cowell et al., 1986). This interaction of the isomaltase with the brush border membrane is mediated by a hydrophobic sequence in the extreme N-terminus (Spiess et al., 1982).

ii) Peptidases

The final stages of protein digestion occurs at the brush border (Fig. 7) (Holmes and Lobley, 1989). Peptide hydrolases catalyzing the hydrolysis of di- or tripeptides into respective amino acids are present in the small intestinal mucosa (Kim et al., 1972). These enzymes have been reported to occur both in brush border membranes and the soluble cytoplasmic fraction (Heizer et al., 1972). A major proportion of the true peptidase activity was found in the cytosol (Josefsson and Sjostrom, 1966; Dolly and Fottrell, 1969). From a biochemical point of view, there are three groups of peptidases.

a) Dipeptidases: Several dipeptidases glycy1-glycine peptidase, leucyl-glycine peptidase, glycy1-leucine peptidase, and imino- and imido-peptidases—which hydrolyse proline containing peptides (Smith, 1955a). These peptides generally require the presence of metal ions for full activity and are inhibited by EDTA (Ugolev, 1965).

b) Aminopeptidase: An aminotripeptidase hydrolyses the N-terminal residue from tripeptides. Although this enzyme has not been purified from the intestinal mucosa, in other tissues the tripeptidase has negligible activity against di- or tetra-peptides (Smith, 1955b; Schwabe, 1969). It is not inhibited by EDTA, and at least a proportion of this peptidase is localized to the brush border region of the enterocytes (Peters, 1968).
c) **Aminooligopeptidase**: An aminooligopeptidase hydrolyses longer peptides (Light, 1967). This enzyme is at least partially localized to the brush border, and although it has not been purified from intestinal mucosa, it is known that brush borders can hydrolyze peptides of at least six amino acid residues (Peters *et al.*, 1969). The enzyme cleaves the aminoacid residues sequentially from the N terminal end of the peptide (Peters *et al.*, 1970). The aminooligopeptidase of the brush border is apparently not inhibited by EDTA and has not yet been shown to be distinct, in the intestinal mucosa, from aminoo-
tripeptidase or leucyl B naphthylamidase (the "leucine aminopeptidase of the histochemists.

iii) Alkaline phosphatase

Intestinal alkaline phosphatase is a glycoprotein anchored in the apical brush border membrane by a glycosyl phosphatidyl-inositol linkage (Engle et al., 1995). Intestinal AP can hydrolyze different substrates, including phosphatidates with various fatty acyl chains, inorganic phosphate provided by the diet as polyphosphates, and phosphate residues of nucleotides, including adenosine triphosphate (ATP) (Wilkes et al., 1987). Intestinal AP can also catalyze the synthesis of diphosphate from inorganic phosphate and transphosphorylate thiamine to thiamine monophosphate (Millan, 2006). Intestinal AP is strategically located at the interface between the food, the microbiota, and the host.

Intestinal alkaline phosphatase (IAP) plays multiple biological roles in the maintenance of intestinal homeostasis (Lalles, 2010). IAP regulates lipid absorption across the apical membrane of enterocytes, participates in the regulation of bicarbonate secretion and of duodenal surface pH, limits bacterial transepithelial passage, and finally controls bacterial endotoxin-induced inflammation by dephosphorylation, thus detoxifying intestinal lipopolysaccharide. IAP has a pivotal role in intestinal homeostasis, and its activity could be increased through the diet. This is especially true in pathological situations (e.g., inflammatory bowel diseases) in which the involvement of commensal bacteria is suspected and when intestinal AP is too low to detoxify a sufficient amount of bacterial lipopolysaccharides (Lalles, 2010).

Longitudinally, AP mRNA and activity in rodents are present at very low levels in the stomach and the colon (Koldovsky, 1969) and found to be the highest in the duodenum, and decrease sharply from there to the jejunum and ileum (Moog, 1953). Therefore, AP distribution runs inverse to bacterial
colonization in the intestines. In that respect, the AP activity gradient is opposite (ileum > duodenum) in germ-free pigs, and it normalizes only after bacterial colonization.

II. Absorption/Transport

Monosaccharides such as glucose and galactose were transported across the brush border membrane by a carrier mechanism which co-transport Na$^+$ (Crane et al., 1961) (Fig. 8). The energy required for sugar transport is derived from the flux of Na$^+$ at high concentration outside to low concentration inside the cell, the gradient being maintained by the Na$^+$-ATPase pump in the basolateral membrane. Na$^+$ dependent transport has been demonstrated for a wide variety of substances including sugars, amino acids, bile salts, and some vitamins and ions. Transport of some macromolecules is affected by receptor-mediated endocytosis. Thus vitamin B12- intrinsic factor complex binds in a Ca$^{2+}$ dependent manner to an ileal microvillar receptor protein before absorption by endocytosis (Robertson and Gallagher, 1985). A similar mechanism is thought to be responsible for the absorption of immunoglobulin-G in the neonate (Mackenzie, 1984).

