# Section 7

## LECTURE

### Intestinal Pathophysiology – Parts I and II

Anatomy, Physiology, and Biophysical Chemistry of terminal Digestion

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## INTRODUCTION

### A. The intestinal epithelium performs 4 major tasks:

Establishes a barrier that separates two distinct compartments
Terminal digestion of nutrient solutes
Vectorial transport of solutes and solvents
Innate and aquired mucosal immunity

### B. Structural and Functional Compartmentalization of the Epithelial Lining.

1) Linear distribution of gross structure and function:
Small intestine: 6-7 meters in length

a) Duodenum--immediately distal to pylorus that demarcates change from gastric to intestinal histology, site of entry for pancreatic juices and hepatic secretions, large surface area to linear length.

b) Jejunum and ileum--distal to ligament of treitz, remainder of the small intestine; site of transition from jejunum to ileum is not based on anatomic landmarks, rather the terms describe a gradual transition in gross anatomy and microscopic histology with progressively decreasing surface area to unit length ratios. The jejunum has clear submucosal folds, termed plicae circularis, which can be distinguished by contrast radiography. The distal ileum exhibits prominent lymphoid nodules, termed Peyers Patches.

c) The functional unit of the small intestine is the crypt-villus unit.

i.)The epithelial cells on the villus exhibit a phenotype dedicated to "terminal digestion" and absorption of solutes and water.

ii.) The epithelial cells of the crypt exhibit active cell division and a phenotype dedicated to secretion of solutes and water.

iii) Six crypts surround each villus (like the barrel of a "six-shooter"), and each crypt harbors a monoclonal stem cell that gives rise to all cell lineages along the crypt villus axis. Thus, the villus contains epithelial cells derived from six different stem cells, which differentiate as they move up the crypt villus axis. One cell lineage, the Paneth cell, moves downward to the base of the crypt.

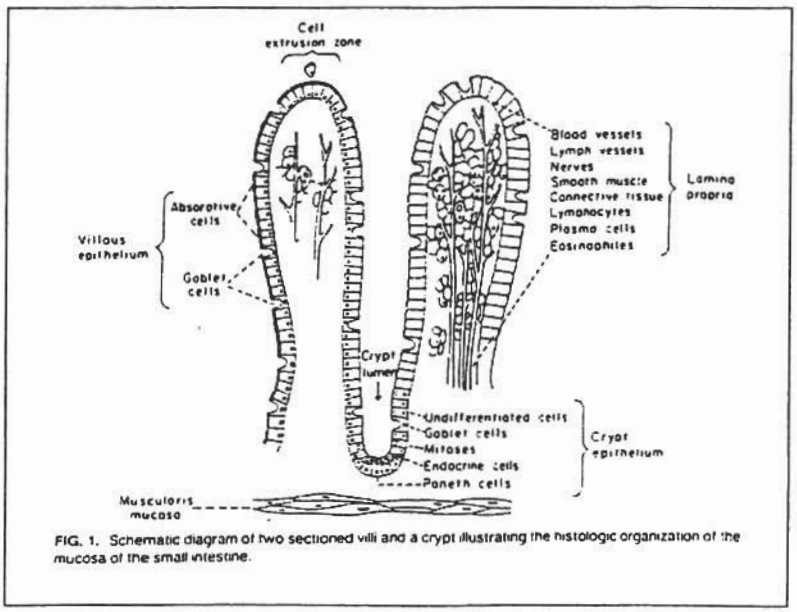


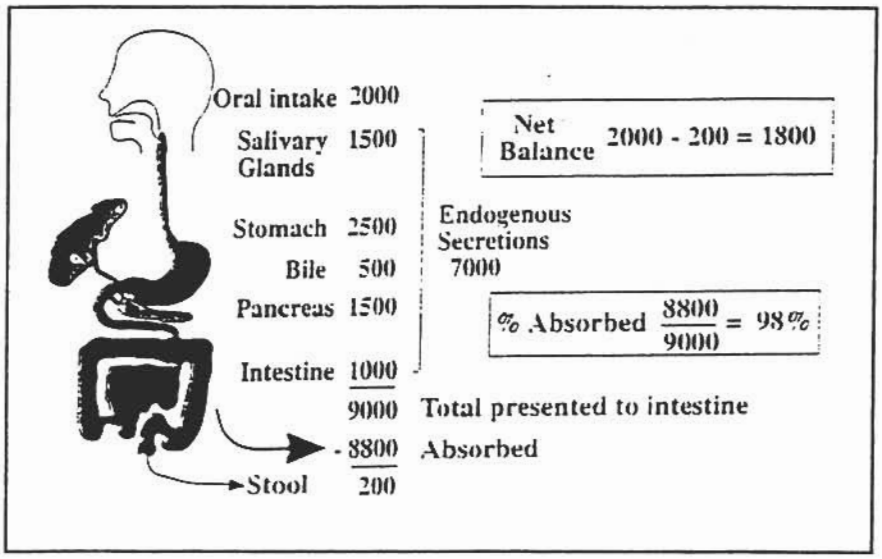

FIG. 1. Schematic diagram of two sectioned villi and a crypt illustrating the histologic organization of the mucosa of the small intestine.

d) In the ileum, the focally scattered sites of follicular dome epithelium represent a minor structural variant of the crypt-villus unit. Here the functional unit is in the shape of a dome rather than a villus. The dome epithelium contains a specialized cell type termed the M-cell.

d) The ileocecal valve demarcates the transition from small intestine to colon and regulates the discharge of lumenal contents into the colon. It also acts as a barrier to prevent reflux of colon contents (which contain much higher density of "normal microbial flora") into the small intestine.

Colon: 1.5 meters in length. The colon has no plicae circularis or villi. The functional unit is the crypt and surface epithelium.

**C. Net intestinal absorption is the sum of two <u>larger</u> unidirectional solute and water fluxes:** inside to out, and outside to in.



**D.** Under physiologic conditions the intestine is highly efficient in the net absorption of solutes and water. As shown in the table below, the absorptive efficiency of water, ions, protein and fat, is greater than 95%. These are actually gross underestimates of the mass of water and solutes absorbed by the intestine. This is due the fact (as stated above) that the intestine not only actively absorbs but also secretes water and solutes.

| | Input (food, drink, intestinal secretions) | Output (stool) |
|---|---|---|
| Protein: | ~200 gm | ~1-2 gm |
| Diet ~100 gm | | |
| Epithelial Sloughing ~50 gm | | |
| Secretions: ~50 gm | | |
| Fat: | ~170 gm | ~2-6 gm |
| Diet ~100 gm | | |
| Epithelial Sloughing ~20 gm | | |
| Bile lipids ~50 gm | | |
| Water: | ~10 L | ~0.05 L |
| Diet ~2 L | | |
| GI tract secretions ~8 L | | |
| Ions: | ~2.6 Mol | ~0.015 Mol |
| (Na+, K+, Cl-, Ca+2, Fe+2) | | |
| Diet ~200 mMol | | |
| Secretions ~2.4 Mol | | |
| Total Mass: | ~22 Kg | ~0.2 Kg |

