The pathophysiology of diarrhea in pigs

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It is important for the clinician to have an understanding of the different pathophysiological mechanisms of the various diarrheal diseases. This knowledge provides a rational basis on which to institute therapeutic measures.

A complete review of the normal anatomy and physiology of the gastrointestinal tract of pigs is beyond the scope of this paper. However, it is pertinent to note a few points because diarrhea occurs as a result of altered intestinal function, which in turn is usually a consequence of structural and/or biochemical change.

Anatomy and physiology of the small intestine

The total surface area is increased by the mucosal folds, the villi, and most significantly by the microvilli. A 10-day-old, 3 kg pig has a small intestine with a total absorptive surface area of 114 m^2 (almost half the size of a tennis court)!

The functional unit of the small intestine is the villus, which is covered by the columnar epithelial cells (enterocytes) joined by well-developed junctional complexes (tight junctions). The epithelium is continually being renewed. It has the fastest rate of turnover of any tissue, lasting a mere 3-4 days in mature animals. Migration is faster in the ileum than the jejunum because the villi are shorter. Mature enterocytes are extruded at the tip of the villus. In neonatal animals, this process of cell turnover takes 7 to 10 days. Consequently, if epithelial cells are damaged in neonates they tend to take longer to recover than in older pigs.

The functional cell of the villus, the enterocyte, originates from the division of undifferentiated crypt cells. In the crypt the enterocyte is secretory. As it moves up the sides of the villus it matures into an absorptive villus cell – the microvilli become long, thin and numerous, and digestive enzymes develop. Consequently, if the tips of the villi are damaged the mature absorptive cells are lost and secretion is the net result. Selective damage to the immature crypt cells would, in theory, have the converse effect, but in fact causes severe disruption of normal villus cell replacement. The net effect is therefore similar to villus tip damage. Histologically, the villi are shortened and fused. This is called villus atrophy. It results in a reduction in the functional surface area of the mucosa.

There is a three-component barrier protecting the epithelium against damage, and hence dysfunction:

1. An outermost mucus layer. Mucus is a gel of water and glycoproteins (i.e. carbohydrates covalently linked to proteins) called mucins. The functions of mucus are: lubrication; protection against dehydration, absorption of macromolecules, physical and chemical injury, enzyme action, and parasites, microbial agents and their toxins; maintenance of intestinal flora; binding of cations; digestion of proteins.

After release from the goblet cell, mucin forms a continuous layer over and between villi. Secretion of mucin is probably controlled by neuronal and hormonal mechanisms. It is stimulated by prostaglandins. The mucus layer is degraded by mechanical forces, the indigenous microflora, pancreatic enzymes, bile, hydrochloric acid, pepsin and bacterial neuraminidases. This degradation probably allows increased absorption of macromolecular antigens, and attachment of microorganisms and toxins to enterocytes.

2. An epithelial layer which includes the glycocalyx, tight junctions, lysozymes and basement membrane. The glycocalyx is a carbohydrate coating on the outer surface of the plasma membrane over the microvilli (brush border). It contains hydrolytic and synthetic enzymes. Its functions are thought to be: ion exchange; cellular recognition and adherence; protection; physical barrier to large molecules and microorganisms.; protect membrane proteins from breakdown by pancreatic enzymes; bind antigens and hold them for digestion; attachment cups for indigenous microorganisms.

3. A mucosal lymphoreticular system which includes the gut-associated lymphoid tissue (GALT)

The intestine is a high-flow fluid system. Most of the fluid within the intestine is secreted into it from salivary glands, gastric mucosa, pancreas, liver, small intestine and colon. The fluid in the lumen of the intestine is in a state of flux. Each day, a volume of fluid approximating the animal's extracellular fluid volume is cycled in and out of the small and large intestine. This far exceeds the amount consumed by the animal and what is excreted. In the normal animal, absorption exceeds secretion. In animals with diarrhea, the flux balance

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is tipped in favor of secretion and rapid dehydration, electrolyte depletion and shock, and death may occur. Because of the large volumes in the flux, only a slight imbalance results in diarrhea.

Absorption and secretion

Water moves passively along osmotic gradients created by the active transport of electrolytes (principally Na^+, and via the "sodium pump" which is described below). It is this complex movement of ions that controls the water flux and maintains the gut-dependent section of the vital homeostatic mechanisms for water and electrolytes. Most of the passive water flow occurs between cells. Tight junctions between adjacent enterocytes regulate the flow of water (and some electrolytes) between the lumen and the intercellular spaces (containing the nonvascular extracellular fluid). Absorption of water is greater in the cells on the villus, whereas secretion occurs mainly from the immature cells in the crypts.

