General Session

Bovine Intestinal Tract - Large and Small

Dr. Ben Norman, Chairman

Basic Structure, Function, Pathophysiology and Immune Mechanisms of the Bovine Gut

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The bovine intestine can be thought of as a tube. Food goes into one end and, after a few changes, comes out as feces at the other end. We never worry much about what's going on in that tube unless something goes wrong. When something goes wrong with the intestine, the most common clinical sign is diarrhea. Unfortunately, it is often difficult to determine the what and the why of the problem, and one is forced to treat all diarrheas in a similar manner, largely with antibacterial drugs and/or parasympatholytic anticholinergic agents. In order to prevent and treat diarrheas, other than symptomatically, we must develop an understanding of what is happening in the gut and what are some of the mechanisms by which bacteria and viruses can cause diarrhea.

The bovine intestine is comprised of three major parts, the small intestine, colon, and cecum. They have a tremendous surface area whose purpose it is to absorb nutrients, water, and electrolytes. The mucosal cells lining the 130 feet of small intestine are the most specialized for the function of absorption. Not only are they arranged into finger-like villi, but each cell also has numerous microvilli on its absorptive surface. The mucosal cells of the small intestine also produce important enzymes for digestion of sugars. When these cells are damaged in neonatal diarrhea, they may become deficient in production of lactase, the enzyme responsible for cleaving the milk sugar lactose to glucose and galactose. There is undigested lactose passing into the colon where bacterial fermentation occurs, further complicating and worsening the original diarrhea problem by producing a fermentative diarrhea.

Below the mucosa is the submucosa, containing Peyer's patches and plasma cells believed to be responsible for local immune mediation. Next are the two smooth muscle layers responsible for motility, one arranged longitudinally and the other circularly. Surrounding all of these layers is the serosa.

The small intestine of the cow is important for protein absorption. It receives most of the protein it digests and absorbs in the form of rumen bacteria and protozoa. In the ruminant, of course, a large part of the energy requirements are met by the volatile fatty acids absorbed from the rumen. The small intestine is responsible for absorbing only small quantities of fatty acids and carbohydrates, although the amount of carbohydrate entering the small intestine for digestion and absorption increases when grain is fed. The fact that the small and large intestine of the bovine are important is obvious when one looks at a cow suffering from chronic disease of the intestine, such as Johne's.

The columnar epithelial cells forming the folded mucosa of the 35-foot colon and 3-foot cecum are less specialized for digestion and absorption than those of the small intestine. The mucosa is arranged in flattened ridges. Microvilli are present, as they are in the small intestine (7). More mucus-producing goblet cells responsible for the mucoid appearance of some diarrheas are found in this part of the gut. Although less specialized for digestion than the mucosal cells of the small intestine, the mucosa of the colon and cecum is important in absorption of water and electrolytes.

Intestinal motility is of two main types: peristalsis and segmentation. Peristalsis and segmentation are controlled by mucosal receptors (intrinsic-myenteric plexuses), vagal tone, and inherent smooth muscle activity. Segmentation is responsible for holding back ingesta to allow for mixing and absorption. In diarrhea, the bowel lacks resistance to flow and acts like a flaccid tube due to a decrease in segmentation, while peristalsis may be increased or may actually be normal or hypomotile. The lack of segmentation allows ingesta to pass through the bowel more rapidly

than is normal, resulting in decreased absorption (4). Only the narcotic analgesics are known to be effective in stimulating rhythmic segmentation to increase resistance to the flow of ingesta, while decreasing peristalsis (1,5,13). Anticholinergics reduce motility in both circular and longitudinal smooth muscle in many species (2), although clinically they seem to be helpful in giving symptomatic relief to some animals with mild diarrheas of certain etiologies.

It is helpful to think of diseases of the intestines as diarrheic and obstructive. This paper will consider only diarrheic. It is clinically useful to further subdivide the clinical sign of loose feces into diarrhea and dysentery: Diarrhea is most commonly associated with large volumes of watery feces and small intestine involvement; dysentery is associated with smaller volumes, increased frequency, tenesmus, presence of mucus, blood and pus, and is mainly of large intestine origin.

We now know that diarrheas can be due to many factors other than simply decreased transit time of ingesta. In acute bacterial diseases such as acute salmonellosis, invasion of the mucosa results in massive destruction of mucosal cells, bleeding, and large amounts of mucus production by the goblet cells. We see this clinically as dysentery. In chronic diseases such as Johne's disease, malabsorption is due to invasion of the gut wall by mononuclear cells attempting to control the mycobacterial infection, an example of cell-mediated immunity (CMI). The reovirus and coronavirus infections of neonatal calves have been demonstrated to cause villus atrophy and cuboidal to squamous mucosal cells replace normal columnar epithelial cells (8,9) with resulting malabsorption and diarrhea. Small intestine lesions are similar to those seen in pigs with transmissible gastroenteritis. In the colon of coronavirus-infected calves, atrophy of the colonic ridges occurred (8).