## E. Clinical Manifestations of Intestinal Failure

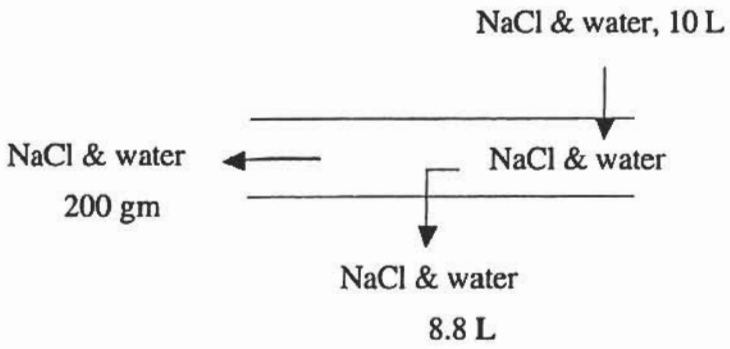
Diarrhea: too much water in the stool

Secretory Diarrhea: Large volume, high salt content, continues in the presence of fasting

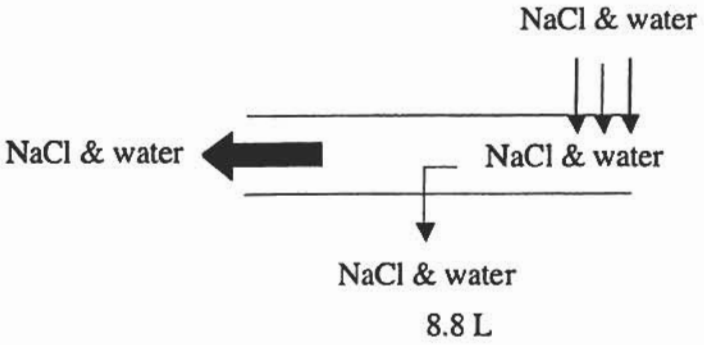
Osmotic Diarrhea: low salt content, small volume to bulky sized stools

Inflammatory Diarrhea: typically small to moderate volume, leukocytes and possibly blood in stool

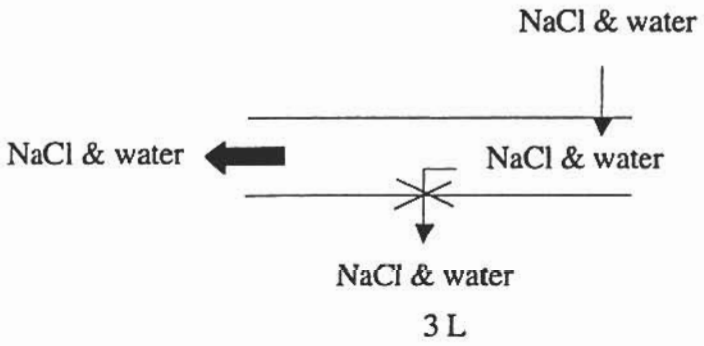
Malabsorption: nutrient deficiency that cannot be explained by abnormal dietary intake. May occur with or without diarrhea. May occur with or without growth failure in children or loss of weight in adults.



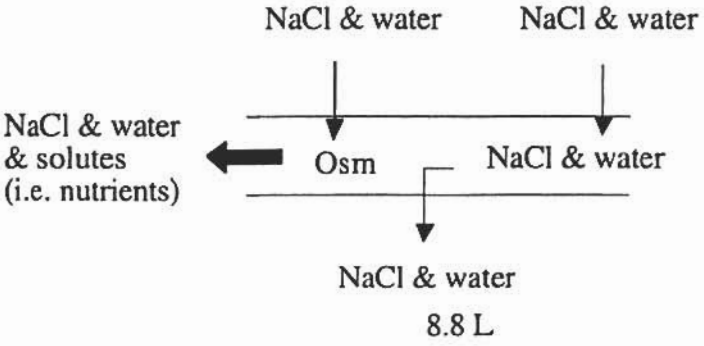

**Normal**




**Secretory Diarrhea I**
(ie Cholera)




**Secretory Diarrhea II**
(ie Intestinal Damage)




**Osmotic Diarrhea**
(ie Lactose Intolerance)

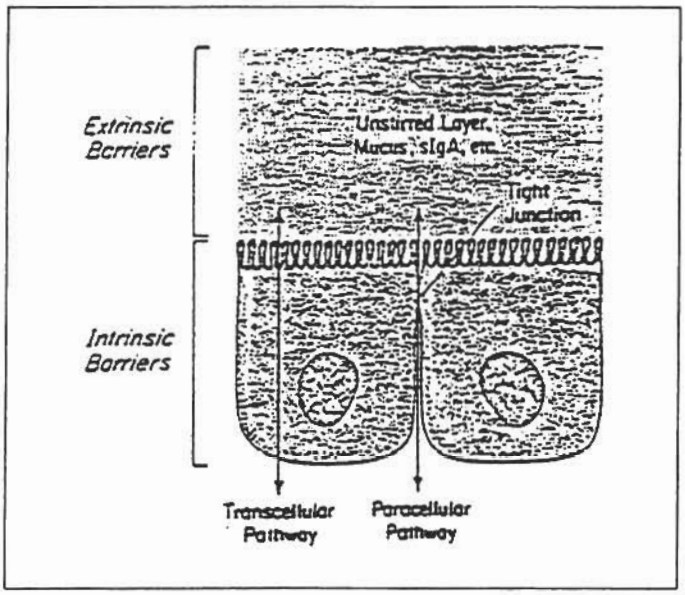
## BARRIER FUNCTION

**A)** For efficient vectorial transport of nutritive substances, the intestine must first prevent the unrestricted passive uptake of nutritive solutes and water. The intestine must also prevent unrestricted passive uptake of noxious compounds.

Such potential threats present in the lumen include:
1) Acid ($H^+$) discharged from the stomach into the proximal duodenum.
2) Intact food Proteins (small intestine) - oligopeptides containing 7-11 amino acids can be antigenic
3) Digestive enzymes: for example, pancreatic elastase can degrade laminin - a key element of the basement membrane needed to support epithelial cells and vascular endothelial cells in the intestinal wall.
4) Pathogenic and non-pathogenic bacteria and Pro-inflammatory compounds (ileum and colon) such as:
   a) Lipopolysaccharide (Endotoxin) from bacterial cell walls which activate inflammatory cells
   b) N-formylated peptides (chemotactic compounds) derived from bacteria
   c) V. cholera, Salmonella, Shigella, Yersinia

### B) General Structural features Promoting Barrier Function

The intestinal barrier can arbitrarily be divided into one of two general categories: Those extrinsic to the epithelial cell (although often produced and maintained by the epithelium itself), and those intrinsic to the epithelial cell.