III. Regulatory functions

In addition to its role in digestion and absorption, the brush border is associated with some regulatory functions. The brush border is known to contain at least two Ca$^{2+}$ and cyclic nucleotide-sensitive transport systems, viz Na-Cl cotransport (by linked Na/H' and Cl /HCO$_3$ exchange) and an electrogenic Cl channel (Donowitz and Walsh, 1987) while a number of potential regulatory proteins have been found there. These include guanylate cyclase, cyclic AMP-dependent protein kinase, a brush border specific, 86 kDa G-kinase, 25 and 21 kDa phosphoproteins acting as cosubstrates for the A- and G-kinases, several phosphoprotein substrates for a Ca$^{2+}$ and calmodulin-dependent kinase and the Ca$^{2+}$ and phospholipid-dependent protein kinase C; several G-proteins including the oncogene-related p21 ras; and phospholipase
A (De Jonge et al., 1975). There is thus the potential for both direct regulation - for example, by the cyclic AMP- or GMP-stimulated phosphorylation of a component of a transporter molecule, and for indirect processes mediated through the release of second messengers such as Ca\(^{2+}\) acting, for example, via protein kinase C and the phosphatidylinositol cascade system (Donowitz and Walsh, 1987; De Jonge et al., 1975). Except in certain enteropathic diarrhoeas, however, it is unclear which of these mechanisms operate *in vivo*.

Intestinal motility and transit time

Most of the alimentary canal of both humans and rats is surrounded by at least two layers of smooth muscle. The muscle fibres of the inner layer are arranged circumferentially relative to the lumen; those of the outer layer are arranged parallel to the long axis of the canal (DeSesso and Jacobson, 2001). The coordinated, rhythmic contractions of these layers of smooth muscle cause the intestinal motility which is responsible for the thorough mixing of chyme, the continual rejuxtaposition of chyme with the brush border of the enterocytes and
the propulsion of food through the GI tract in a net aboral direction (peristalsis). In addition to the external layers of smooth muscle, there is a thin layer of smooth muscle associated with the lamina propria (muscularis mucosae) that causes the intestinal villi to undulate, thereby agitating the layer of fluid that is associated with the brush border of the enterocytes (DeSesso and Jacobson, 2001).

Gastrointestinal (GI) motor activity consists of an intricate group of functions that are essential for life (Huizinga and Lammers, 2009). A number of techniques have been employed to evaluate GI motility and measuring intestinal transit rate is one of the widely followed techniques. GI transit can be quantified in rats, by measuring the movement of charcoal, dye, radiopaque markers or other non-absorbable materials (Enck and Wienbeck, 1989; Baggio et al., 2003). In our study the intestinal motility was determined in terms of intestinal transit rate by measuring the movement of dye front according to the method of Anitha et al., (2006).

Transit time is the amount of time taken for a bolus of food or chyme to traverse a region of the alimentary canal. The time for chyme to traverse the small intestine of rats is approximately 3-4 h. The velocity of transport is faster in the proximal segments of the small intestine (duodenum and proximal jejunum) than in the distal segments (Marcus and Lengemann, 1962). The transit time through the large intestine of rats has been reported to be approximately 15 h (Enck et al., 1989). The reported values for transit time are subject to great variations depending on many factors including health status, age and fasting state.

Most of the absorption of substances takes place during the time that chyme is in the small intestine. Absorption of some fluid and electrolytes, as well as products of bacterial digestion of otherwise indigestible materials, takes place while chyme remains in the large intestine (DeSesso and Jacobson, 2001). In general, an increase in the intestinal transit time will increase the absorption of poorly or incompletely absorbed substances. However, this is not
always true. For instance, some substances increase transit time by inhibiting intestinal smooth muscle motility. While inhibition of peristalsis does increase intestinal transit time, it also inhibits the movements of the intestine that mix chyme and agitate the unstirred layer of fluid. These movements are especially important for the absorption of lipophilic substances because without them, the unstirred layer forms a barrier between the brush border and the micelles that contain the lipophilic substances (DeSesso and Jacobson, 2001).

**Oxidative stress in the small intestine**

The intestine being situated at the interface between the organism and its luminal environment, it symbolizes a crucial defence barrier against luminal lethal agents. Hence, in addition to being exposed to luminal nutrients, the intestinal mucosa is continuously challenged by diet-derived mutagens, oxidants and carcinogens in addition to endogenously generated reactive oxygen species (Ames, 1983). The intestine possesses several defence mechanisms to maintain cellular integrity and tissue homeostasis, such as the ability to maintain high antioxidant concentrations, to up-regulate antioxidant enzymes systems, and to provoke cell death by apoptosis to dispose of injured or depleted enterocytes (Aw, 1999).