Movement of ions

In contrast to water absorption, absorption of electrolytes mainly occurs *through* the mature enterocyte on the villus tips.

The sodium pump has a central role in the whole process. It is located on the basolateral membrane of enterocytes and is energy dependent, utilizing K^+ activated ATPase. Three molecules of Na^+ are "pumped" out of the cytoplasm into the intercellular space in exchange for two molecules of K^+. This induces an electronegativity in the cytoplasm and increases the concentration of Na^+ in the intercellular spaces. It also results in the passive movement of water and other ions (Na^+, Cl^-, K^+, HCO_3^-) along concentration or electrical gradients.

There are three types of mechanisms for the transport of electrolytes across the membranes of enterocytes:

1. A unidirectional transport of a single ionic species (called a uniport);

2. A unidirectional co-transport of two ionic species in the same direction (symport); and

3. An ion exchange mechanism in which two ionic species of the same charge move in opposing directions, e.g. Na+ entry in exchange for H^+ exit (antiport). The sodium pump is a Na^+–K^+ antiport.

Sodium entry

Sodium enters the cell through the apical membrane by two mechanisms:

1. A uniport system — Na^+ enters along the electrochemical gradient created by the sodium pump;

2(a). a symport system controlled by a protein carrier — Na^+ and Cl^- enter together (it is inhibited by lack of luminal Cl^- and by intracellular regulators — see below); and

2(b). a second symport system coupled to the uptake of glucose or amain. Each molecule of glucose carries with it a molecule of sodium. Water follows, bringing further Na^+ and Cl^- ions by "solvent drag." This symport is not inhibited by intracellular regulators. This fact is utilized in the treatment of hypersecretory diarrhea.

Sodium exit

Sodium leaves the enterocyte at two locations:

1. at the basolateral membrane via two antiport systems:

(a) A Na^+/H^+ antiport in immature crypt secretory cells, and

(b) the sodium pump; and

2. At the apical membrane via two symport systems:

(a) a Na^+/Cl^- symport, and

(b) a Na^+/HCO_3^- symport.

These 2 symports appear to be stimulated by the intracellular regulator cyclic adenosine monophosphate (cAMP), and are thus involved in hypersecretory diarrhea.

Chloride entry

Chloride enters through the apical membrane by two mechanisms:

1. The Na^+/Cl^- symport (as for sodium above); and

2. A Cl^-/HCO_3^- antiport system mediated by a protein carrier

Chloride exit

Chloride exits through both the basolateral and apical membranes by diffusing along electrochemical gradients.

Potassium entry

Potassium enters through the basolateral membrane via the Na^+ / K^+ antiport (sodium pump — see above).

Potassium exit

Potassium leaves cells by diffusing through the basolateral membrane

Regulators

Absorptive and secretory processes are regulated by intracellular "regulators." These regulators are important in the pathogenesis of diarrhea because some substances (such as bacterial enterotoxins from the lumen acting on the apical membrane or hormones acting on the basolateral membrane) can affect them, thus altering the normal control process of the absorptive/secretory balance (see Pathophysiology below).

The regulators are:

1. Cyclic adenosine monophosphate (cAMP): cAMP influences both absorption and secretion. In villus cells, cAMP inhibits the Na^+/Cl^- apical membrane symport but not the Na^+/glucose/amino acid symport or the sodium pump. In crypt cells, cAMP increases the permeability of the apical membrane to

Cl^- and stimulates the Na^+/Cl^- and/or the Na^+/HCO_3^- symports across the apical membrane;

2. Cyclic guanosine monophosphate (cGMP): cGMP probably has similar mechanisms to cAMP;

3. Calcium: Ca^{++} appears to be a regulator but its role in disease is uncertain; and

4. Secretagogues: Some of the gut hormones such as serotonin, neutrotensin and carbachol apparently stimulate the secretion of NaCl and/or $NaHCO_3$ by crypt cells, thus potentially being able to exacerbate diarrhea.

Anatomy and physiology of the large intestine

Anatomical differences from the small intestine include:

1. no villi, but microvilli are present,

2. long straight crypts,

3. many goblet cells — presumably the reason for mucus in the feces during chronic diseases of the large intestine,

4. an increased number of lymphatic nodules and

5. extension of some crypts into the submucosa in association with lymphoid tissue.

Both 4 and 5 are often visible grossly and are presumably the result of the enormous microbiological flora of the large intestine.