1) **Extrinsic barrier**: the microenvironment overlying epithelial cells.
   a) Mucus, sIgA, IgG, cryptdins, lysozyme, bacterial barrier, glycocalyx
   b) Unstirred layer: a water layer devoid of convection, important to consider when performing kinetic measurements of uptake of a molecule by absorptive cells, of unclear physiologic significance
   c) Secreted $HCO3^-$ (duodenal surface cells): a buffer barrier to $H^+$
   d) Hydrophobic layer: a putative extracellular phospholipid layer imparting surface hydrophobicity. Questions remain as to the extent to which this potential barrier is induced by the method used to demonstrate it (e.g., measurement of contact angle between blotted intestinal surface and a droplet of water)

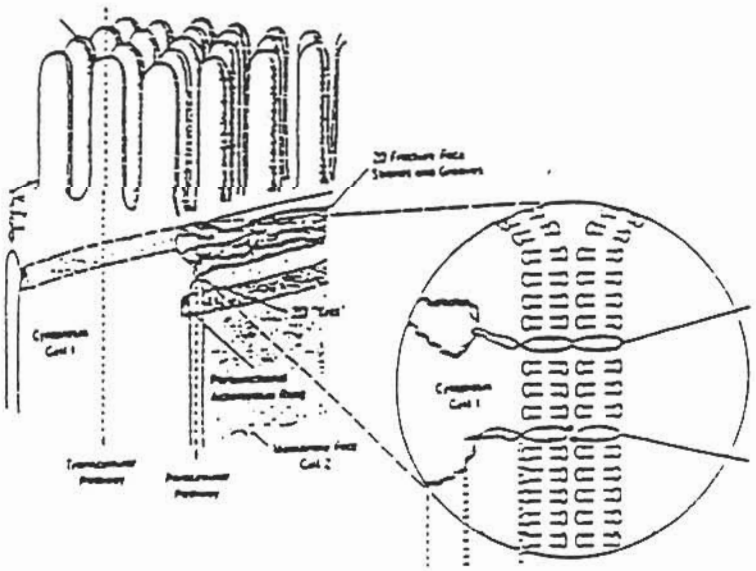
2) **Intrinsic barrier** (i.e. the epithelial monolayer itself): the continuous monolayer of epithelial cells represents the major restrictive barrier to passive diffusion of water, solutes, and microorganisms across the intestinal mucosa.

3) There are two ways for solutes and water to cross the epithelial barrier: by "passive" flow around cells (the paracellular pathway), and by passive or active flow through cells (the transcellular pathway). These pathways are fundamentally different. Both pathways are regulated.

**C) The Transcellular pathway:** very restrictive to passive flow of hydrophilic solutes. This pathway across the epithelial cell crosses not only the cytoplasm of the epithelial cell but also two plasma membranes (apical and basolateral). The plasma membranes consist of a lipid bilayer. Such bilayers are highly impermeable to hydrophilic solutes. For example, the resistance to passive ion flow across model lipid bilayers ranges from $10^6$-$10^9$ ohm $cm^2$. With the addition of integral membrane proteins (biomembrane) the resistance falls to $10^3$-$10^4$ ohm $cm^2$ - still a much higher value than the range of epithelial resistance seen across the composite epithelial monolayer ($5x10^1$ - $3x10^2$ ohm $cm^2$). The reflection coefficients of these biological membranes to inert solutes with Stokes radii >4Å, approximates one (i.e. almost totally impermeable). The ability of these membranes to retard passive flow of hydrophilic solutes is the basis of the ability of the cell to maintain the transmembrane ion gradients, generated by pumps, which are important for active transport.

Mechanisms of transport across the transcellular pathway can be passive, active (i.e. dependent on an ATP-ase pump), or "secondarily active (i.e. dependent on the potential energy stored by the cell created "secondarily" by the action of the Na/K ATPase pump.

**D) The Paracellular (shunt) pathway:** the major transepithelial passive permeation pathway. Consists of apical tight junction and subjunctional paracellular space - ~85% passive transepithelial ion permeation occurs through paracellular route - Almost all passive transepithelial permeation of hydrophilic solutes >4Å Stokes radius takes place via the paracellular route. The barrier to unrestricted diffusion across the paracellular pathway is represented by the intercellular junction that forms a circumferential "gasket" around each epithelial cell, sealing one cell to another.



1) Molecular structure of intercellular junctions: the paracellular pathway:

a) Zonae Occludens (the tight junction): is the structure that represents the rate-limiting barrier for passive diffusion of solutes and water across the paracellular pathway. The tight junction consists of a series of "kisses" or fusions between lateral membranes of adjacent cells. The molecular structures include: review refs: *Am J Physiol Gastrointest Liver Physiol.* 279:G250-254, 2000. *J Cell Biol.* 149:13-16, 2000. .

1) Occludin ≈ 65 kDa essential but not sufficient
2) Claudins ≈ 23 kDa (18 different isoforms) essential component
3) JAM not essential

3) ZO1, ZO2, ZO3
4) Cingulin 140 kDa
5)Actin

b) Molecular hypothesis of tight junction structure:

1) specific claudins for specific tissues in specific combinations
2) tightness (ie permeability) of TJ determined by the number/type of species of claudins and their mixing ratio in strands
3) Hypomagnesemia 2° to claudin-16/paracellin gene defect *Science.* 285:103-106, 1999

b) Zonae Adherens (Intermediate Junction or Belt Desmosome). The biogenesis and maintenance (and thus function) of the tight junction depends on formation and maintenance of the intermediate junction.

a) E-cadherin (uvomorulin)
b) a and b catenins
c) f-Actin and myosin II

c) Spot Desmosome

a) Desmoglein/Desmoplakin
b) cytokeratins, tonofilaments

2) Physiology of the tight junction and transport across the paracellular pathway:

a) the tight junction separates the apical from the basolateral membrane domains of the intestinal epithelial cell, and thus contributes fundamentally in the biogenesis and maintenance of cell polarity that is essential for vectorial transport.
b) the tight junction is cation selective (important for intestinal secretion, see below) $PK^+:PNa^+:PCl^- = 1.14:1.00:0.55$
c) tight junctions are more restrictive (i.e. less permeant) as one proceeds distally: in the small intestine 80-90% of passive ion permeation is paracellular. In the colon 50-60% is paracellular due to greater paracellular restriction. (Effective radii for solutes crossing tight junctions in jejunum, ileum and colon - 7.5-8, 3- 3.5, and 2-2.5 Å, respectively)
d) permeability through tight junctions can be regulated (see below).
e) Tight junctions interface with the cytoskeleton, probably via tight junction specific proteins (ZO-1, cingulin), and the cytoskeleton likely mediates tight junction function.

**E) Clinical correlates**: Breakdown of barrier function occurs with any disruption of the epithelial monolayer.

1) Protein Losing Enteropathy: Virtually any insult if severe enough can manifest in protein losing enteropathy

a) Exaggeration of the normal secretion of serum proteins, particularly albumin, into the intestinal lumen. Intraluminal digestion of serum proteins is followed by absorption of amino acids and subsequent resynthesis of serum proteins. When loss into gut is sufficiently great, synthesis fails to maintain serum protein levels. The result is a contracted body protein pool that is turning over rapidly.

b) Laboratory studies show:
- i) Hypoproteinemia, particularly hypoalbuminemia, without proteinuria
- ii) Rapid removal of $I^{131}$-labeled albumin from serum
- iii) Increased fecal radioactivity following intravenous injection of $Cr^{51}$-labeled protein
- iv) In severe cases, lymphopenia and impaired immune responses

c) Protein losing enteropathy may be observed in a wide variety of diffuse gastrointestinal disorders but is particularly seen in intestinal lymphangiectasia, hypertrophic gastritis (Menetrier's syndrome), severe right-sided heart failure, non-topical sprue, Whipple's disease.