Importantly, a number of gastrointestinal diseases are associated with reactive oxygen species (ROS) and oxidative stress (Grisham et al., 1994; Parks, 1989; Pavlick et al., 2002). ROS-mediated damage to the small intestine has been confirmed in several conditions such as inflammatory bowel disease and ischemia/reperfusion (Halliwell and Gutteridge, 1999), surgical stress (Prabhu et al., 2000) and, radiation enteritis (Mutlu-Turkoglu et al., 2000). In fact, the gastrointestinal mucosa is repetitively exposed to luminal oxidants from ingested foods (Cross et al., 1984; Grisham et al., 1987; Parks, 1989) and, despite the antioxidant properties of its mucus lining, there is a continuous production of oxidative stress (Grisham et al., 1994). Evidently, the ingestion
and/or occurrence of peroxides may have implications for human health, mainly in the long term.

Glutathione is a tripeptide (γ-Glu-Cys-Gly) present in high concentrations in tissues (Kaplowitz et al., 1985; Shan et al., 1990), including the intestine (Shan et al., 1992; Aw and Williams, 1992; Aw, 1994). Cellular glutathione homeostasis is maintained through de novo synthesis (cysteine and methionine), through regeneration from glutathione disulfide (Kaplowitz et al., 1985; Shan et al., 1990), and through glutathione uptake from exogenous sources. The ability of intestinal cells to transport luminal glutathione has important implications for the control of intestinal thiol redox balance, especially under oxidative conditions.

Cells are susceptible to oxidation by the excessive accumulation of intracellular glutathione disulfide during oxidative stress and this altered thiol-disulfide status together with the resultant oxidation of protein sulphhydrals has intense effects on metabolic processes. These include distorted specific cell cycle responses, damaged functions of a variety of enzymes and proteins, and activity of transcription factors, such as NF-kB (Brigelius, 1985). It is obvious that the loss of redox balance matches directly to the development of cellular oxidative stress and the resultant redox imbalance could have detrimental consequences for cell integrity, metabolic regulation, and organ homeostasis. These conditions are particularly applicable to the intestine given that the intestinal epithelium is often challenged by dietary oxidants. Imposition of a severe oxidant stress normally results in cytotoxicity. Sub toxic oxidative stress can induce the phase transition of a cell from a quiescent state to a proliferative, apoptotic, or necrotic state. It has long been recognized by scientists studying cell cycle responses that the entry of cells into proliferation or death is governed by regulatory genetic or environmental barriers (Beach et al., 1988).

In recent studies, it was reported that human intestinal cells respond differentially to the amount of oxidative stress. At mild oxidant concentrations (<10 mmol/L), intestinal proliferative activity increases up to a point; at higher
oxidant stress (10–50 mmol/L), cells die by apoptosis. At even higher concentrations of lipid peroxides (>100 mmol/L), significant necrotic cell death was observed (Cepinskas et al., 1994). The concentrations of lipid peroxides found in foods cooked in oils or fats (2–15 mmol/L) can generate mucosal oxidative stress and redox imbalance, which can have far-reaching effects on intestinal metabolic homeostasis. Together, these results reveal that the lipid peroxide–induced intestinal cell proliferation or death exhibits a clear bell-shaped function that is directly dependent on cellular redox status (Aw, 1999).

**Small intestine and inflammation**

In the gastrointestinal tract, inflammation and oxidative stress are the significant routes highly concerned in many disorders (Grisham, 1994). Intestinal inflammation is a chronic condition of the intestine with indefinite etiology involving various immune, genetic and environmental factors. A good correlation between the action of free radicals (O₂, H₂O₂, OH) in the intestine and the clinical disease has been established indicating the importance of these factors in the inflammatory process (Nielsen and Ahnfelt-Ronne, 1991). The role of oxidants in arbitrating inflammatory reactions in an array of experimental models is well recognized and has been intensely studied. Lipid peroxidation is proposed to be a major mechanism involved in inflammatory bowel diseases (Thomson et al., 1998).

In the intestine, damage to the epithelium ensuing from an inflammatory response is usually considered as a secondary event. The primary event is the systematic inflammatory surge of neutrophil adherence to vascular endothelial cells, interruption of the endothelial barrier, and following infiltration of inflammatory cells into the intestinal interstitium, where proteases and oxidants are released and induce mucosal injury (Aw, 1999). The extent to which diet-derived lipid peroxides contribute to systematic inflammation is not known.

