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The *in vitro* effects of *Escherichia coli* heat-stable enterotoxin on porcine small intestine

by

Wara Panichkriangkrai

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

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INTRODUCTION

Colibacillosis produced by enteropathogenic strains of *Escherichia coli* is the principal cause of neonatal diarrhea in piglets and calves. Swine colibacillosis is characterized by high morbidity, induction of a secretory state in the anterior small intestine and marked fluid and electrolyte loss which is frequently fatal in the neonate. The resulting serious economic losses have stimulated investigations of the pathogenesis, prevention and treatment of this syndrome.

The cellular events involved in the development of the diarrheal state are poorly understood, especially those caused by *Escherichia coli* heat-stable enterotoxin (ST). Large unidirectional fluxes of water and ions occur across the epithelium of the small intestine and it has been proposed that the mature villus cells are primarily absorptive and the crypt cells are primarily secretory in nature. The locus of ST action on these cell types has not been determined but it has been postulated that the toxin inhibits absorption of neutral coupled sodium-chloride influx at the brush border of gut epithelial cells.

Antidiarrheal drugs are frequently employed in an attempt to reduce fluid and electrolyte loss in diarrheic states. Drug effects are frequently attributed to inhibition of intestinal motility but there is increasing evidence that drug action on ion transport mechanisms may be more important. Investigation of these actions may increase our understanding of normal epithelial transport and the derangements of transport in diarrhea.

The objective of the present study was to determine the effects of ST and selected drugs on unidirectional chloride transport in isolated pig jejunum and on chloride efflux (secretory) rates in isolated mature and immature enterocytes.

LITERATURE REVIEW

Organization and General Architecture of Small Intestinal Mucosa

The mucosa of the small intestine can be divided into three layers, the absorptive layer, the lamina propria and the muscularis mucosa. The muscularis mucosa consists of a thin sheet of smooth muscle generally 3-10 cells thick and separates the mucosa from submucosa (Trier, 1968). The middle layer, the lamina propria, is heterogeneous in composition and contains various cell types (Levitan and Wilson, 1974) including fibroblasts, macrophages, plasma cells, lymphocytes, eosinophils, and mast cells, as well as noncellular connective tissue elements including collagen and reticular fibers. Blood and lymph vessels, unmyelinated nerve fibers and strands of smooth muscle are also regularly present (Trier, 1968).

The lamina propria provides important structural support for the intestinal epithelium and its vascular and nervous elements. In addition, the lamina has been shown to contain significant amounts of immunoglobulin (Rubin et al., 1965). IgA seems to be the predominant immunoglobulin in external secretions of mammals and is locally derived from cells of the lymphoid series situated near glandular epithelium (Tomasi and Bienenstock, 1968). Studies on porcine intestinal tissue have shown a similar secretory immune system to be operative in the pig and IgA has been demonstrated in both the crypt epithelium and lymphoid cells of the lamina propria (Allen and Porter, 1970). Thus, this antibody is probably synthesized locally in the lamina propria by plasma cells and secreted by crypt cells (Allen et al., 1973; Moon, 1976). The lamina propria together with

the epithelium forms the numerous fingerlike villi which project from the surface of the mucosa into the intestinal lumen as well as the many pit-like indentations, the crypts, which are found between adjacent villi (Trier, 1968). In humans, the villi measure 0.5-1.0 mm in height and increase the absorptive surface eightfold (Friedman and Cardell, 1972).

The third layer of the intestinal mucosa consists of a continuous sheet of a single layer of columnar epithelium attached directly to the underlying lamina propria (Levitan and Wilson, 1974). The surface of the villi consists of absorptive, goblet and enterochromaffin cells. The crypts contain undifferentiated, goblet, enterochromaffin and, in most mammalian species, Paneth cells (Trier, 1968).

Intestinal epithelium is in a constant state of migration and replacement (Moon, 1976). The loss of the epithelial cells occurs regularly at the tip of the villus. Constant proliferation of new cells from the crypts is necessary to maintain normal function of epithelium. The crypts seem to be the proliferative pool which contain highly undifferentiated cells which then differentiate during migration from crypts onto villi. Thus, there is a gradient within the epithelium such that the oldest, most differentiated epithelial cells are at the tip of the villus, the younger and less differentiated cells are on the proximal portions of the villus and the undifferentiated cells are only in the crypts (Trier, 1968; Moon, 1976). Although the pattern of cell renewal is the same in different species, the time required for cells to migrate from the crypt to the villus varies among mammals. In man, it takes about 5-7 days before cells can reach the tip of the villus in the duodenum and jejunum

(MacDonald et al., 1964; Shorter et al., 1964), and about 3 days in the ileum (Lipkin et al., 1963). The migration rate of jejunum epithelium of rats and mice is only 1-3 days as shown by Leblond and Messier (1958) and Quastler and Sherman (1959).

Fine Structure of Intestinal Epithelial Cells

Many different structural and functional cell types occur in intestinal epithelium. The major cell types seem to be the absorptive epithelial cells and their precursors, the undifferentiated crypt epithelial cells. These cells are present throughout the intestinal tract and the major digestive and absorptive functions of the epithelium depend upon the integrity of these two highly integrated cell types (Moon, 1976).

Undifferentiated crypt cells

Undifferentiated cells are the predominant cell type in the crypts and are generally confined to these areas (Levitan and Wilson, 1974). They are the progenitors for other epithelial cells. In addition to their location and proliferative activity, undifferentiated crypt cells can be recognized by their sparse, short, irregular microvilli, straight lateral membranes with multiple desmosomes at their apex, numerous free ribosomes and polyribosomes, and numerous membrane-bound secretory granules (Moon, 1976).

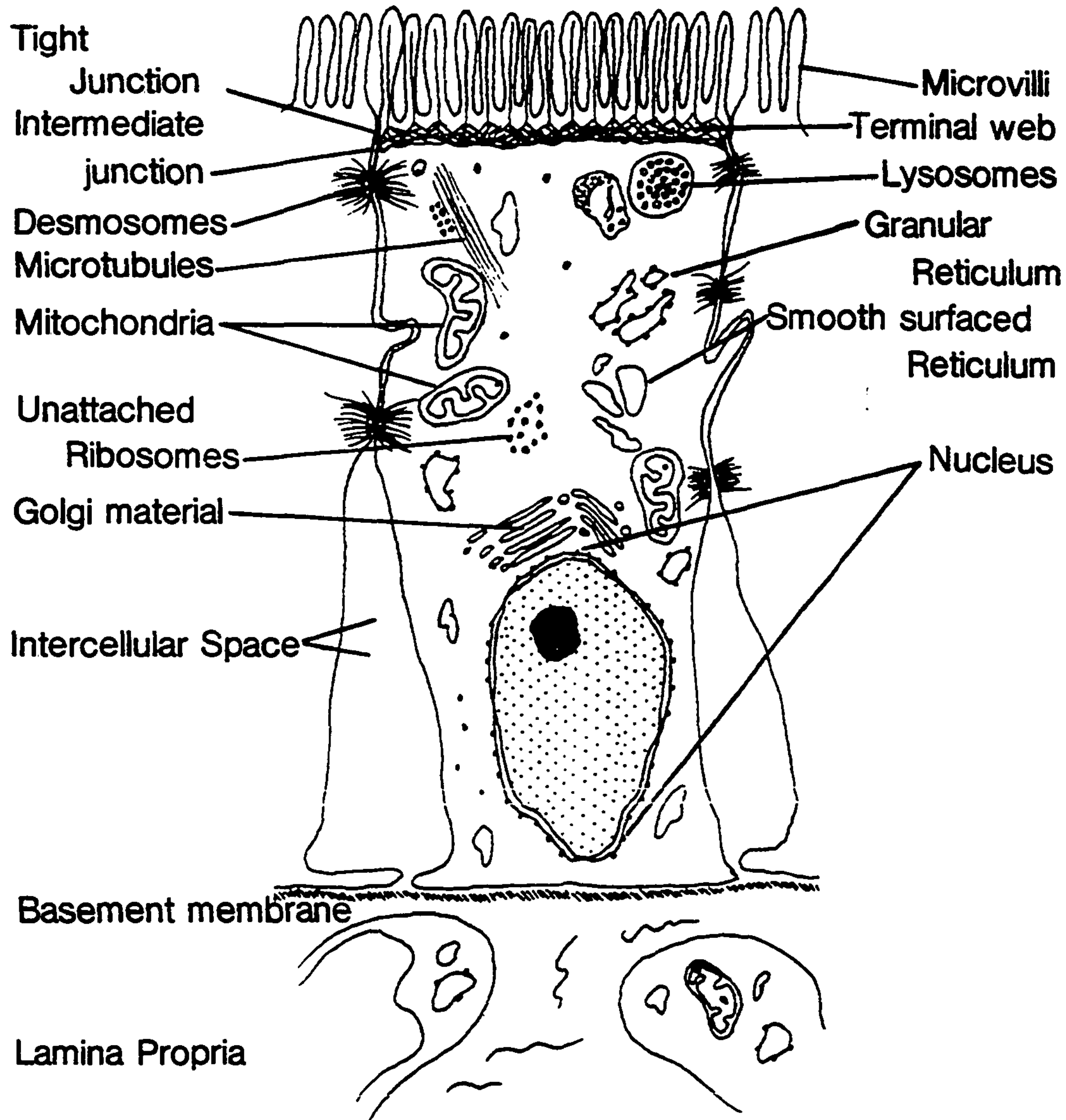
Hendrix and Bayless (1970) pointed out some differences of the undifferentiated cells from the villus cells as follows: 1. Greater affinity for basophilic dyes because of an abundance of ribosomes with