2). Clostridium difficile toxin; ZOT toxin; V. cholerae HA protease

3) Na+/glucose transport

4) ulcer, inflammation

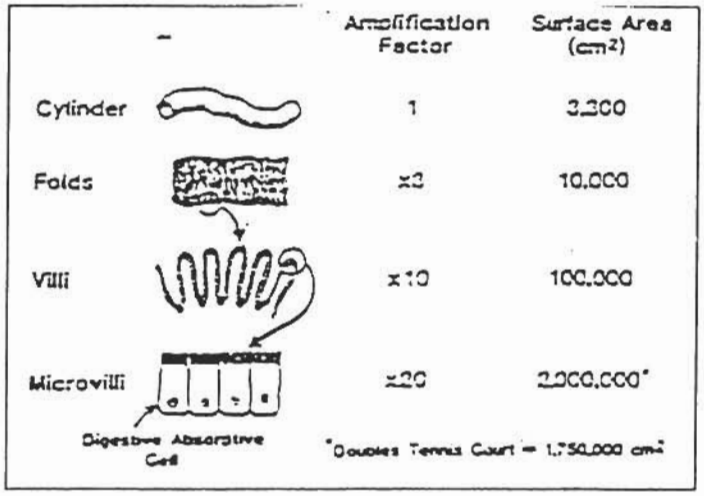
# TERMINAL DIGESTION, ABSORPTION, and SECRETION

## General Comments on Nutrient Digestion

Many essential functions of the GI tract can be viewed in terms of enzyme kinetics (substrate specificity, enzyme and substrate concentration, time, and temperature).

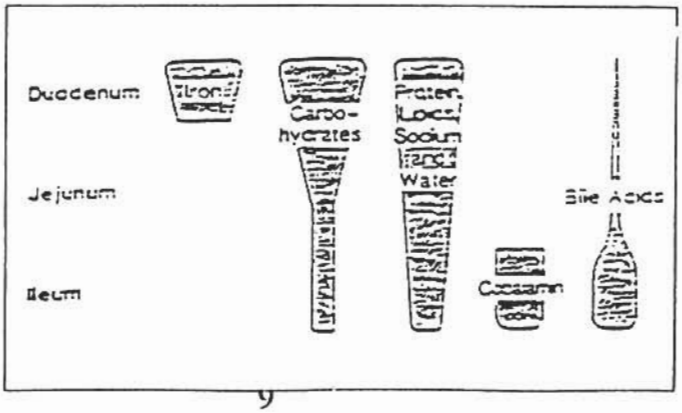
### A) General structural features promoting absorption and digestion:

1) Expansion of surface area (see Figure)
   a) The surface area of the small intestine (6-7 meters long) is highly amplified by folds (plicae circularis), microscopic villi, and microvilli on cells to a total surface area of 2 million cm2 (larger than a doubles tennis court).
   b) The colon, ~1.5 meters in length, has no plicae circularis or villi. The major expansion to the surface area here is the microvilli of the surface absorptive cells.




2) Regional specializations promoting absorption (Fig)
   a) Specializations having gross or ultrastructural correlates: jejunum has less structurally complex tight junctions than ileum. This is thought to be the basis for the greater effective pore radius of the paracellular pathway in the jejunum (see below). This increased "leakiness" proximally promotes passive bulk uptake by solvent drag (discussed below) that accompanies active transport and thus permits the jejunum to be the major site of bulk transport.

   b) Specializations with structural correlates at the molecular level: Regional specializations of absorption occur due to regional variation in expression of integral membrane proteins of intestinal absorptive cells.



3) Intestinal Motility: a general physiologic feature promoting absorption and digestion:

In many ways, intestinal absorption and digestion can be viewed as a variation on enzyme kinetics. Thus the concentration of "enzyme" (i.e. density of hydrolases and transporters on brush border together with the surface area of intestine) and the TIME of incubation between substrate and "enzyme" are fundamental determinants of the reaction. Thus, intestinal motility, which determines transit time as substrates flow through the intestine, modulates net intestinal absorption or secretion.

**Clinical correlates:**

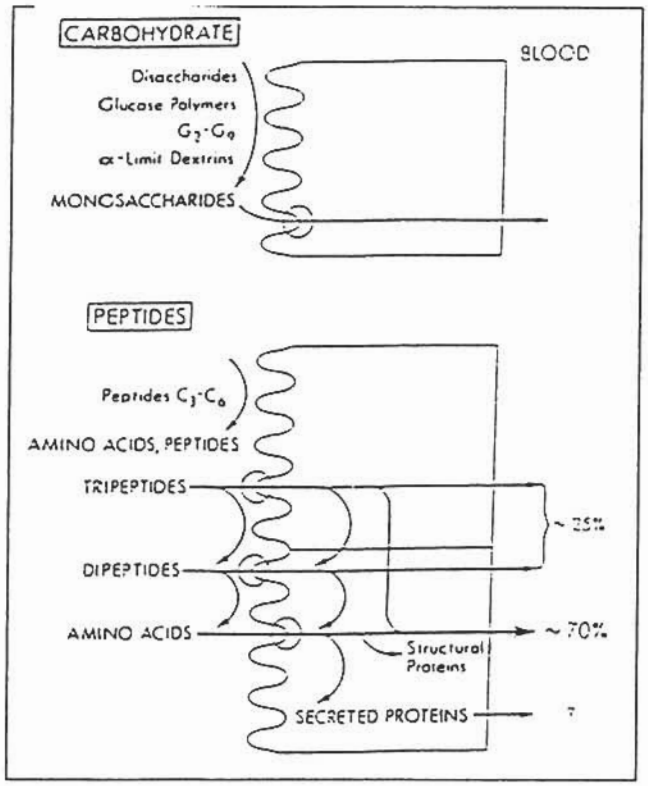
Loss of surface area by surgical resection
Loss of enzyme concentration by infection or celiac disease
Loss of "time" due to hypermotility

**LUMINAL DIGESTION** (salivary glands, stomach, pancreas, bile salts)

Clinical correlates: cystic fibrosis

## TERMINAL DIGESTION

**A) Protein and carbohydrates**: Proteolytic enzymes, oligopeptidases, and nucleotidases are present on the brush border of absorptive villus epithelial cells and are also found intracellularly. These enzymes complete the hydrolysis of proteins and complex carbohydrates to single amino acids, di- or tri-peptides, and simple monosaccharides that are transported across the cell membrane. Disaccharides, such as sucrose and lactulose, do not have an intralumenal phase of digestion, and hydrolysis of these substrates is entirely dependent on brush border enzymes.




**B) Clinical correlates**:

1) *Sucrase - isomaltase deficiency:* Inherited recessive disorder, manifested in infancy with pain, bloating, watery diarrhea, acidic and gassy stool from bacterial fermentation of unabsorbed sucrose and maltose. Enzymes absent from brush border.

2) *Lactase deficiency:* two forms, congenital and acquired.
   a)*Constitutional* - heritable in children and adults - 50% of certain population (Bantu, American Blacks, Greeks, etc); 10-15% of healthy white Americans
   b)*Acquired* - Secondary disaccharidase deficiency and, therefore, impaired monosaccharide transport in any small intestinal mucosal disease (e.g. celiac sprue or surgical reduction in digestive-absorptive surface). Clinical presentation similar to above exhibited with milk and milk products (e.g. ice cream). Diarrhea in each case can be corrected by dietary substitution of monosaccharides for offending disaccharide. Laboratory findings include:
      1) flat blood glucose curve (<25 mg%) after ingestion of 50 g of disaccharide but normal curve after ingestion of 50 g of component monosaccharides
      2) Low fecal pH (organic acid production)
      4) Elevation of breath $H_2$ after disaccharide ingestion
      5) Low enzyme activity in jejunal mucosal biopsy

## ABSORPTION and SECRETION

**A) Water**: Mechanism(s) remain incompletely defined. Water moves across both transcellular and paracellular pathways. Water channels may be involved but thus far no evidence for direct effect on intestinal physiology