The small intestine is overwhelmingly a net secretor of water. Hence, the large intestine has a "lifesaving" role. It must be a net absorber of water and achieves this by being an excellent Na^+ scavenger. As in the small intestine, there are two routes for absorption:

- transcellular, via the active sodium pump across the basolateral membrane which creates an electrochemical gradient for the movement of Na^+ through the apical membrane, and
- paracellular, via the tight junctions and dependent on the passive forces, viz. the electrical, chemical, osmotic and hydrostatic gradients.

In the large intestine, this is the main route for water and ion absorption. This is achieved by having "very tight" tight junctions, which reduce the exit of Na^+ ions. Two features of the large intestine's tight junctions make them "tighter," thus increasing its absorptive capacity for water over that of the small intestine:

1. a high electrical resistance which counters the steep electrical gradient created by the sodium pump (which tends to force anions into the lumen) and

2. a low hydraulic conductivity which counters the high osmotic pressure created in the intercellular spaces by the sodium pump. The basolateral spaces distend and the pressure in them increases, but the tight junctions result in this pressure, forcing the water and solutes across the basement membrane into the capillaries.

An intact large intestine is able to compensate for some "oversupply" of water from the small intestine, but damage to the large intestine can result in the loss of large amounts of Na^+ ions and hence water.

There is no Na^+/glucose symport in the large intestine, so glucose cannot be used to stimulate water absorption in its diseases. In fact, excess sugars in the large intestine are broken down by bacteria to lactate resulting in a lowering of the pH, which in turn may inhibit the absorption of some ions.

Pathophysiology

All diarrheal diseases are associated with the presence of increased number of osmotically active particles in the intestinal lumen. Water then moves into the luminal fluid.

Simple osmotic diarrhea may be illustrated by the mechanism of action of the saline cathartics, such as magnesium sulfate, which are poorly absorbed from the intestinal tract.

Mechanisms for diarrhea

1. Overfeeding of young animals with milk or milk replacers can cause a maldigestion diarrhea. The material ingested is incompletely absorbed by the small intestine. This increases the number of osmotically active particles in the lumen and draws in water. When digesta reach the large intestine, the resident bacteria ferment the lactose. This further increases the osmotic overload and more water enters the luminal fluid. The large intestine has a large reserve capacity and is able to reabsorb three to five times the volume of fluid that enters the small intestine in the healthy animal. It is probable, however, that when compounds like lactose are fermented, the local buffering systems are overwhelmed and the pH in the luminal fluid falls. The large intestine is then no longer able to absorb to its normal capacity and diarrhea is the result.

2. A reduction in the intestinal epithelial surface area decreases the ability of the intestine to digest and absorb material in the luminal contents. Situations in which this occurs are also associated with an osmotic diarrhea and are often referred to as maldigestion / malabsorption diseases. For example, transmissible gastroenteritis virus and rotavirus have a predilection for the mature enterocytes at the villus tip in young pigs. As described earlier, this results in shortening and fusion of the villi, referred to as villus atrophy, and a significant reduction in the surface area of the intes-

tinal epithelium. The net effect of this is an osmotic diarrhea similar to that which occurs with the overfeeding of milk or milk replacers.

Normally, lactose is split into glucose and galactose in the jejunum but their rapid absorption prevents them from increasing the osmotic pressure in the lumen. When mature enterocytes are lost (e.g. the virus enteritides), there is a disaccharidase deficiency, and sugars plus an osmotic equivalent to water pass undigested to the colon where bacterial fermentation converts them to volatile fatty acids. Whether diarrhea occurs depends on the balance between the substrate (sugar) input, microbial digestive capacity and mucosal absorptive capacity. If the colon is well developed, the mucosa healthy and the input small (proportional to the dietary intake and the amount of damage to the small intestine), the VFAs will be absorbed along with Na^+ and H_2O. In very young animals, the colon is poorly developed, the sugar remains undigested, water stays in the lumen and diarrhea occurs. Even in mature animals, a sudden increase in large amounts of sugar in the colon can result in hyperfermentation and the production of lactic acid. This is poorly absorbed, water is drawn into the lumen by osmosis, and diarrhea occurs.

Malabsorptive or maldigestive diarrhea is reduced or stopped by fasting. Feces in this type of diarrhea have a high osmolality, their volume is smaller than with hypersecretory diarrhea, and they may be acidic because of the secretion of H^+ and Cl^-. This helps in their diagnostic differentiation from hypersecretory diarrhea.