high content of ribonucleic acid; 2. Frequent mitotic figures; 3. Less well-developed microvilli and absent terminal web; 4. Secretory granules in the apical third of the cells. As these cells migrate from the crypt onto the villus, they differentiate into absorptive cells, losing all morphologic evidence of secretory activity and the ability to divide (Levitan and Wilson, 1974). By the time these cells arrive at the base of the villus, they show histochemical evidence of enzyme activity (Johnson and Kugler, 1953). It has been demonstrated by Fortin-Magana et al. (1970) and Nordström et al. (1967) that the epithelial cells of the villus have many specialized enzymatic functions such as alkaline phosphatase and disaccharidases which are concerned with the processes of digestion and absorption. In contrast, the progenitor cells of the crypt have none of these specialized activities, but as would be expected of proliferating tissue, they contain high activities of enzymes, such as thymidine kinase, involved in the synthesis of DNA (Imondi et al., 1969; Herbst et al., 1970).

Absorptive epithelial cells

Mammalian absorptive cells are tall, columnar cells with basally located nuclei. The individual morphologic features of the intestinal absorptive cell, such as the microvilli, mitochondria, endoplasmic reticulum, and lysosomes, seem to be shared by other epithelial cells but its general appearance is distinct and permits its identification (Trier, 1968). A schematic diagram of an intestinal absorptive cell is shown in Figure 1. These cells are characterized by numerous long, regular microvilli along their luminal borders (Moon, 1976). The microvilli increase

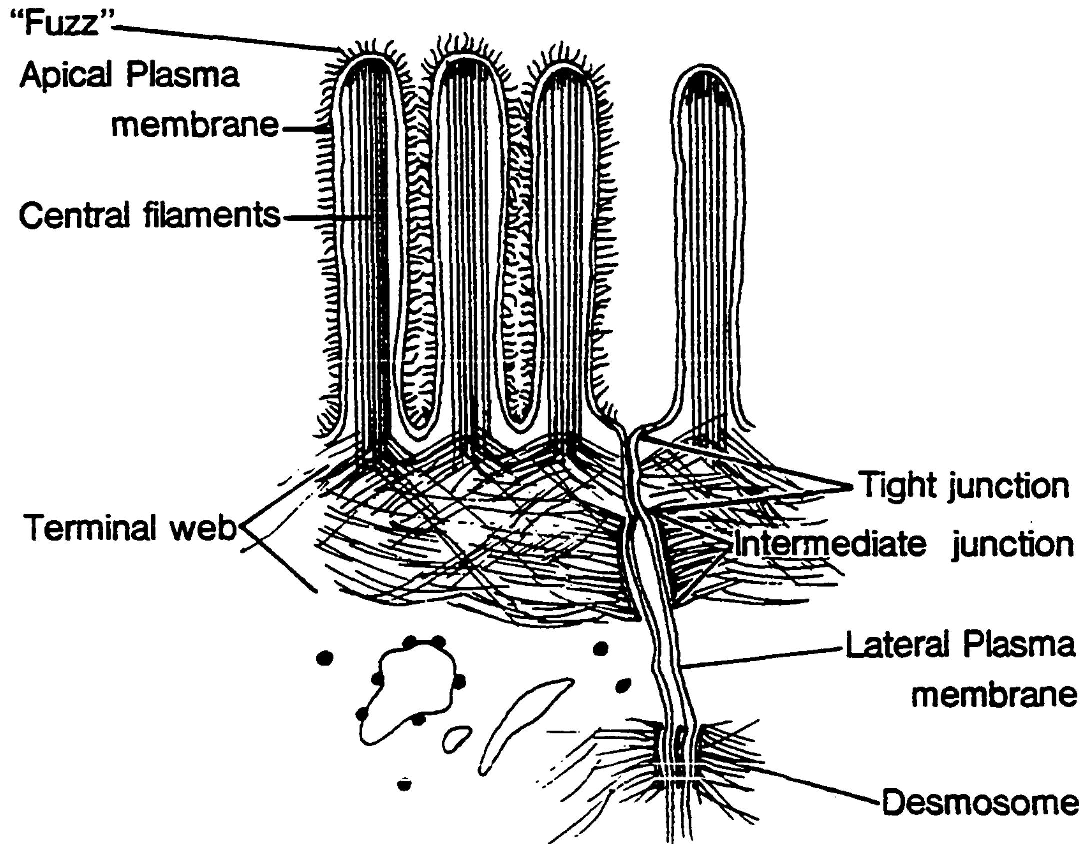
Figure 1. Schematic diagram of an intestinal absorptive cell (redrawn from Trier, 1968)


Tight
Junction
Intermediate
junction
Desmosomes
Microtubules
Mitochondria
Unattached
Ribosomes
Golgi material
Intercellular Space
Basement membrane
Lamina Propria
Microvilli
Terminal web
Lysosomes
Granular
Reticulum
Smooth surfaced
Reticulum
Nucleus

the absorptive surface of the intestine about 30-40 fold (Brown, 1962). The microvilli are approximately 0.75-1.5 μ in length and 0.1 μ in width (Curran and Creamer, 1963). The organization of the microvillus is complex, perhaps relating to its intimate role in so many aspects of nutrient movement (Sabesin, 1980). The outer surface of the microvilli is covered by a fuzzy coat or glycocalyx (Figure 2). The glycocalyx is composed of fine filamentous material which is synthesized by the individual epithelial cells (Ito and Revel, 1964). It seems to be remarkedly stable since it cannot be removed by washing with ethylenediaminetetracetate (EDTA) or with a number of known mucolytic or proteolytic agents (Ito, 1969). Functionally, the glycocalyx may serve as a barrier against the absorption of potentially noxious substances, but more important, it is the site containing enzymes involved in the terminal digestion of carbohydrates and proteins (Sabesin, 1980). Ito (1969) and Johnson (1969) have shown that the glycocalyx consists in part of both digestive and absorptive enzymes. Among these are disaccharidases such as lactase and sucrase, peptidases, and alkaline phosphatase (Sabesin, 1980; Weiser, 1973).