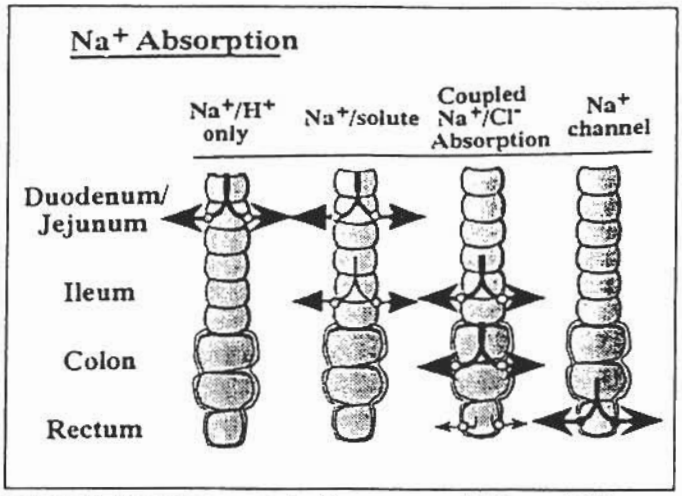
> Ma, T., and A.S. Verkman. 1999. Aquaporin water channels in gastrointestinal physiology. *J Physiol (Lond)*. 517:317-326.
> Fluid transport is a major function of the gastrointestinal (GI) tract with more than 9 litres of fluid being absorbed or secreted across epithelia in human salivary gland, stomach, the hepatobiliary tract, pancreas, small intestine and colon. This review evaluates the evidence that aquaporin-type water channels are involved in GI fluid transport. The aquaporins are a family of small (approximately 30 kDa) integral membrane proteins that function as water channels. At least seven aquaporins are expressed in various tissues in the GI tract: AQP1 in intrahepatic cholangiocytes, AQP4 in gastric parietal cells, AQP3 and AQP4 in colonic surface epithelium, AQP5 in salivary gland, AQP7 in small intestine, AQP8 in liver, pancreas and colon, and AQP9 in liver. There are functional data suggesting that some GI cell types expressing aquaporins have high or regulated water permeability; however, there has been no direct evidence for a role of aquaporins in GI physiology. Recently, transgenic mice have been generated with selective deletions of various aquaporins. Preliminary evaluation of GI function suggests a role for AQP1 in dietary fat processing and AQP4 in colonic fluid absorption. Further study of aquaporin function in the GI tract should provide new insights into normal GI physiology and disease mechanisms, and may yield novel therapies to regulate fluid movement in GI diseases.

1) Water transport is strictly dependent on solute transport. The motive force is osmolarity and high rates can be driven with motive forces of 1 mOsm or less

2) Recent evidence indicates that water may move together with certain solutes as they are transported across the cellular membranes via protein transporters -- SGLT1 as a water pump.

3) Colon: $Na^+$ and water reabsorption: stool dehydration

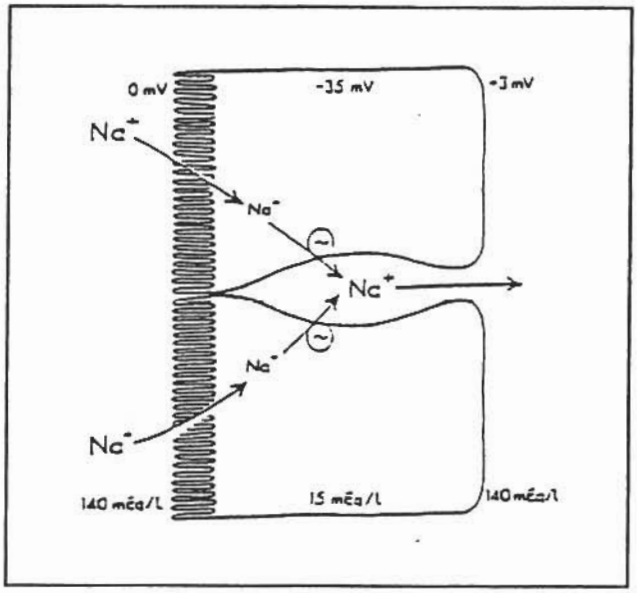
**B) $Na^+$ Transport**: (secondary active transport)




a) $Na^+$ is most important ion transported in GI tract. It is essential for the absorption of water, $Cl^-$, bicarbonate, sugars, amino acids, dipeptides, and perhaps most $H_2O$-soluble nutrients including water soluble vitamins.

b) Transcellular $Na^+$ transport is dependent on asymmetry of the transport proteins in the plasma membrane. (i.e. on cell polarity)

c) As shown in Figure xx, driving force for transmembrane $Na^+$ transport is supplied by $Na^+$ pump that is located at basolateral membrane, requires metabolic energy, ATP, and Mg and is inhibited by ouabain, a cardiac glycoside.




d) $Na^+$-$K^+$-ATPase is the $Na^+$ pump. It is located almost entirely in the basolateral membrane and is not found in the brush border. Inhibition of $Na^+$-$K^+$-ATPase by ouabain (cardiac glycoside) abolishes net $Na^+$ transport both in vivo and in vitro.

e) The pumping of $Na^+$ out of the cell lowers the intracellular $Na^+$ concentration. Three moles of Na are probably pumped out of the intestinal epithelial cell for every two moles of $K^+$ pumped in and this helps explain the electronegativity of the cell and the transepithelial potential difference. Movement of $Na^+$ across the brush border into the cells thus occurs passively down its electrical (-35 mV) and chemical gradient (125 mM). This is referred to as the "$Na^+$ gradient hypothesis".

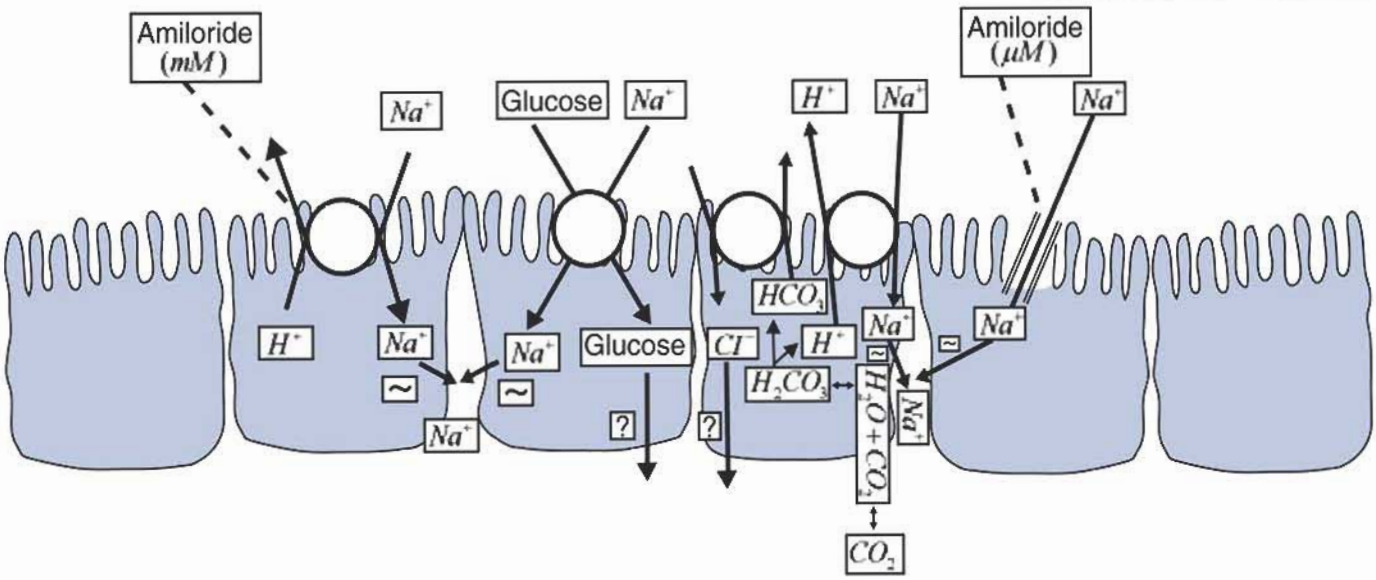
f) Movement of $Na^+$ across the brush border is facilitated by brush border carriers. $Na^+$ moves across the brush border in at least 4 ways (see Figure):