Histologically, the intestinal inflammation is characterized by the infiltration of macrophages, monocytes and polymorphonuclear leukocytes.
They are activated by different intermediaries including cytokines, leukotrienes prostaglandins and platelet-activating factor to produce and release reactive oxygen metabolites (Simmonds and Rampton, 1993; Lih-Brody et al., 1996). Among the mediators involved in gastrointestinal inflammation, NO has been found to play a decisive role in several animal models. For example, treatment with the inhibitor of NO synthases (NOS), NG-nitro-L-arginine methyl ester (L-NAME), attenuates iodoacetamide (Rachmilewitz et al., 1995) or peptidoglycan (polysaccharide) (Grisham et al., 1994) induced colonic inflammation. Further, it has been shown that murine inducible NOS (iNOS) deficient mice do not develop colitis after instillation of the hapten trinitrobenzene sulfonic acid (TNBS) (Zingarelli et al., 1999).

Myeloperoxidase (MPO) is an enzyme present in neutrophils and its activity in the intestine and colon is directly proportional to the infiltration of neutrophils. The measurement of MPO activity is well established for quantification of intestinal inflammation (Krawisz et al., 1984). In the case of inflammatory conditions like IBD, the content of neutrophils in inflamed tissues, and subsequently MPO enzyme, increase. Acetic acid-induced colitis is an easily inducible model of IBD, and the similarity of the inflammatory mediators report to IBD indicated that the inflammatory phase bears some similarity to human intestinal inflammation (Elson et al., 1995).

**Paraoxonase 2 (PON 2) in intestine**

Paraoxonase (PON) was initially identified as an enzyme capable of hydrolyzing organophosphate compounds; now there are evidences that it plays an antioxidant and anti-inflammatory role (Aviram and Rosenblat, 2005). Its distinct members (PON 1, PON 2, and PON 3) are believed to be powerful attenuators of oxidative damage and highly atheroprotective (Getz and Reardon, 2004; Li et al., 2003). PON 1 and PON 3 are expressed in the liver and excreted in the blood where they are linked with the high-density lipoprotein (HDL) particle (Reddy et al., 2001; Mackness et al., 1985). PON 2 is not present
in blood, but is expressed widely in a number of tissues, including the liver, lungs, brain, heart and intestine (Mochizuki et al., 1998). The distribution of PON 2 in many tissues implies the likelihood of its playing an antioxidant role (Draganov et al., 2000). PON 2 appears to be cell-based and, thus, is a good nominee for preventing oxidative stress within cells (Horke et al., 2007). Despite the growing interest in PON 2, there is modest information about its characteristics and functions in the gastrointestinal system.

PON 2 may be particularly involved in fighting the potentially proinflammatory flora and prooxidant diet that confront the intestinal epithelium, since the addition of purified PON 2 to permeabilized intestinal Caco-2/15 cells protected against iron/ascorbate (Fe/Asc)-induced oxidative stress (Levy et al., 2007). However, whether PON 2 is an antioxidant or anti-inflammatory player in the digestive tract remains unclear.

Studies have been conducted to highlight the role of the ubiquitously expressed PON 2 in the small intestine by generating a consistent intestinal model of PON 2 knockdown (PON 2 KD) in the human intestinal Caco-2/15 cell line that ablated the expression of PON 2 gene and protein by 80 % (Précourt et al., 2012). PON 2 silencing did not affect cell integrity, viability, tight junctions, and differentiation, as an expression of sucrase, villin, and occludin was not affected. However, oxidative balance seemed to be disturbed in PON 2 KD cells, given the increase in SOD activity and a fall in CAT activity. As a result, H$_2$O$_2$ levels were found to be increased in the culture medium of PON 2 KD cells. Cells with PON 2 KD were also more vulnerable to oxidative stress, as demonstrated by simultaneous higher MDA level and a reduced GSH-to-GSSG ratio. The inflammatory response was aggravated in PON 2 KD cells, since TNF-α and IL-6 expression was increased after Fe/Asc-induced oxidative stress compared with Mock cells. The proinflammatory transcription factor NF-kB was also over activated after LPS challenge. In fact, the involvement of PON 2 in the antioxidative defense and anti-inflammatory response in the intestinal cell would
suggest a potential role for PON 2 in pathophysiological conditions such as IBD (Précourt et al., 2012).

**Role of Acetylcholine (ACh) in the small intestine**

Activities of the motor neurons in the gut wall are coordinated by the enteric nervous system to generate functional movement, such as peristalsis. Acetylcholine (ACh) is the most common neurotransmitter and in the gastrointestinal tract, ACh is released from the primary excitatory motor neurons and mediates an immediate smooth muscle contraction (Goyal and Hirano, 1996; Furness, 2000). Although the excitatory enteric neurons co-release other transmitters, such as tachykinins (Holzer and Holzer-Petsche, 1997), ACh is believed to be functionally predominant in inducing contractions (Goyal and Hirano, 1996; Furness, 2000).