Microvilli and glycocalyx become more extensive as the cell matures in its migration toward the villus tip. Microvilli are more numerous, regular and elongated on villus absorptive cells of small intestine than on surface absorptive cells in the colon (Moon, 1976). The area immediately beneath the microvilli is called the terminal web and is relatively free of organelles except for filaments that extend into the cores of the microvilli. The major function of the terminal web might be to stiffen and

Figure 2. Schematic illustration of the specializations of the apical cytoplasm of the plasma membrane of intestinal absorptive cells (redrawn from Trier, 1968)


"Fuzz"
Apical Plasma membrane
Central filaments
Terminal web
Tight junction
Intermediate junction
Lateral Plasma membrane
Desmosome

stabilize the cell surface (Palay and Karlin, 1959). The structural stability of the microvillus-terminal-web complex is demonstrated by its ability to resist osmotic and mechanical shock of sufficient magnitude that can result in disintegration of the remainder of the absorptive cell (Miller and Crane, 1961).

The apical portion of the intestinal epithelial cells contains the usual complement of subcellular organelles found in most mammalian cells such as smooth and rough endoplasmic reticulum, mitochondria, occasional lysosomes, and vesicles. The intestinal epithelium is not particularly active in protein synthesis and this may be implied indirectly by the observation that the rough endoplasmic reticulum is not as extensively developed as it is, for example, in pancreatic acinar cells in which pancreatic enzyme synthesis occurs (Sabesin, 1980).

Absorptive cells interdigitate extensively along their lateral borders especially in fasting cells where the intercellular space is insignificant. During active nutrient transport, the entrance of absorbed nutrients into the intracellular space is associated with a marked distension of this space such as during active lipid absorption (Sabesin, 1980). There are various junctional complexes which provide a mechanism for the adherence of cells to each other and also provide structural devices which prevent the leakage of nutrients and ions but allow cell to cell communication. Specialized attachment zones have been noted between adjacent intestinal epithelial cells for many years. Apparent thickenings with increased stain affinity at the extreme apical portion of adjacent lateral plasma membranes were named "terminal bars" by light micro-

scopists since 1898 (cf. Trier, 1968). Farquhar and Palade (1963), using the electron microscope, noted that the "terminal bar" of the light microscopists is indeed a "tight junction" resulting from the fusion of the outer leaflets of adjacent plasma membranes. This fusion obliterates the intracellular space and forms an encircling belt that provides an impermeable barrier to the exit of nutrients contained within the intercellular space (Hull and Staehelin, 1976). The lateral intercellular space, between the apical junctional complexes and the basement membrane of absorptive cells, does not occur in normal crypt epithelium. This space becomes progressively more prominent as cells move toward the villus tip (Moon, 1976). Most absorbed materials, such as water or lipid, move into the lateral intercellular space to blood and lymph (Loeschke et al., 1970; Tomasini and Dobbins, 1970).

Also binding adjacent epithelial cells are two types of desmosomes (Sabesin, 1980). The belt desmosome located near the tight junction is made up of protein fibrils which occupy the intercellular space between adjacent lateral membranes adding to the syncytial chracteristics of the epithelium. The spot desmosome is composed of areas of electron-dense fibrils in the intercellular spaces extending into the surrounding cytosol.

Another junctional complex called a "gap" junction connects the exterior membranes of adjacent cells but permits the passage of molecules from cell to cell through connecting channels of some 15 Å in greatest width (Hull and Staehelin, 1976). This site is sufficient to permit passage of molecules of the size of sucrose from cell to cell. Freeze

fracture studies have shown that the gap junction is formed by a mosaic of cylindrical particles (Sabesin, 1980).

The basal surface of intestinal epithelial cells is in close proximity to its basement membrane (Trier, 1968) which is a continuous sheet of homogeneous material of intermediate density measuring approximately 300 Å wide (Sabesin, 1980). The basement membrane separates the basal pole of the epithelial cells from the underlying lamina propria. Rubin (1966) has shown that interruptions occur in the basement membrane during migration of materials such as chylomicrons and mononuclear cells from the intercellular space to the lamina propria. Nutrient absorption is enhanced by the rich vascular and lymphatic supply of the lamina propria which provides a ready means of access of absorbed nutrients into the vascular lymphatic system (Sabesin, 1980).

Under some conditions, absorptive cells develop an invagination of their apical membranes that connect with tubules in the terminal web and eventually with vesicles and large vacuoles in the cytoplasm (Moon, 1976). This system is used, during passive immunization of the newborn, to take up macromolecules and colostral and milk antibody from the intestinal lumen. Worthington and Graney (1973a,b) have shown that epithelial cells can absorb bacteria and viruses by pinocytosis during the neonatal period.

Goblet cells

Goblet cells are found both in the crypts and along the villi of the small intestine and become progressively more numerous from the midjejunum to terminal ileum (Hendrix and Bayless, 1970). They possess a few apical

microvilli in addition to their characteristic mucous granules and prominent rough endoplasmic reticulum. The morphology of the goblet cells of the small intestine is very similar regardless of their location in crypts or villi and in proximal or distal small bowel (Trier, 1968). Presumably the only important function of goblet cells is mucus secretion (Moon, 1976).

Paneth cells

Paneth cells occur at or near the bases of the crypt in small intestine. They are prominent in primates and rodents but do not occur in cattle, cats, dogs or pigs (Moon, 1976). These cells are highly differentiated and have an elaborate and well-organized rough endoplasmic reticulum which suggests great secretory potential (Hendrix and Bayless, 1970). Their functional significance is not well-understood, however, they are both phagocytic (Erlandsen and Chase, 1972a,b) and secretory (Staley and Trier, 1965). Moon (1976) described the ultrastructure of Paneth cells as containing large secretory granules which nearly fill their cytoplasm, nuclei, basal and prominent lamellar arrays of rough endoplasmic reticulum, numerous lysosomes, and a few irregular microvilli. Troughton and Trier (1969) have shown that Paneth cells persist in crypts longer than most intestinal epithelial cells.

Enterochromaffin cells (argentaffin cells)

Enterochromaffin cells occur throughout the alimentary tract, especially in the crypts (Moon, 1976). They are endocrine cells which cannot be considered as a single cell type but vary depending on their granule type and content, and whether they contain serotonin, catecholamine, gastrin,