i.) Via $Na^+$ channel (in colon, Figure xx) - electrogenic

ii) Via $H^+$ exchange coupled to $Cl^-$-$HCO_3^-$ exchange (small intestine, Figure 8b) - electroneutral

iii) Via nutrient-coupled uptake (Figure xc, small intestine) - electrogenic

iv) Via $Na^+$ $H^+$ exchange (Figure xd, throughout, prominent in jejunum) - electroneutral




(D) (C) (B) (A)

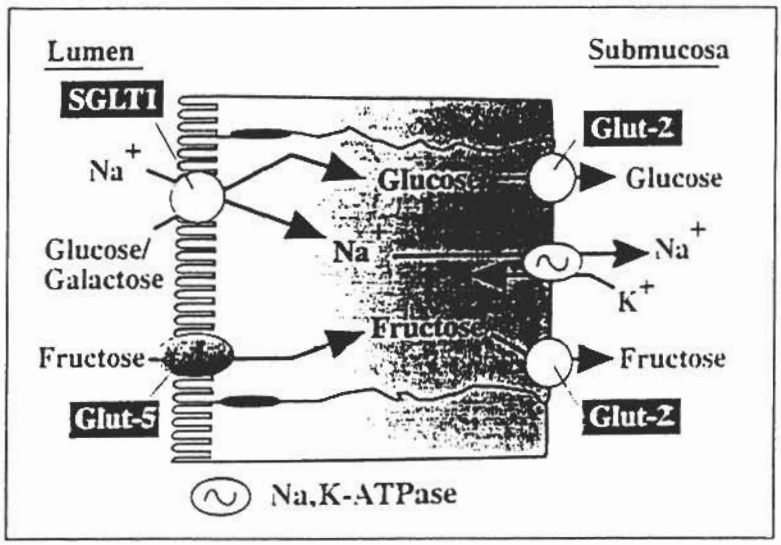
g) Na+ also moves across the epithelial barrier via the paracellular shunt by diffusion and "solvent drag" (see explanation below); $Na^+$ does not move across the basolateral membrane into the epithelial cell (no carrier protein).

h) Regional differences of intestinal $Na^+$ transport in man:

| | Jejunum | Ileum | Colon |
|---|---|---|---|
| Active transport | + | + | + |
| Solvent drag (major importance) | + | + | ? |
| Glucose increases $Na^+$ absorption | + | + | no |
| $Na^+$ xchanged for $H^+$ | + | + | + |
| Minimal $Na^+$ concentration developed (mEq/L) | 130 | 75 | <20 |
| Aldosterone increases $Na^+$ absorption | no | no | + |
| Methylprednisone increases $Na^+$ absorption (in rat) | no | no | + |

**C) Glucose transport**: (secondary active transport)

1) SGLT1 (70-75 kD) transports glucose and galactose across the apical brush border membrane. SGLT1 has distinct $Na^+$ and glucose binding sites each with distinctive kinetics (glucose Km ≈0.75 mM; $Na^+$ Km ≈80 mM). Transport stoichiometry is 2Na : 1 Glucose.




a) Galactose also is a substrate

b) Binding of $Na^+$ and glucose results in conformational change which results in internalization of these substrates

c) The energy for this conformational change is provided by the electrochemical gradient favoring $Na^+$ entry into cells (intracellular $Na^+$ <20 mM and intracellular compartment -40 to -60 mV relative to cell exterior)

d) This electrochemical gradient favoring $Na^+$ entry is generated by the basolateral $Na^+$-$K^+$ ATPase pump (3 : 2 stoichiometry). Thus, by ATP expenditure on the basolateral pump, energy is provided, largely in the form of a transmembrane voltage, which drives $Na^+$ into the cell. As will be shown, many nutrients are absorbed by $Na^+$-linked co-transport process

2) Glucose transport out of the cell across the basolateral membrane occurs by facilitated transport across GLUT2 (the GLUT family of glucose transporters includes the insulin-sensitive glucose transporter GLUT4). (passive transport)

a) GLUT 2 exhibits broad substrate specificity and will transport nearly all monosaccharides (galactose, fructose, but not α-methyl D-glucopyranoside)

b) Km for glucose is 20 – 80 mM

c) driving force is glucose chemical gradient inside>outside cell, passive equilibration, no energy required

d) when no glucose is in the lumen and interstitial (i.e. blood) glucose is higher than the intracellular concentration, glucose passes into cell via GLUT2 (facilitated pathways in the basolateral membrane are routes to provide metabolic substrate in the interprandial state)

3) Fructose transport into the cell across the apical brush border membrane occurs by facilitated transport across GLUT5

a) GLUT 5 also transports Xylose, which is commonly used clinically to assay for intestinal function (see below).

b) driving force is the chemical gradient for fructose.

4) Clinical correlates

a) *Glucose-galactose malabsorption:* Very rare congenital defect in monosaccharide transport. Watery diarrhea with acid stools in infants with flat blood glucose curves following oral ingestion of glucose, galactose, lactose or maltose. Normal biopsy with normal disaccharidase activity. Diarrhea corrected by dietary substitution of fructose (facilitated transport).

b) *Xylose absorption:* Very useful lab test to detect mucosal disease of small intestine. Xylose, like other pentoses, does not share in the glucose-galactose pathway. At high luminal concentrations, xylose is absorbed by diffusion. Absorption relatively inefficient so that only about one-half of a 25 gm oral load is absorbed. Tissue, especially hepatic, metabolism of xylose is also slow so that only about one-half of the absorbed is degraded; the remainder is excreted in the urine. Thus, following a 25 gm oral load, 5-8 gm is normally excreted in the urine.

c) Oral Rehydration Salts

d) celiac disease

**D) Concept of Solvent Drag**: Contribution of Solvent Drag to the paracellular absorption electrolytes, nutrients, and water

1) As a consequence of activation of $Na^+$-nutrient co-transporters on the apical membrane, absorptive cell tight junctions become "leaky" and exhibit increased permeability to nutrient and peptide-sized molecules.

2) Since water crosses the tight junctions in response to the intercellular osmotic pressure generated by active transcellular transport of $Na^+$, nutrients and electrolytes will also be absorbed by solvent drag.