ACh can act as an intercellular messenger in parallel of its task as a neurotransmitter (Yajima et al., 2011). At epithelium level, ACh also controls proliferation, mucus secretion, cytokine production and movement. ACh regulates gut motility, blood flow, and immune cell activation within the lamina propria. The situation becomes even more intricate when one considers that not only the epithelium can produce ACh, but other cells, like immune cells and fibroblasts, also have this capability. Hence, ACh cannot be considered merely as a neurotransmitter but rather as a universal intercellular messenger that is likely to be significant in integrating many different aspects of intestinal physiology in health and disease (Keely, 2011). Certainly, the cholinergic nervous system is frequently referred to as the 'cholinergic anti-inflammatory system' since ACh has been revealed to play a protective role in animal models of inflammatory bowel disease (Tracey, 2007). Interestingly, short chain fatty acids (SCFAs) have similar advantageous effects, and the question arises as to exactly how much of a role non-neuronal ACh plays in mediating these actions.
Factors affecting intestinal health

In addition to exposure to skin and respiratory system, the gastrointestinal tract provides a source of contact to a variety of chemicals (Pfeiffer et al., 1977). The kind of substances include materials derived from ingested foods and liquids, food additives, food contaminants, pesticides, drugs and bacterial and fungal species which proliferate within the intestine. Epithelium is a primary membrane exposed to pesticides when these compounds enter the body through the oral route with foods (Schulenburg et al., 2004). There is a quickly growing body of knowledge concerning the effects of ingested substance on intestinal function (Spicer, 1975). Epidemiological data has been able to identify intestinal illness associated with gross contamination and in occasional instances there is evidence for many hundreds of chemical substances, ranging from insecticides, pesticides to heavy metals causing contamination of the environment and entering the digestive tract from the mouth (Lu et al., 2006). Their target effects on the small intestine are, if any, unknown.

Pesticides and intestinal health

There is continuous debate concerning the role of pesticides in many chronic human health effects. In spite of strict legislation to limit the presence of insecticide residues in food, there is growing concern about their safety and how their residues in food may affect human health. Exposure of the general population during the consumption of foodstuffs treated improperly with pesticides or harvested prematurely before residues have declined to tolerable levels or from domestic use have been reported in several countries. Epidemiological studies indicate that, despite premarket testing, current pesticide exposures are associated with risks to human health (Lu et al., 2006). The acute adverse human health effects of insecticides include neurological, dermatological, gastrointestinal and respiratory manifestations. Continual effects thought to include neurotoxicity, reproductive, carcinogenesis, and
developmental effects which are more difficult to demonstrate. The residues of insecticides present in the crops, water and soil after use get into the human food chain. Ingestion of pesticide residues through food and water has been linked to birth defects, toxicity to the fetus, genetic defects, cancers, blood disorders, neurotoxicity and endocrine disruption (Bhushan et al., 2013).

A study conducted on farm gate samples of commonly consumed vegetables comprising brinjal, okra, cauliflower, cabbage, knol-khol, summer squash, smooth gourd, cucumber, pea and potato revealed that 26% samples contained residues above MRL values (Kumari et al., 2004). The contamination was mostly with organophosphates subsequently synthetic pyrethroids and organochlorines. Among organophosphates, residues of monocrotophos, chlorpyrifos and quinalphos exceeded the MRL value in 23% samples. Residues of monocrotophos were elevated than MRL value in 3 samples of brinjal and one sample each of cauliflower, okra and smooth gourd. Similarly, chlorpyrifos residues were found in higher amounts in 3 samples of cauliflower and 8 of cabbage and quinalphos in one sample each of okra and cauliflower. Amongst synthetic pyrethroids, cypermethrin was the key contaminant and its residue exceeded the MRL value in one sample each of brinjal, okra and cucumber.

In another study, vegetable samples (Cabbage, Brinjal, Beans and Carrot) grown in Kolar district of Karnataka, India were tested for 20 pesticide residues (Ananda Gowda and Somashekar, 2012). All the samples were found to be contaminated. The contamination was mostly with organochlorines (OC) (97%), followed by organophosphates (83%) and pyrethroids (60%). The residues of OPI exceeded the maximum residue limits (MRL) value in 58% samples i.e. 2% dichlorvos, 6% monocrotophos, 14% chlorpyrifos and 36% Phorate. OCs and SPs did not exceed the MRL value in any sample. This exhibits the shift from OC to OP and SP insecticides and the restricted use of OC insecticides.
Chronic ingestion of pesticide residues has been reported to induce inflammation of the gastrointestinal mucosa, which represents the first protective barrier of the organism against the effects of ingested noxious compounds. Such a hypothesis has already been put forward for Ulcerative Colitis (Crotty, 1994). Hence, even the small amounts of pesticides that we are constantly being exposed to are probably toxic to the alimentary tract. If this toxicity produces diseases of the intestine, then like all toxicity syndromes the effects must range from acute to chronic with consequences that may be immediate or long-term. Some of these toxic effects may be very common, others exceedingly rare, occurring only in the genetically susceptible individuals. Several studies established relationship between gut inflammation and infections (Nakajima et al., 1997), toxins (Geboes and Ectors, 1995), or genetic factors (Satsangi et al., 1997), but the involvement of environmental and dietary factors, while often suggested (Bing et al., 1998) has never been clearly demonstrated. Recently it has been shown that repeated low level exposure to a food contaminant, diquat, at doses possibly found in food (0.1 mg/kg/d per os) induces a mild gastrointestinal inflammation associated with mast cell hyperplasia in rats (Anton et al., 2002).