In the last few years, much attention has been given to enterotoxins and how they act to produce diarrhea. Where we once thought that pathogenic *E. coli* caused diarrhea by invading the small intestinal mucosa (much as salmonella do in the colon and cecum), it is now known that many enteropathogenic *E. coli* possess the ability to produce enterotoxin (10). A plasmid, which is a transferable extranuclear piece of DNA, controls whether or not a bacterial cell will be able to produce enterotoxin (15). *E. coli* with this plasmid can produce enterotoxin, those without it cannot. The toxin acts very similarly to that produced by *Vibrio cholera,* the agent causing cholera in man. The enterotoxin causes an increase in movement of fluid into the lumen of the bowel by a biochemical change involving adenyl cyclase and cyclic AMP (Figure 1) (3). Instead of the normal pump moving a large gradient from lumen to blood, the cells actually secrete large volumes of fluids and electrolytes into the lumen, resulting in diarrhea. No visible gross or histologic changes occur in many cases (11).

To be effective at producing diarrhea, enterotoxin producing enteropathogenic *E. coli* must also be able to colonize the small intestine effectively. To do this, some strains possess a plasmid-controlled factor which allows them to stick to the microvilli of the small intestinal mucosal cells (12). This factor is called K99 or Kco in the bovine and is like K88 in the pig.

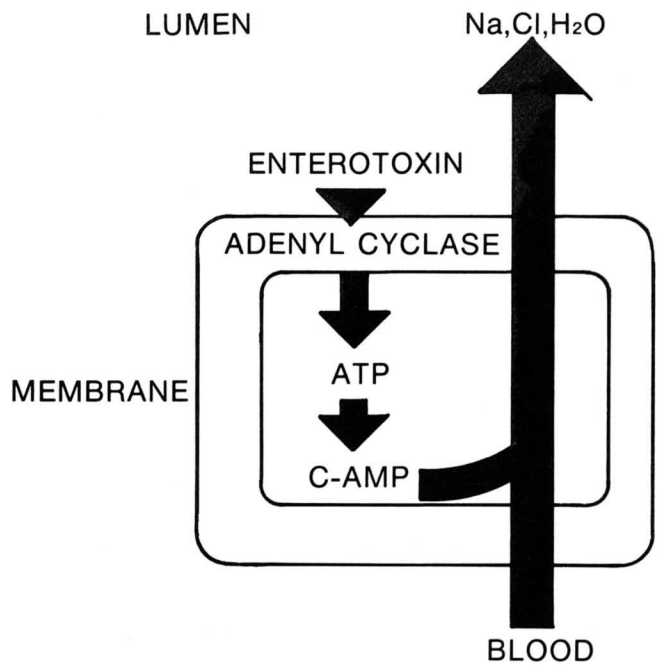

Figure 1. Mechanism of action of enterotoxin on a mucosal cell. Enterotoxin attaches to the membrane, activates adenyl cyclase which in turn activates cyclic-AMP to increase secretion of sodium chloride and water into the lumen of the bowel (3).

Exactly what it is and how it allows bacteria to stick is still not known. Bacteria that do not possess the K99 plasmid do not stick and are washed posteriorly with the ingesta. Those *E. coli* that have both plasmids (K99 and enterotoxin) and are thus able to stick and produce enterotoxin are the most enteropathogenic. It is interesting to note that many of the traveler's diarrheas in man are now believed to be due to enteropathogenic *E. coli* (3).

The mechanisms by which helminths and protozoa produce diarrhea and dysentery are usually more obvious. It is largely the direct mucosal invasion and tissue damage caused by stages emerging from or entering the gut wall that cause bleeding, mucus secretion, and irritation. Intense lymphocytic and eosinophilic infiltration of the lamina propria may occur in infections such as with *Cooperia spp.* This may be followed by necrosis of the mucosa in severe cases. Resistance to both coccidia and *Trichostrongylus spp.* is known to occur.

Diarrhea is often observed in cattle secondary to a severe toxic disease such as metritis. Although the mechanism for this is not known, it may be mediated by histamine and other autocoids, with gut smooth muscle contractions occurring as a result of histamine

release at the site of toxin production. This area is poorly understood at present.