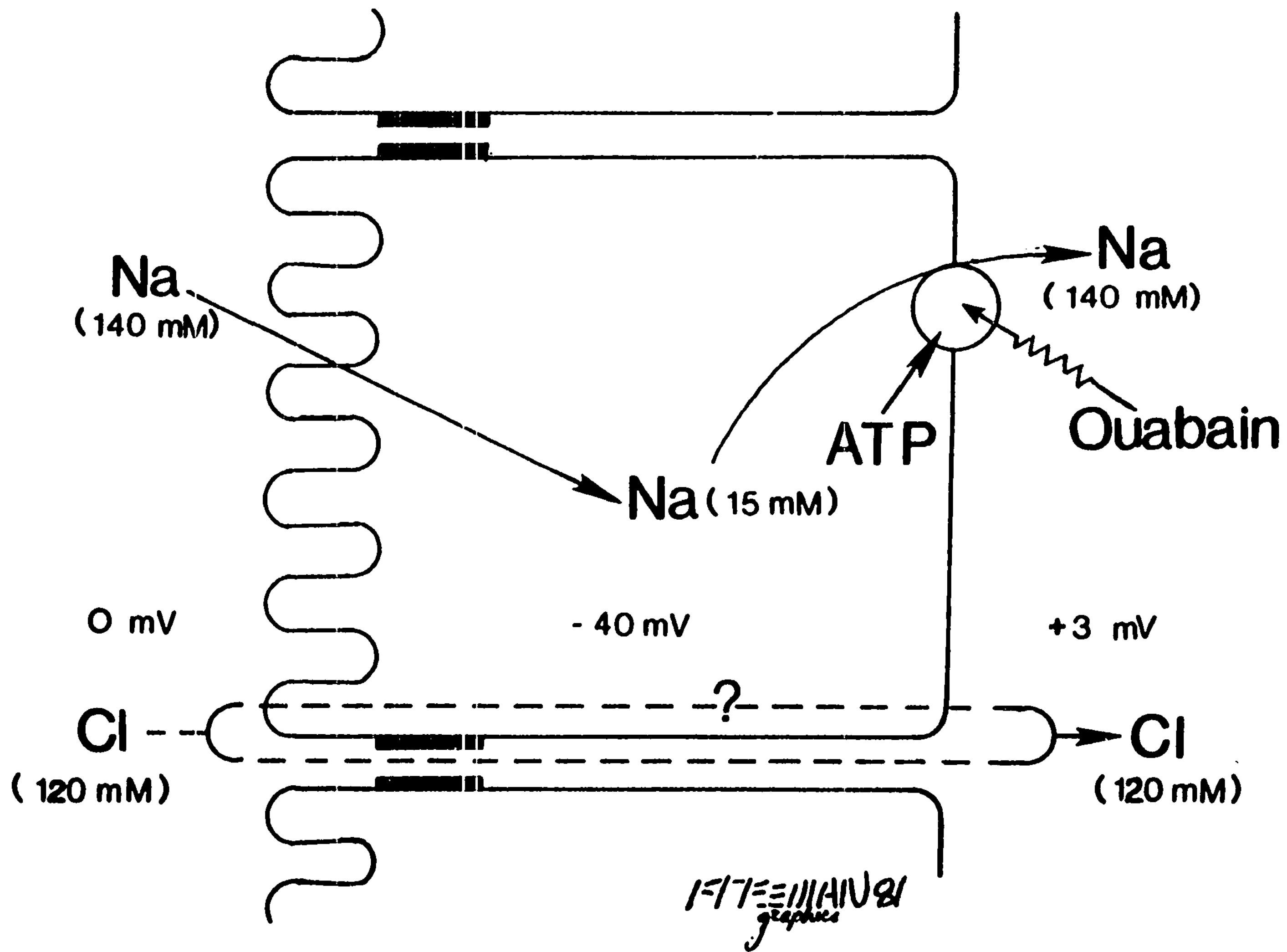
secretin or enteroglucagon (Forssmann et al., 1969). Moon (1976) described enterochromaffin cells as triangular in outline, with most of their small heterogeneous secretory granules between the nucleus and broad aspect of the cell which is near the basement membrane. It has been suggested that the secretory products are liberated into tissue and enter the blood in contrast to other intestinal epithelial cells that secrete their products into the intestinal lumen. Patients with enterochromaffin cell neoplasms have diarrhea in association with excessive blood levels of serotonin and bradykinin (Oates et al., 1964). The diarrhea is attributed to alterations in intestinal motor function. Whether any alteration of intestinal fluid transfer occurs independently of the motility alteration has not been determined (Hendrix and Bayless, 1970).

Transepithelial Sodium and Chloride Transport

Three processes of sodium and chloride absorption by mammalian small intestine *in vitro* have been described by Schultz (1980) as follows:

1. Uncoupled, "electrogenic" sodium absorption--The cell interior is at least 40 mV negative with respect to mucosal solutions and intracellular sodium activity is lower than the surrounding solutions. Sodium can passively move from the mucosal solution across the apical membrane into the cell because of these differences in both chemical concentration and electrical potential, as illustrated in Figure 3. However, sodium exit from the cell across the basolateral membrane into the serosal solution or plasma is actively transported against a chemical and electrical potential difference. The energy required for this transport is derived from ATP

Figure 3. Cellular model of "electrogenic" Na transport by small intestine accompanied by passive, "electrically coupled" Cl absorption (redrawn from Schultz, 1980)


Na
(140 mM)
Na (15 mM)
Na
(140 mM)
ATP
Ouabain
0 mV
- 40 mV
+3 mV
?
Cl
(120 mM)
Cl
(120 mM)

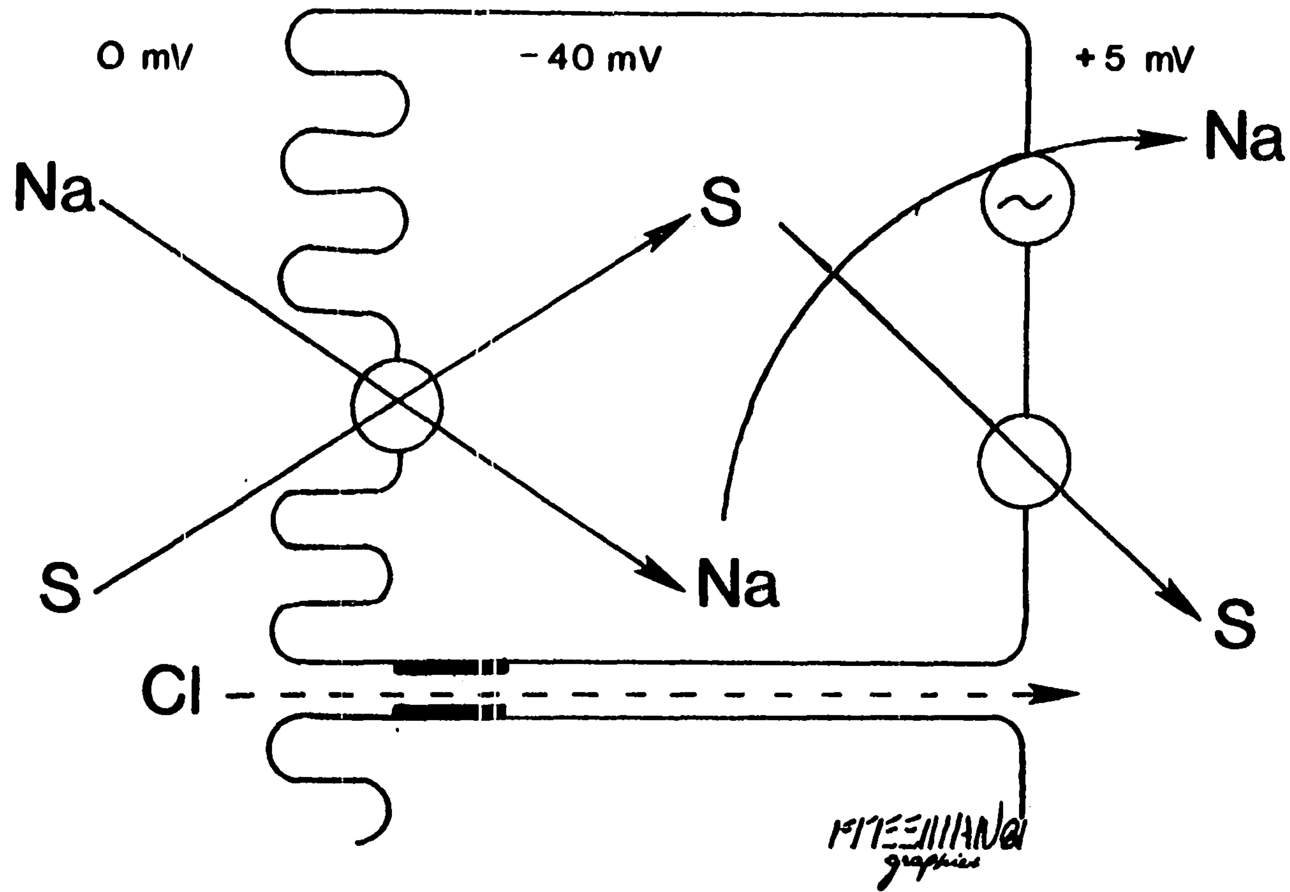
hydrolysis and the enzyme responsible is the ubiquitous sodium-potassium adenosine triphosphatase (Na-K-ATPase). Schultz (1978) has shown by autoradiographic studies and enzymatic analyses of isolated small intestinal cell membranes that the ouabain-sensitive ATPase is localized at basolateral membrane and no activity is found in the apical membrane.