Listed below are few pesticides/ insecticides (organochlorine, carbamate, pyrethroid class) and their effects on the intestinal epithelium and/or brush border enzymes.

**a) DDT:** Studies have shown that single oral dose of DDT (100 mg/kg b.w) induced considerable alteration in the functional and chemical architecture of brush border membrane (Dudeja and Mehmood, 1982). This study showed that activities of brush border sucrase, alkaline phosphatase and Na\(^+\), K\(^+\) - ATPase were significantly reduced in DDT treated rats. DDT treatment was found to enhance the synthesis of lipid constituents of the membrane in particular to that of phospholipids. A considerable enhancement of total lipids, phospholipids and triglyceride contents was evident in the microvilli membranes of DDT treated rats.
b) Lindane: A considerable decrease in weight gain was observed in chickens injected with lindane at 30 mg/kg b.w. (Moreno *et al.*, 1995). In the intestinal mucosa of treated chickens, total lipids especially phospholipids were increased. Lindane treatment also decreased alkaline phosphatase activity in all intestinal segments whereas, maltase and sucrase activities were decreased. Histological observations revealed a significant rise in the number of crypt mitotic cells in animals treated with pesticide. In addition, lindane treatment induced an increase in intestine mucosal DNA and RNA levels.

c) Endosulfan: Single oral dose of endosulfan administration (5 mg/kg b.w) to rats was reported to significantly elevate the uptake of glucose and alanine (Wali *et al.*, 1982). The activities of brush-border sucrase and alkaline phosphatase were significantly enhanced while the activity of Na\(^+\), K\(^+\)-ATPase was reduced. There was a major decrease in cellular LDH and GOT activities with no change in GPT activity.

d) Carbofuran: Carbofuran, a carbamate, when administered to rats (4 mg/kg b.w for 7 days and 2.8 mg/kg b.w for 30 days) was found to increase the activities of sucrase, alkaline phosphatase, leucine aminopeptidase and \(\gamma\)-glutamyl transpeptidase in the intestine (Gera *et al.*, 2009). Intestinal histology showed disruption of the villi and comet assay showed disintegration of DNA in enterocytes of animals exposed to carbofuran. The above findings suggest that carbofuran toxicity may alter digestive functions in rat intestine.

e) Esfenvalerate: Intragastric administration of esfenvalerate to rats showed functional alterations in the gastrointestinal tract at different time points after a single oral administration (Varró *et al.*, 2014). Effects on the GI tract were studied by analyzing the motility and excitability of isolated ileum segments. Both spontaneous and acetylcholine (ACh)-elicited contractions were modified by treatment. Esfenvalerate increased the amplitude of contractions in the low ACh concentration range. From the study, it can be concluded that a relatively high, single oral dose exerted mild and temporary effects on the intestine functions of the rat.
Organophosphorus insecticides (OPI)

In recent times though OPI are the most widely used class of insecticides, very limited studies have been done on their impact on the small intestine. Impact of few OPI like malathion, chlorpyrifos, diazinon, DDVP on the small intestine is summarized in Table 1.

Table 1 Effect of different OPI on small intestine

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Animal Model</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazinon</td>
<td>Guinea pig</td>
<td>Total protein &amp; general carbohydrates ↓ Histology (changed)</td>
<td>Rady, 2009</td>
</tr>
<tr>
<td>Malathion</td>
<td>Rat</td>
<td>Sucrase &amp; ALP ↑ Na⁺, K⁺ - ATPase ↓ LDH, glucose-6-phosphatase ↑ Transaminases ↓</td>
<td>Wali et al., 1984</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Caco-2</td>
<td>Barrier integrity affected</td>
<td>Tirelli et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Caco-2</td>
<td>MDR gene expression ↓ Efflux transporter function ↓</td>
<td>Cetinkaya and Baydan, 2010</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>Interaction study between chlorpyrifos (CPF) and ethephon (ETF), a plant growth regulator - Agonistic interactions with regard to potency of ACh</td>
<td>Agarwala et al., 2004</td>
</tr>
<tr>
<td>DDVP</td>
<td>Fish</td>
<td>Proteolytic activity ↓ Carbohydrase activity - no change</td>
<td>Golovanova et al., 1999</td>
</tr>
</tbody>
</table>