To look at how the animal can respond immunologically to infectious agents injuring the intestine, it is helpful to look at the basic immune mechanism operating in the bovine. Immunity to viral and bacterial diseases can be divided into two main categories (Table 1), namely, antibody-mediated or humoral immunity and cell-mediated immunity (CMI). Antibody is very important in

Table 1

Humoral and Cell-Mediated (14) Immune Responses

	Humoral (IgG, IgM, IgA)	Cellular
Passive Transfer	Immune Serum	Sensitized Leukocytes
Circulating Antibody	Present	Absent
Time of Max. Response	Rapid (usually minutes)	24-48 hours
Effector Cell	B-cell and Plasma cell	T-cell
Examples	Antibacterial antibodies; some antiviral antibodies	Resistance to viral disease; tuberculin skin test

protecting against bacterial diseases and plays a role in immunity to viral diseases. CMI is most important in protecting the animal from viral, fungal, mycobacterial, and protozoal diseases. This is illustrated by the fact that animals with defective cell-mediated immune mechanisms often succumb to viral rather than bacterial diseases. Phagocytosis is also an important aspect of the immune response. Neutrophils (PMN's) and macrophages have the ability to phagocytize and digest foreign material. These cells can ingest an antigen, often with the help of complement, and process this antigen so that it will then "turn on" T and B lymphocytes. This processing is an important part of the immune response.

There are two types of lymphocytes, T and B cells. Humoral immunity is mediated by B lymphocytes, CMI by T lymphocytes. Although both are of bone marrow stem cell origin and look similar with light microscopy, they have different abilities and perform different functions. Bone marrow stem cells which become B lymphocytes (B cells) are processed through lymphoid tissues (possibly Peyer's patches in the bovine) to become antibody producers. T cells have become specialized (by migration through the thymus) to defend against diseases caused by viruses, fungi, mycobacteria, brucellosis, and protozoa, as well as playing a role in controlling neoplasms and participating in delayed hypersensitivity and graft rejection. T lymphocytes also have the ability to liberate lymphokines, substances which enhance the immune response in a variety of ways, such as increasing vascular permeability, stimulating multiplication of lymphocytes, activating the macrophages to enhance phagocytosis, and directly lysing an antigen.

T and B cells can be identified and tested *in vitro* to look for immunodeficiency states. There is evidence that some infectious agents, such as BVD virus, can cause a depression of the immune response. Some animals chronically infected with BVD have a depressed CMI and never mount a serological (titer) response. The newborn calf is similarly in an immune depressed condition due to high corticosteroid levels.

When B cells are stimulated by an antigen, they become plasma cells which produce antibody of three major types: IgG, IgM, and IgA. Table 2 lists some of

Table 2

Some Properties of Bovine Immunoglobulins (6)

	IgG	IgM	IgA
Molecular wt.	140,000	900,000	160,000
Half Life (days)	23	5	6
Antibody Activity	Most Bacterial & Viral Antibody	First Antibody Formed, Mainly Intravascular	Found in External Secretions
Serum Concentration (mg/100 ml)	1840	250	30
Intestinal Concentration (mg/100 ml)	31	Trace	24
Nasal Secretion Concentration (mg/100 ml)	6	Trace	195

the properties of each immunoglobulin (14). We now have the ability to quantitate each of these immunoglobulins. Although IgG predominates in serum, IgA predominates in nasal secretions. In the gut, IgG and IgA are present in roughly equal concentrations (6). Why is IgA relatively higher in nasal and gut secretions than in serum? IgA is the antibody class responsible to a large degree for local immunity, i.e., it may help to protect mucous membranes from invasion by pathogens. IgA is produced locally by submucosal plasma cells. It is then carried through a mucosal cell where one secretory piece is attached to each pair of IgA units to form a dimer. Secretory piece apparently aids in making IgA more resistant to destruction by enzymes in the gut. Stimulation of local IgA is thought to be important in the intranasal bovine virus vaccines, and probably plays a role in oral vaccination with such vaccines as reovirus and corona virus calf diarrhea agents. Exactly what role IgA plays in helping to control intestinal infections is not currently known. It has recently been shown that fetal calves vaccinated orally with killed *E. coli* bacterin had IgG and IgM fluorescent plasma cells in the intestinal submucosa at birth (16). IgA was not examined in that study. We have been unable to

detect IgA in intestine or serum of orally vaccinated fetal calves.

As our understanding of the diseases of the bovine intestine increases, we may find that still other mechanisms operate to cause diarrhea and dysentery. Humoral immunity and cell-mediated immunity probably play roles of differing importance in different diseases. Most likely, many bacterial diseases are best controlled by antibody, while CMI is relatively more important in some diseases such as Johne's (paratuberculosis) and coccidiosis. Viral diseases are probably brought under control or prevented by both antibody and CMI mechanisms.

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