The active absorption of sodium makes the serosal solution electrically positive compared to the mucosal solution. Even though the electrical potential difference is low, only 3-5 mV, it is enough to provide a driving force for diffusional flow of chloride from mucosa to serosa across the intercellular junctions of the epithelial cells. However, this might not be the only mechanism responsible for chloride absorption in the small intestine.

2. Sodium absorption coupled to absorption of organic solutes--The model proposed by Schultz and Curran (1970) and Schultz (1977) is illustrated in Figure 4. Sodium is absorbed by a cellular mechanism resembling the uncoupled process mentioned above. A wide variety of water-soluble organic solutes are actively absorbed by using the energy indirectly derived from the sodium pump at the basolateral membrane which maintains low intracellular sodium concentrations. Thus, the entrance of organic solutes, such as glucose or amino acids is coupled to the downhill movement of sodium into the cell in a carrier-mediated facilitated diffusion. Solute exit from the cell may occur by sodium independent facilitated diffusion (Schultz, 1980).

3. Neutral sodium chloride cotransport--Frizzell et al. (1979a) have presented a model for a neutral sodium chloride cotransport process

Figure 4. Cellular model of Na-coupled absorption of organic solutes (S) by small intestine (redrawn from Schultz, 1980)



0 mV
-40 mV
+5 mV
Na
S
Na
S
Na
S
Na
S
Cl

(Figure 5). This mechanism seems to be a major route for sodium and chloride absorption by the small intestine. The existence of a mechanism capable of mediating electrically neutral (one-for-one) sodium chloride absorption was first suggested as a result of studies in fish and rabbit gallbladder by Diamond (1962, 1964), Wheeler (1963), and Dietschy (1964). Later, its presence was confirmed in epithelial cells of the mammalian intestine, including human ileum (Turnberg et al., 1970), rabbit ileum (Nellans et al., 1973), rat colon (Binder and Rawlins, 1973) and bovine rumen (Chien and Stevens, 1972).

The central features of this mechanism as explained by Frizzell et al. (1976, 1979a) and Schultz (1980) are as follows:

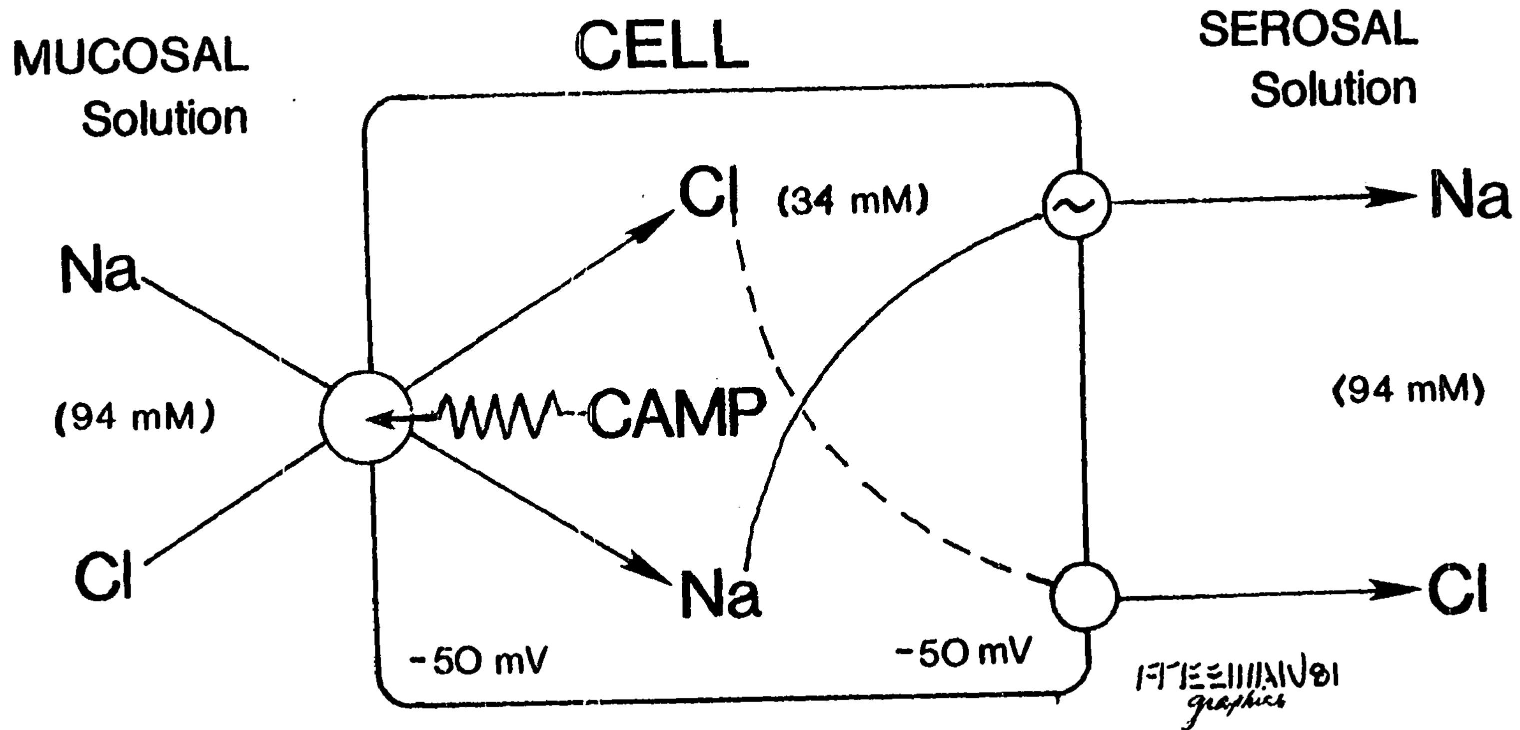
1. A carrier mechanism located at the mucosal membrane mediates the one-for-one, neutral entry of sodium and chloride into the cell. This entry process is inhibited by elevated levels of cell cyclic adenosine 3',5'-monophosphate (cAMP) as shown by Frizzell et al. (1975, 1979a) and Nellans et al. (1973).

2. Sodium passively moves into the cell due both to the differences in chemical concentration and electrical potential. The movement of sodium energizes the uphill flow of chloride against its electrochemical potential difference.

3. Sodium is extruded from the cell by the energy dependent, ouabain-sensitive ATPase to maintain low intracellular sodium concentrations and the electrochemical potential difference for sodium across the mucosal membrane.