Diseases of the small intestine

In recent times, gastrointestinal (GI) disorders are on the increasing globally. However, the etiological factors contributing towards most of the disorders of the intestine such as, irritable bowel syndrome, Crohn’s disease, ulcerative colitis, inflammatory bowel disease and GI bleeding are not evidently understood. Altered food habits, increased stress levels, and environmental factors are hypothesized to play major roles (Blumberg and Strober, 2001).
**GI bleeding:** GI bleeding is a symptom than a disease and can happen anywhere in the digestive system. Common grounds of bleeding in the small intestine include ulcerations due to inflammation and ulcers in the duodenum from ulcerative colitis or Crohn’s disease (Thompson et al., 1999).

**Crohn’s disease:** Crohn’s disease is a persistent condition that induces inflammation in the lining of the small intestinal wall. It occurs in the lower part of the small intestine – ileum but can have an effect on any part of the digestive tract. Symptoms include abdominal pain, weight loss, diarrhea and rectal bleeding. The cause is unidentified, but the most accepted theory is that the immune system is reacting to a virus or bacterium that induces inflammation (Thompson et al., 1999).

**Ulcerative Colitis:** Ulcerative Colitis induces inflammation and ulcers in the upper layers of the lining of the large intestine, although it can occur in the small intestine. Symptoms like rectal bleeding, weight loss and diarrhea are similar to Crohn’s disease. Inflammation occurs in the rectum and lower part of the colon, but can influence the entire colon. The cause is not known, but the immune system is speculated of reacting to a virus or bacterium which causes inflammation in the intestinal wall (Thompson et al., 1999).

**Irritable Bowel Syndrome:** Irritable Bowel Syndrome (IBS) is one of the main general functional GI disorders. Though the symptoms of IBS may appear analogous to ulcerative colitis and Crohn’s disease, it does not induce intestinal bleeding, inflammation or lead to cancer. IBS is exemplified by persistent abdominal pain, bloating and altered bowel function such as constipation and diarrhea, or irregular pattern between the two. It mainly affects people in their late-teens to early 40s and may run in families (Thompson et al., 1999).
Other diseases / disorders affecting intestinal health

There are many diseases wherein intestinal structure and functions are altered, and Parkinson’s disease (PD) and Diabetes mellitus (DM) are few among them.

Gastrointestinal dysfunction has been reported as the most commonly seen non-motor feature of PD (Pfeiffer, 2003). Even though PD has conventionally been considered as a disease of dopaminergic neurons in the substantia nigra, pathological examination of brain and gastrointestinal samples from PD patients have recommended neuronal loss in other areas also (Braak et al., 2006). PD patients experience symptoms which cover the whole alimentary tract including dysphagia, abnormal salivation, delayed gastric emptying, constipation, and defecatory malfunctions (Pfeiffer, 2003). Altered motility is the pathophysiological reason underlying many of these symptoms. Dysmotility accounts directly to the morbidity of PD and complicates the clinical management of the disease. The precise mechanism of motility dysfunction in PD is poorly understood. Lack of understanding of the alterations in the GI tract in PD has led to partial success in the treatment of GI dysfunction in PD.

Diabetes mellitus

Diabetes mellitus (DM) which is emerging as a major health problem in the world is a syndrome of disordered metabolism. However, diabetes is also accompanied by several morphological and functional changes in the small intestinal mucosa and elevated levels of digestive enzymes. Experimental diabetes in rats has been reported to increase the enzymatic activity of many brush border hydrolases including the disaccharidases (Caspery et al., 1972), stimulate several transport systems in the membrane, as well as alter normal histology of intestine. DM has also been associated with enhanced intestinal disaccharidase activity and enhanced glucose absorption in humans (Tandon et al., 1975). Studies have also demonstrated the occurrence of oxidative damage in the intestine during experimental diabetes in rats (Bhor et al., 2004).
Diabetes mellitus and gastrointestinal tract

Diabetes mellitus usually associated with hyperglycemia, which affects multiple organs particularly if it remains for a prolonged period either in type 1 or type 2 diabetes mellitus (Rodrigues and Motta, 2012). Since diabetes mellitus affects every organ system, it affects the gastrointestinal tract as well. Complications involving the GI tract are the main reason for morbidity in patients with diabetes mellitus (Talley et al., 2001). Even though there are many differences in type 1 and type 2 diabetes, studies have shown that the gastrointestinal tract function is compromised in both the types, affecting the digestive process, the motility and nervous control of the entire digestive system (Bener et al., 2012; Bernstein, 2000). Mechanisms that may lead to GI complications in diabetes include autonomic neuropathy, poor glycemic control, diabetic micro angiopathy, altered production of insulin and glucagon and increased susceptibility to GI infections (Taub et al., 1979; Goyal and Spiro, 1971). Hyperglycemia damages the nerves and also disrupts the blood supply to the nerves in the GI tract (Ordög et al., 2009; Kashyap and Farrugia, 2010). The damage to the nerve functions ultimately affects the motility of the gut inducing incomplete emptying of the different parts of the GI tract. In addition, it can also cause malabsorption.