3. Severe diarrhea occurs when the effective osmotic pressure of the intestinal luminal contents is increased by the active secretion of ions. This is referred to as hypersecretory diarrhea and is due to an enhancement of the normal secretory processes. The enterotoxigenic strains of *Escherichia coli* are the most common causes of this type of diarrhea. These organisms do not invade or cause histological lesions in the intestinal mucosa but exert a profound biochemical effect by producing two toxins.

A heat-labile toxin (LT), structurally and functionally similar to cholera toxin, stimulates cGMP activity in intestinal epithelial cells. A heat-stable toxin (ST) stimulates cGMP activity in intestinal epithelial cells. Current evidence suggests that both toxins stimulate the absorption of sodium and chloride by the villous tips, and stimulate bicarbonate secretion, although the pathway for this is unclear. It is also probable that these effects are mediated through intracellular calcium, which acts as a second messenger to the cyclic nucleotides in the cell (Fig 1). These toxins act by "switching on" some of the normal mechanisms which have net loss of water. As long as these toxins are present, the

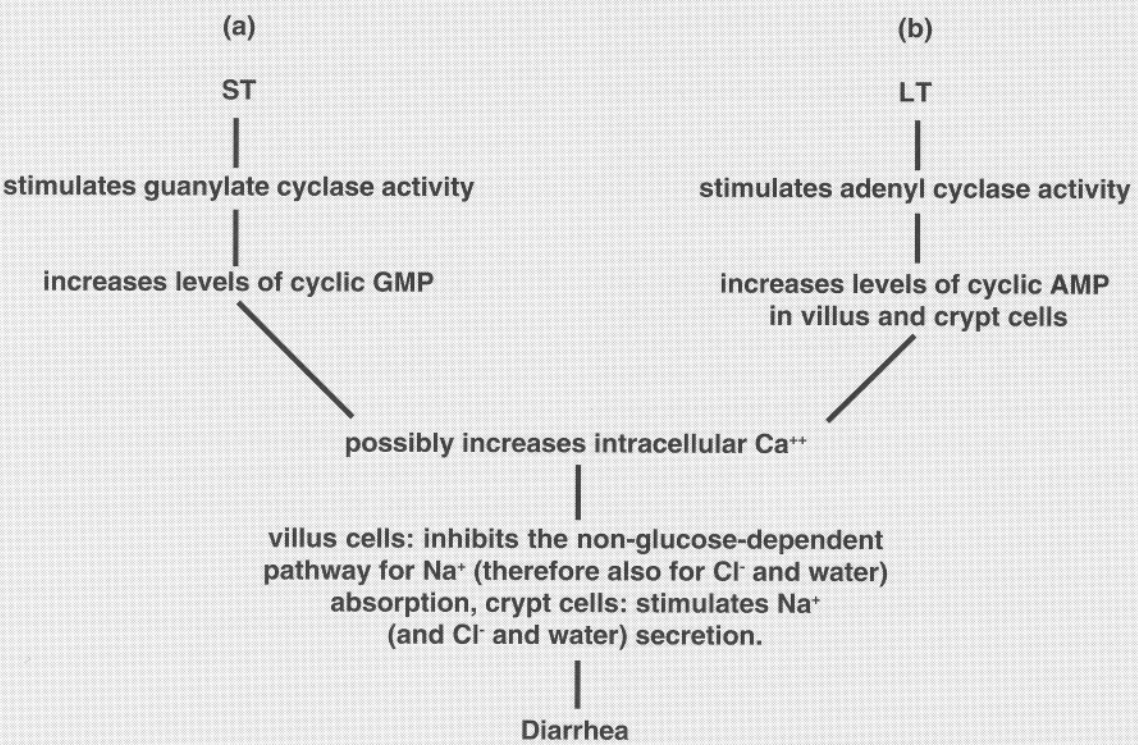

Fig 1. — Probable pathways for the creation of diarrhea by the heat-stable (ST) and heat-labile (LT) toxins from *E. coli*. The overall effect is to increase water loss to the lumen by blocking its absorption at the villus tips and to increase its secretion from the crypts, resulting in acidosis and rapid dehydration.

piglet cannot prevent water loss, which accounts for the devastating effects of enterotoxic colibacillosis in neonates. Hence ST and LT increase secretion of ions and thus water and, in addition, prevent their replacement except by the glucose-dependent pathway. This is important for the treatment of pigs with enterotoxic colibacillosis.

Prostaglandins have also been shown to stimulate secretion in the small intestine in association with increases in the cAMP concentration in the epithelial cells. Produced in association with inflammation, prostaglandins mediate the secretogenic effects of invasive bacteria such as some strains of salmonellae.