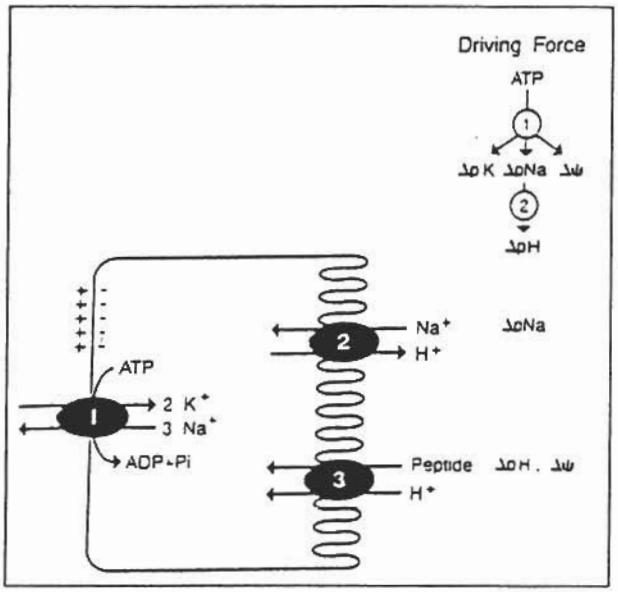
3) Convective movement of molecules across a pore (here a tight junction) by solvent drag is likely to be a major contributor to net absorption. This is due to the fact that while diffusive movement relates to the $r^2$ of a pore, convective movement relates to the $r^4$ of a pore (from Fick's First law of diffusion and Poiseuille's law for laminar flow respectively).

4) It is likely at least 50% of water absorption occurs across tight junctions and as a result of the related solvent drag, approximately 50% of $Na^+$ absorption and a sizable fraction (unclear how much) of nutrient absorption also occurs by this route.

**E) Amino acid and peptide absorption**: (secondary active transport)

1) Peptides. Oligopeptides (di, tri and perhaps tetra peptides) can be taken up across the brush border of absorptive cells, hydrolyzed intracellularly, and released as free amino acids. The transporter has recently been cloned, PepT1 and transport is dependent on an inwardly directed proton gradient. Transport of small peptides across the basolateral membrane may also occur. However, oligopeptides can also cross tight junctions by solvent drag during absorption and in two species of mammals oligopeptides have been detected in portal blood (representing 20-50% of absorbed amino acids).

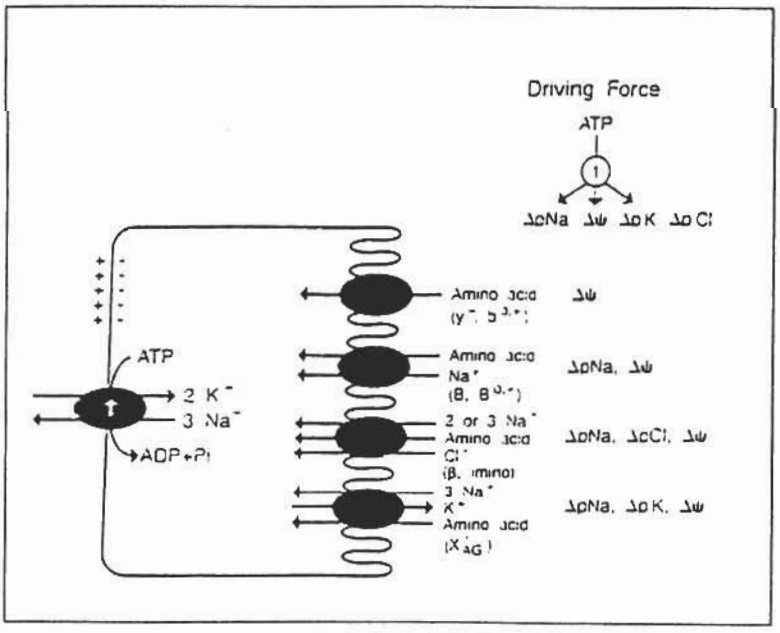
2) Amino acid transport:




a) There are several different classes of amino acid cotransporters. The driving forces for most are $Na^+$ dependent, but this may be indirect. For example, some transport is coupled directly to Na uptake (as for SGLT1), but others are driven by membrane potential alone, and various combinations of $Na^+$, $Cl^-$, $K^+$, $H^+$ electrochemical gradients and membrane potential.

b) Multiple integral membrane proteins (functionally defined) are likely to participate. For example, in non-intestinal cells five types of $Na^+$-dependent systems for neutral amino acids uptake have been defined. Current concepts would include intestinal transportation for:

i) neutral amino acid transport system
ii) a phenylalanine and methionine transport system
iii) Imino acid transport system - very uncertain area




c) Movement of amino acids across the basolateral membrane
1) Tends to be

$Na^+$-independent, facilitated-type transport as for glucose across GLUT2

2) Exception for L-glutamine. L-glutamine is the preferred metabolic substrate for absorptive cells and may be absorbed basolaterally by a $Na^+$ dependent process. A $Na^+$ independent facilitated exit pathway is also present to allow glutamine absorption under conditions in which intracellular glutamine is higher than necessary for metabolic needs (e.g. after a meal)

**TABLE 2.** *Classification of amino acid transport systems in brush-border membrane of the small intestine*

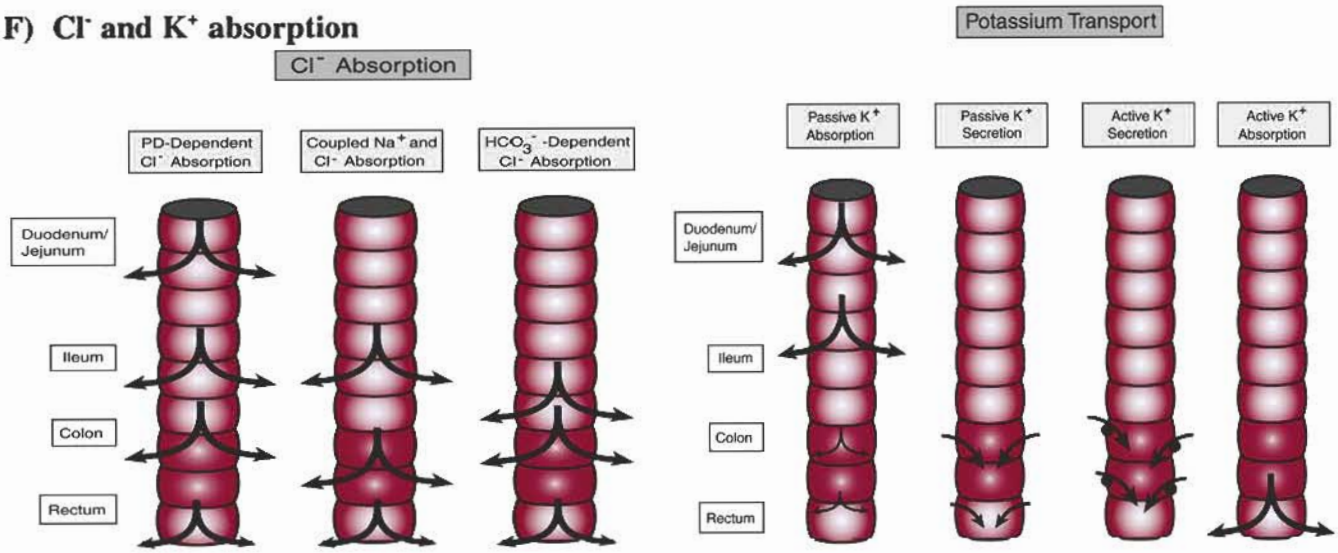
| Transport system | Substrates | Dependence on $Na^+$ gradient | Involvement of other ions |
|---|---|---|---|
| B | Dipolar α-amino acids | Yes | None |
| $B^{0,+}$ | Dipolar α-amino acids | Yes | None |
| | Basic amino acids | | |
| | Cystine | | |
| $b^{0,+}$ | Dipolar α-amino acids | No | None |
| | Basic amino acids | | |
| | Cystine | | |
| $y^+$ | Basic amino acids | No | None |
| IMINO | Imino acids | Yes | $Cl^-$ |
| β | β-Amino acids | Yes | $Cl^-$ |
| $X^-_{AG}$ | Acidic amino acids | Yes | $K^+$ |

(From Ganapathy V, Brandsch M, Leibach FH. Intestinal transport of amino acids and peptides. In: Johnson LR, ed. *Physiology of the Gastrointestinal Tract.* 3rd ed. New York: Raven Press; 1994:1773–1794.)