Relatively little is known about the effect of diabetes on intestinal epithelium in the absence of autonomic neuropathy. It is well recognized that diabetes induces oxidative stress and that the resulting oxidative damage has a key role in the development of diabetic complications (Son et al., 2004). Within the small intestine, diabetes is associated with numerous changes, including hyperplasia and hypertrophy of epithelial cells (Zoubi et al., 1995), increased absorption of sugars and amino acids (Fedorak, 1990) and increased endogenous cholesterol synthesis (Feingold et al., 1990). Inflammatory cytokines are also increased in diabetes (Esposito et al., 2002).
Diabetic patients are prone to severe gastric injury and impaired ulcer healing (Konturek et al., 2010; Harsch et al., 2003). It has been reported that severe gastric ulcer or inflammation can affect gastric motility in diabetic patients (Boehme et al., 2007). Healing mutilation of persistent ulcers in diabetes mellitus has been credited to release of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and the reduced activity of the mucosal antioxidative system (Brzozowska et al., 2004). *Helicobacter pylori*, an organism associated with ulcer has been observed to have a high prevalence amongst diabetics (Tseng, 2012).

**Diabetes mellitus and small intestine**

Small intestinal dysfunctions are reported to be common in diabetes compared to esophageal or gastric dysfunctions. A decrease in the intestinal tone has been reported in diabetic patients that is due to an increase in cholinergic activities and decrease in adrenergic receptor activation (Anjaneyulu and Ramara, 2002). The result is seen as rapid transit of the small intestine and is well recognized in animal studies (De Freitas et al., 2008). Structural and functional changes observed in the small intestine of diabetic patients are responsible for the impairments of motility, altered transit time and compromised secretory and absorptive functions. Studies show that increase in the sorbitol production leads to changes in the intracellular osmolality, which finally leads to neuronal death (Zanoni et al., 2002). Diabetes stimulates the functional activity of the intestinal brush border membrane with enhancement of both hydrolytic enzyme activity and membrane transport systems.

Experimental diabetes is reported to be associated with a variety of functional changes in the intestinal brush border membrane. There is reportedly an increase in the total and specific enzymatic activity of several membrane-bound hydrolases including sucrase, maltase, lactase, trehalase, leucyl naphthylamidase, and alkaline phosphatase (Olsen and Rogers, 1971; Younoszai and Schedl, 1972; Caspary et al., 1972). In addition, enhancement of
active transport mechanisms for monosaccharides (Pauls and Drury, 1942; Crane, 1961), amino acids (Olsen and Rosenberg, 1970), sodium (Rosenberg and Schultz, 1971), and bile acids (Caspary, 1973) has been reported; that this enhancement is also the result of a membrane effect is suggested by the recent observation of increased glucose transport by isolated brush border membrane vesicles prepared from diabetic animals (Hopfer, 1975).

From animal studies it is established that, diabetes induced rats show hyperplasia and hypertrophy of mucosal and submucosal layers of the small intestine (De Freitas et al., 2008). In addition, hyperplasia and hypertrophy of the villi epithelial cells has been reported (Zoubi et al., 1995). Increased levels of digestive enzymes (Sharma and Sivakami, 1998), elevated absorption of sugars, amino acids (Fedorak, 1990), increased endogenous synthesis of cholesterol and triglycerides (Feingold et al., 1990) and reduced fluidity of the brush border membrane are some of the changes noticed in the small intestine under diabetic condition. Also, the occurrence of oxidative stress in the small intestine during diabetes has been reported (Bhor et al., 2004).

Clinical examinations have revealed that, the histological alterations observed in diabetic condition are because of angiopathy in the intestinal mucosa of diabetic patients and are thus associated with autonomic neuropathy. These alterations that are deeply associated with decreased mesenteric perfusion are atrophic in nature with thick walls and possible change in permeability (De Las Casas and Finley, 1999; Kandemir et al., 1995). Any disruptions in the intestinal mucosal cell integrity will modulate its functional ability as a surface barrier to avoid the passage of possible harmful agents. The increase in permeability of the small intestine to potentially dangerous substances in diabetic condition has also been documented in human studies and is related to its functional abnormality (de Kort et al., 2011).