Hypersecretory diarrheas occur despite fasting. The feces are characteristically profuse and watery. The osmotic gradient generated by the luminal accumulation of sodium and chloride causes the movement of large quantities of water from the blood to the lumen. Feces are isotonic with plasma, and they are alkaline (this is an aid to differentiation from maldigestion / malabsorption diarrhea, caused for example by rotavirus) because of the secretion of large quantities of bicarbonate. The net effect is that the affected animal becomes rapidly dehydrated and acidotic. Acute hypersecretory diarrhea is always caused by a bacterial infection. For practical purposes, the binding of bacterial enterotoxin to the luminal surface of intestinal epithelial diarrhea therefore depends on replacement of the affected cells by the turnover process. While this is occurring, the animal must be maintained by replacing the large amounts of lost water and electrolytes. An important finding is that the mechanism coupling sodium absorption with that of non-electrolytes such as glucose and amino acids is unaffected in hypersecretory diarrhea. Affected animals can therefore be maintained cheaply and effectively by the oral administration of fluids containing sodium and dextrose.

4. Increases in the permeability of the intestinal tract may also cause diarrhea. Increases in permeability usually occur in those conditions in which inflammation may damage the enterocytes, cause a loss of integrity of the tight junctions between the enterocytes and increase the effective tissue pressure in the lamina propria. In the normal intestine, sodium is actively pumped into the spaces between the enterocytes. This creates a region of high sodium concentration and water moves from the lumen into the intercellular spaces, through the tight junctions. The intracellular spaces distend, creating a hydraulic force which squeezes fluid into the circulation. The tight junctions prevent backflow from the intercellular compartment into the intestinal lumen. In inflammatory diseases such as swine dysentery and salmonellosis, permeability is increased to the extent that, in addition to the loss of sodium and water, protein is also lost into the gut lumen. Such diseases are referred to as "protein losing enteropathies." In swine dysentery and proliferative hemorrhagic enteropathy, the mucosal damage may be so severe that blood may be lost into the lumen.

5. Motility disorders of the small and large intestines are poorly understood but as far as is known, an increase in small intestinal motility per se cannot result in diarrhea. Changes in motility have been demonstrated in the diarrhea that occurs with both enterotoxigenic *E. coli* and transmissible gastroenteritis. In both cases, however, the changes in motility contribute to the diarrhea rather than being the cause.

Physiological Consequences

Extracellular fluid

The most significant effect is the loss of extracellular fluid (ECF). A loss of 15% leads to clinical signs, and of 30% to death.

In some animals, the equivalent of the total ECF may normally be secreted into the gut every 24 hours, so an absorption secretion imbalance can be fatal very quickly. Rehydration is the first priority in the treatment of diarrhea.

Intravenous rehydration will be necessary in severe cases, but is generally impractical with sucklers. The success of oral rehydration will vary according to the type of diarrhea. In hypersecretory diarrhea caused by bacterial endotoxins, the Na/glucose symport is unaffected and electrolyte solutions containing glucose are very effective. In mild virus diarrhea, there may be sufficient functional mucosa for oral rehydration to work but this is often not the case in very severe virus diarrhea.

The fluid should be isotonic with plasma and contain sufficient K^+ and HCO_3^- to replace losses, plus Na^+ and glucose in equimolar amounts. An appropriate recipe is:

- 3.5 g NaCl
- 2.5 g $NaHCO_3$
- 1.5 g KCl
- 20.0 g glucose

per liter of clean water.

The changes that occur in plasma and cells tend to be similar regardless of the cause of diarrhea, and they tend to occur in a consistent sequence. However, the fluid volume and metabolite concentrations in plasma may not reflect those in the cell, and thus are not useful as a guide to the stage of the disease process.

The reduced plasma volume causes a fall in arterial pressure which in turn stimulates peripheral vasoconstriction, localized ischemia and reduced metabolic activity, and lower temperatures of peripheral tissues. Rectal temperature increases until near death, when it falls rapidly to below normal.

Electrolyte concentrations

A severe acidosis results from several factors but mainly because of loss of HCO_3^- in the feces. The intracellular pH parallels that in plasma. The movement of H^+ into cells forces

K^+ or Na^+ to move out or Cl^- in, in order to maintain electrical neutrality.

The most significant effect is the development of a hyperkalemia. As plasma K^+ concentration increases, the heart rate falls. The changed ratio of extracellular : intracellular K^+ reduces the resting membrane potential of cells, which affects cardiac muscle function and causes death.

Metabolic changes

Hypoglycemia frequently occurs in acute severe diarrhea as a consequence of anorexia, decreased absorption of nutrients, inhibited gluconeogenesis and increased glycolysis.

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