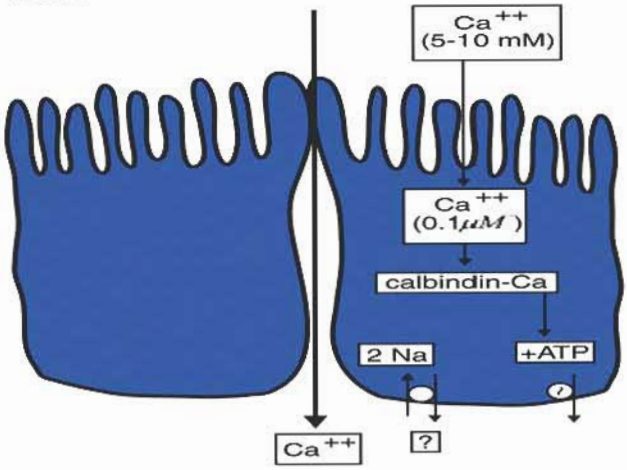
3) Clinical correlates related to amino acid absorption

1) Hartnup disease - defective renal tubular and intestinal resorption of tryptophan and other neutral amino acids
2) Cystinuria - defective intestinal transport for basic amino acids

## F) $Cl^-$ and $K^+$ absorption




## G) Ca++




## H) Iron

Iron is an essential transition metal, potentially toxic and thus tightly regulated. Iron is not excreted. Thus total body iron stores depend entirely on regulation of iron absorption in the intestine.

Recent studies have identified the apical membrane metal transporter likely responsible for iron uptake across the brush border membrane, DCT1 or NRAMP2. The transporter is coupled to H+ (i.e. dependent on an inwardly directed proton gradient for driving force, like peptide and amino acid transporters), and will transport Fe+2, Cd+2, Co+2, Cu+2, Ni+2, Mn+2, Pb+2, Zn+2, and NOT Ca+2. Thus, iron in the diet, usually in the water insoluble oxidized state Fe+3 must be converted to Fe+2 possibly by an apical brush border ferri-reductase. DCT1 contains in its promoter an Fe-response element under the control of the MHC class I-like molecule HFE (which is the protein defective in hereditary hemochromatosis). HFE is expressed in the intestinal crypt. Thus the regulatory system works by HFE reading Fe stores, and inducing the Fe-response element to protect DCT1 mRNA from degradation as the crypt cell migrates up the crypt-villus axis and differentiates into a mature villus absorptive cell.

Transport of Fe out the basolateral membrane occurs by facilitated transport across the recently identified IREG protein. Again, Fe2+ is transported (passively, down its electrochemical gradient), and must be converted back to Fe3+ by a ferro oxidase (possibly Hephestin or ceruloplasmin) for coupling to transferrin and transport throughout the body.

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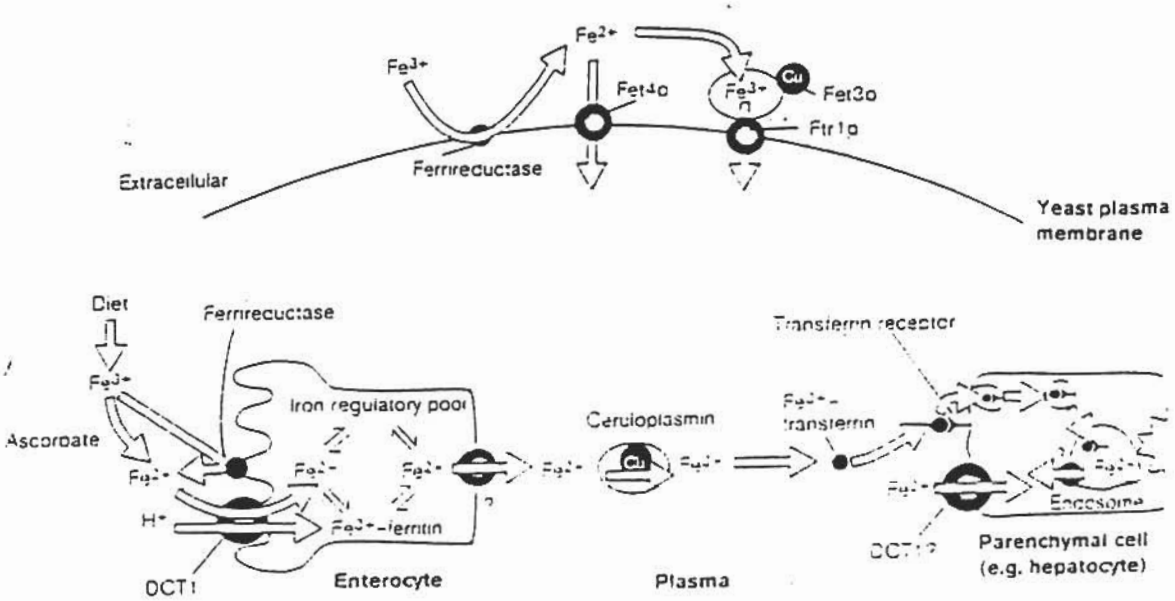


Clinical correlate: hemocromatosis

**I) Vit B12** Transport by receptor mediated endocytosis

Clinical correlate: Ileal resection or crohns disease and B12 defficiency

## J) Intestinal Secretion of water and salts

1) Crypt cells are the sites of electrogenic $Cl^-$ secretion. This is the mechanism by which cells secrete ions and water in response to secretagogues.
2) The mechanism includes (see Figure ):
   a) Chloride entry via a Na-K-2Cl co-transporter on the basolateral membrane (driven by electrochemical gradient favoring $Na^+$ entry (see Figure )
   b) The electrochemical gradient favoring $Na^+$ entry and, thus driving the co-transporter, is the basolateral Na-K-ATPase pump
   c) A basolateral $K^+$ channel(s) which allows equilibration of $K^+$ according to electrochemical potential
   d) An apical chloride channel (chloride exit due to electrochemical gradient)
3) The above mechanism results in a separation of $Na^+$ (beneath) and $Cl^-$ (above) the cation selective tight junction. The resulting transepithelial electrical potential drives passive outward movement of $Na^+$ (and with it water, Cl, and water are secreted by this process.
4) Regulation of $Cl^-$ secretion occurs by $Ca^{++}$, cAMP and cGMP (and perhaps $IP_3$). Regulation occurs at the level of the $Cl^-$ channel and perhaps the $K^+$ channel, due to phosphorylation events.
5) The genetic defect in cystic fibrosis is faulty regulation of the cAMP-dependent $Cl^-$ channel CFTR.
6) Examples of $Cl^-$ secretion:
   a) cholera toxin - via cAMP
   b) *E. coli* toxins:
      heat stable - via cGMP
      heat labile - via cAMP
   c) neurotransmitters - via $Ca^{++}$