4. Chloride passively moves out of the cell down its electrochemical gradient.

Figure 5. Cellular model of neutral NaCl absorption by small intestine (redrawn from Schultz, 1980)

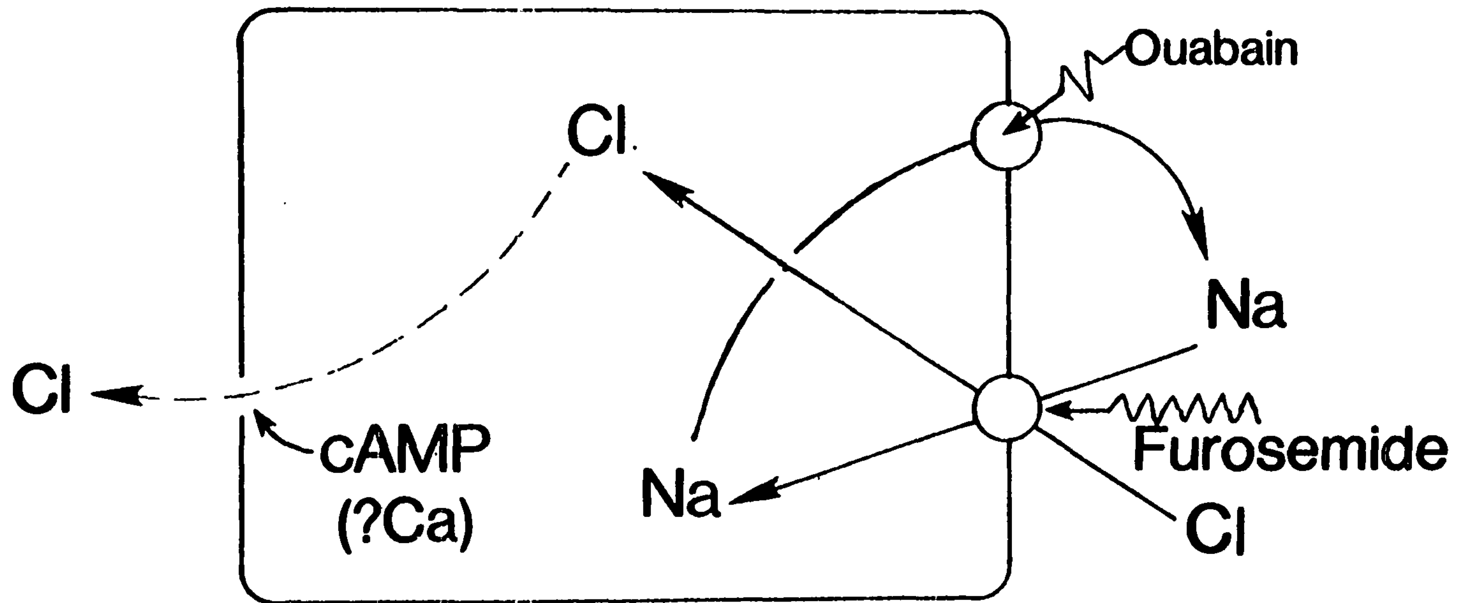

CELL
MUCOSAL
Solution
SEROSAL
Solution
Na
Cl
(94 mM)
Cl
(34 mM)
CAMP
Na
-50 mV
-50 mV
Na
Cl
(94 mM)

The energy necessary for this active transepithelial chloride absorption may be derived from coupling to the sodium gradient across the apical membrane and also indirectly from the energy invested in the sodium pump at the basolateral membrane. However, the precise mechanism is still unknown.

Mechanism of Intestinal Chloride Secretion

A cellular model for active chloride secretion has been proposed by Frizzell et al. (1980) as shown in Figure 6. Sodium and chloride enter the cell from the serosal solution via sodium chloride cotransport at the basolateral membrane. Chloride movement into the cell is against its electrochemical potential difference. By coupling to sodium, which moves down its electrochemical potential difference, chloride can enter the cell. Furosemide interacts with the secretory process by inhibiting sodium chloride cotransport across the basolateral membrane. After sodium and chloride accumulate in the cell, sodium is extruded by an energy dependent, ouabain-sensitive sodium pump located at basolateral membrane. However, the exit of chloride from the cell to the mucosal solution is passive and down its electrochemical potential difference. Thus, any agent that stimulates secretion of chloride can do so by increasing the chloride permeability of the apical membrane. There is a direct evidence from the study by Klyce and Wong (1977) that stimulation of active chloride secretion by rabbit cornea is associated with a marked decrease in the resistance of the apical membrane due to a specific increase in its chloride permeability. A similar increase in membrane permeability to

Figure 6. Model for active Cl secretion by rabbit colon (redrawn from Frizzell et al., 1980)


MUCOSAL
Solution
Cell
SEROSAL
Solution
Ouabain
Cl
Na
Cl
cAMP
(?Ca)
Na
Furosemide
Cl

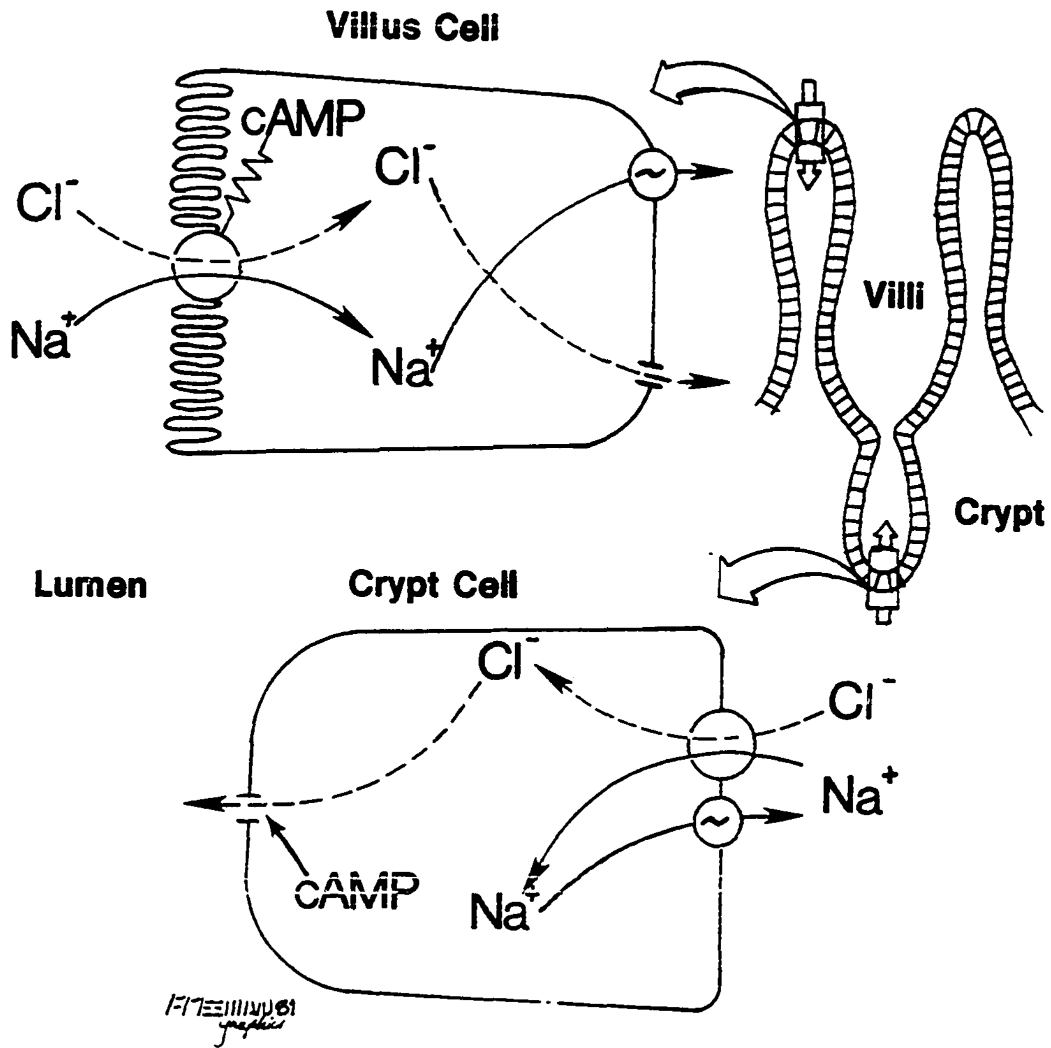
chloride has been observed in insect salivary gland cells (Berridge et al., 1975) and pancreatic exocrine cells (Gardner, 1979).

Cyclic Nucleotides and Small Intestinal Ion Transport

Determination of unidirectional sodium and chloride fluxes and short circuit current across small intestine mounted in Ussing chambers *in vitro* has provided useful information about ion transport across the epithelial tissues. The studies by Field (1971), Field et al. (1972), and Bolton and Field (1977) show that if an agent that increases the concentration of cAMP (e.g., cholera toxin) is added to the tissue, rapid and sustained increases in potential difference and short circuit current develop. The direction of net chloride transport reverses from absorption to secretion due both to a decrease in the unidirectional mucosa-to-serosa flux and an increase in the unidirectional serosa-to-mucosa flux. The direction of net sodium transport is also reversed, though to a lesser degree than chloride transport. Field (1980) postulated that there are two mechanisms responsible for these changes: sodium chloride cotransport across the brush border and sodium-dependent chloride secretion. These two mechanisms are cAMP sensitive.

The model for actions of cAMP on ion transport in intestinal villus and crypt cells is shown in Figure 7 (Field, 1980). Cyclic AMP inhibits sodium chloride cotransport across the brush border, so transepithelial chloride absorption and most transepithelial sodium absorption are inhibited. This was first demonstrated by Nellans et al. (1973) using rabbit ileum. Similar studies on the effect of cAMP have been made by

Figure 7. Postulated separate actions of cAMP on ion transport in intestinal villus and crypt cells (redrawn from Field, 1980)


Villus Cell
cAMP
Cl⁻
Cl⁻
Na⁺
Na⁺
Villi
Crypt
Lumen
Crypt Cell
Cl⁻
Cl⁻
Na⁺
cAMP
Na⁺

Frizzell et al. (1979b) on flounder intestine and on rabbit gallbladder (Frizzell et al., 1975). They concluded that cAMP may inhibit a specific brush border permeability factor that is rate limiting for sodium chloride absorption.

In addition to the antiabsorptive effect of cAMP which plays a significant role in the overall shift from absorption to secretion, the direct stimulation of an electrogenic secretory process has also been suggested (Field, 1980). Chloride is secreted against an electrochemical potential difference which depends on the presence of sodium in the serosal bathing medium and is ouabain-sensitive. Chloride accumulates intracellularly above electrochemical equilibrium, and in the resting secretory cell, there is little or no chloride conductance in the apical membrane and chloride simply recycles to the serosal medium. Chloride secretion is initiated when apical conductance is increased. An important intracellular stimulus for this increased membrane conductance appears to be cAMP in the frog cornea (Klyce and Wong, 1977) and cAMP may act in a similar manner on intestinal epithelium.

Circumstantial evidence suggests that cAMP exerts its antiabsorptive action in villus cells and its direct secretory action on crypt cells (Field, 1980). For example, in rabbit gall bladder (Frizzell et al., 1975) and in flounder intestine (Frizzell et al., 1979b) which contain no crypts or other multicellular gland-like structures, cAMP inhibits salt absorption in both tissues but does not stimulate net secretion in either. On the contrary, in rabbit distal colon, where crypts are prominent, cAMP

can stimulate active chloride secretion but has no effect on coupled sodium chloride absorption (Frizzell et al., 1976).

Cyclic guanosine monophosphate (cGMP), another important cyclic nucleotide, has also been shown to play a role in stimulating secretion in the small intestine. Many studies show that the effect of heat stable *Escherichia coli* enterotoxin (ST) in producing diarrhea is associated with increased level of mucosal cGMP (Field et al., 1978; Hughes et al., 1978; Scoot et al., 1980), and activation of guanylate cyclase (Field et al., 1978; Guerrant et al., 1980). Cyclic AMP levels are unchanged. However, the precise mechanism for the secretory effects of cGMP is unknown. Paradoxically, some drugs, such as adrenergic agonists, which increase absorption of water and electrolytes in the small intestine also produce a short-lived increase in mucosal cGMP (Field and McColl, 1973; Brasitus et al., 1976). It has been suggested that there may be more than one pool of cyclic nucleotide that can be stimulated in the intestinal mucosa (Powell and Tapper, 1979).

Calcium has been shown to stimulate a variety of secretory processes. Addition of Ca ionophore A-23187 to the serosal side of isolated rabbit ileal mucosa produced a secretory response qualitatively identical with those produced by cAMP and theophylline (Bolton and Field, 1977). Many epithelia have been shown to secrete their products in the presence of Ca ionphore: histamine by mast cells (Foreman et al., 1973), fluid by blowfly salivary gland (Prince et al., 1972), catecholamine by cat adrenal (Garcia et al., 1975). Babcock et al. (1976) suggested that the ionophore can increase cytosolic Ca by facilitating Ca entry across the plasma mem-

brane and/or by releasing Ca from mitochondria. Frizzell (1977) found that Ca ionophore when added to mucosal solution of rabbit colon reversed active chloride absorption to active chloride secretion without affecting the rate of active Na absorption. The effect of Ca ionophore was Ca dependent, unlike the cAMP effect which did not need extracellular calcium. The mechanism of calcium-induced active chloride secretion is unknown. However, Frizzell (1977) suggested that the ionophore-induced secretion was not mediated by an increase in mucosal cAMP since A-23187 did not influence the cAMP levels of colonic mucosa. He concluded that Ca might act as a direct activator of the transport process responsible for chloride secretion. The ability of cAMP to increase Ca efflux from fly salivary gland (Prince et al., 1972), isolated kidney cells (Borle, 1974), isolated pancreatic acinar cells (Christophe et al., 1976), and colonic mucosa (Frizzell, 1976) suggested that cAMP might cause electrolyte secretion by releasing Ca from intracellular stores (Frizzell, 1977; Field, 1980).

Frizzell (1977) noted that the effect of A-23187 could be mediated by an increase in cGMP levels since A-23187 has been shown to increase cGMP in rat parotid slices (Butcher, 1975), guinea pig pancreatic acinar cells (Christophe et al., 1976) and human neutrophils (Smith and Ignarro, 1975). Guanylate cyclase is a cytoplasmic enzyme which is Ca dependent (Frizzell, 1977). Many investigators have shown that cholinergic agonists could increase the short circuit current and stimulate chloride secretion (Hardcastle and Eggenton, 1973; Isaacs et al., 1976) and increase cGMP concentration (Brasitus et al., 1976). However, the role of cGMP in electrolyte secretion may not be a direct one since adrenergic agonists which also

increased cGMP caused electrolyte absorption (Brasitus et al., 1976; Tapper et al., 1978).

Secretory Diarrhea of Bacterial Origin

Several enteric bacteria release exotoxins, termed enterotoxins, which are capable of stimulating active secretion by the small intestine (Argenzio and Whipp, 1980). Among these are *Vibrio cholerae*, which is an important cause of epidemic diarrheal disease in man (Carpenter, 1980), and *Escherichia coli*, the most important cause of morbidity and mortality of diarrheal disease in young animals (Wohlgemuth, 1977).

Vibrio cholerae

Vibrio cholerae causes an acute diarrhea in man with no apparent pathologic change in the intestine (Elliott et al., 1970; Sharp, 1973). Since the enterotoxin of *V. cholerae*, choleragen, is the most thoroughly studied and produces the classic example of a pure "secretory diarrhea," it is useful as a model with which to compare other bacterial enterotoxins (Argenzio and Whipp, 1980). *V. cholerae* is a gram-negative, motile, curved rod that grows on simple nutrient media at alkaline pH (Schrank et al., 1973). If large numbers of vibrios are ingested, the most important factor preventing disease is the gastric acid barrier since vibrios are highly sensitive to acid (Carpenter et al., 1974). However, organisms which survive passage through the stomach multiply in the small intestine and produce enterotoxin. Cholera toxin (CT) is a protein enterotoxin with a molecular weight of 84,000 and a well-defined subunit structure (Carpenter, 1980). The three subunits are characterized as A_1, A_2 and B. A_1

subunit is responsible for the toxin activity, while A_2 may serve to stabilize the A subunit complex (Ohtomo et al., 1976) which contains four to six B subunits (Lonnroth and Holmgren, 1973). The B subunit complex lacks toxin activity but is capable of binding to membrane sites (Richards and Douglas, 1978). After the toxin binds to mucosal receptors, G_{M1} monosialogangliosides in the brush border (Fishman and Brady, 1976), a sustained stimulation of cellular adenylate cyclase occurs (Kimberg et al., 1971) with subsequent increased levels of cAMP. Cholera toxin is characterized by a delayed onset of action and a prolonged duration of action (Argenzio and Whipp, 1980). After exposure of small intestinal mucosa to CT, decreased net absorption can be detected within 30 to 60 minutes, but maximum secretion is not observed until 3 hours and is sustained for 12 hours even when the mucosa is washed in an attempt to remove the toxin (Carpenter et al., 1969). The delayed onset of action is thought to be related to dissociation of a toxin subunit from the catalytic site of the adenylate cyclase complex at the intracellular surface of the cell membrane (Argenzio and Whipp, 1980).

The concept that the enterotoxin effect of cholera is mediated by cAMP has been confirmed by identical changes in ion movement (a net secretory flux of chloride and reduced net absorptive flux of sodium) upon addition of cAMP or theophylline (Field et al., 1968). Field et al. (1972) suggested that both cAMP and CT stimulate active secretion by a common pathway. This conclusion is based on the flux data and also the fact that pretreatment of rabbit ileal mucosa with CT greatly reduced the short circuit current response to theophylline and dibutyryl cyclic AMP.

From the investigation of enterotoxin effects on rabbit and swine jejunum, Hamilton et al. (1978) found that, in contrast to rabbit jejunum, the secretory response to enterotoxin in pigs was not accompanied by elevations in mucosal cAMP concentrations. The results are inconsistent with the hypothesis that the adenylate cyclase system is an essential step for enterotoxin induced intestinal secretion. They concluded that the activation of intestinal adenylate cyclase by bacterial enterotoxin may only be an associated and not a necessary event for the stimulation of intestinal secretion.

In contrast to the specificity of most hormone-adenylate cyclase interactions, CT is able to interact with any cell-surface receptor activating adenylate cyclase (Argenzio and Whipp, 1980). When the tissue is exposed to this toxin by artificial means, a response occurs which is characteristic of the particular tissue examined. For example, CT enhances lipolysis in the rat epididymal lipocyte (Vaughan et al., 1970); causes sustained hyperglycemia after intravenous injection in the dog (Pierce et al., 1972); increases steroidogenesis from adrenal cells (Donta et al., 1973). Fortunately, V. cholerae does not have the ability to penetrate the gut mucosa so the intestinal effect appears to be the only one of significance in the pathogenesis of naturally occurring disease (Carpenter et al., 1974; Argenzio and Whipp, 1980).

Escherichia coli

Although Escherichia coli is part of the natural flora of the mammalian gut, certain strains can cause a cholera-like disease in humans and young farm animals (Dallas and Falkow, 1979). The characteristic

feature of enterotoxic colibacillosis is proliferation of E. coli in the anterior small intestine where they produce an enterotoxin which causes the small intestine to secrete fluid (Moon, 1974). Enterotoxic colibacillosis in livestock occurs in the young. Three ages of peak incidence of porcine enterotoxic colibacillosis are: neonatal, 3 weeks, and immediately post-weaning, which were explained by Moon (1974) as periods of relative antibody deficiency leading to disease susceptibility. Resistance to the effects of enterotoxigenic E. coli increases with age (Kohler, 1968; Moon and Whipp, 1970; Smith and Linggood, 1972). However, antibody may not be the sole factor in resistance to enterotoxin production because resistance to both antigenic and nonantigenic forms of enterotoxin occurs (Moon, 1974). Kenworthy and Allen (1966) and Kenworthy et al. (1967) found that in normal, healthy pigs there was a transient increase in fecal fluid content, degenerative changes in intestinal absorptive cells, and malabsorption of some nutrients following weaning. It is possible that a temporary reduction in net fluid absorption at weaning is able to reduce the amount of enterotoxin required to cause net secretion (Moon, 1974). In other words, physiological changes of the intestinal mucosa may predispose the epithelium to the secretory effects of toxin.

Adhesion of E. coli to the intestinal epithelium has been demonstrated (Smith and Halls, 1968a), and it is possible that attachment allows enteropathogenic strains to overcome gut motility and proliferate to large numbers in the small intestine (Jones and Rutter, 1972). Bertschinger et al. (1972) have shown that enteropathogens rapidly form layers near the apical portions of absorptive cells along the villi from

the tip to the base adjacent to the brush border membrane while nonpathogens tend to be in the central lumen. Colonization of the upper intestine in pigs may be enhanced by the presence of K88 plasmid-transmitted fimbrial antigen (Jones and Rutter, 1972). Surface antigen K88 is common to many, but not all, pig enteropathogens and may be involved in the pathogenicity of strains which produce it (Moon, 1974). Jones and Rutter (1972) found that in contrast to conventional pigs, enterotoxigenic K88-negative _E. coli_ did intensively colonize, without adhering to, the anterior small intestine and caused diarrhea in gnotobiotic pigs. The essential contribution of adhesion to the pathogenesis of enterotoxic colibacillosis is in facilitating colonization of the small intestine (Moon, 1974). O'Hanley and Cantey (1978) showed that strains of _E. coli_ that produce diarrhea by enterotoxin synthesis or unknown mechanisms contain pili and flagella while strains that produce diarrhea by mucosal invasion lack both types of surface structure. Pili are thought to mediate adherence to gut mucosal epithelium by bacteria that cause diarrhea (Evans et al., 1978). Flagellum-mediated motility may be involved in chemotaxis towards gut mucosal epithelium (Allweiss et al., 1977) and penetration of the barrier that overlies mucosal epithelium (Guentzel et al., 1977).

Pathogenic strains of _E. coli_ from various species of domestic animals injected into ligated intestinal loops of suitable hosts caused fluid accumulation (De et al., 1956; Smith and Halls, 1967a). Smith and Halls (1967a,b) demonstrated that not only _E. coli_ bacterial cultures but also their cell-free filtrates produced fluid accumulation in homologous

ligated loops of the small intestine. Endotoxin from these cultures did not cause fluid accumulation. Like K88 antigen, enterotoxin production is controlled by plasmids which can be transmitted between strains of E. coli during conjugation (Smith and Halls, 1968b). Smith and Linggood (1971) transmitted both K88 and enterotoxic plasmids into the same recipient E. coli, transforming nonenteropathogenic strains to enteropathogenic strains which could colonize small intestine and produce enterotoxin, and demonstrated that these are controlled by separate plasmids.

Two enterotoxins are known to be produced by E. coli. These two enterotoxins, one heat-labile (LT) and the other heat-stable (ST) have been purified by several groups of investigators (Clements and Finkelstein, 1979; Kunkel and Robertson, 1979; Takeda et al., 1979; Lallier et al., 1980). Comparison of results among laboratories is difficult because different methods of treating and assaying enterotoxin as well as different strains and methods of production have been used (Moon, 1974). However, data from different laboratories indicate that LT, which is closely related functionally, structurally, and immunologically to the enterotoxin of V. cholerae, is a single entity since the LTs from all strains of enterotoxigenic E. coli (ETEC) characterized thus far are similar if not identical (Sack, 1980). LT synthesized in cell-free medium has a subunit structure analogous to cholera toxin, i.e., an active subunit A, with a molecular weight of 23-26,000 daltons associated with 3-4 subunits B, each 11,500 daltons (Rašková and Raška, 1980). Furthermore, the B subunit of LT binds specifically to G_{M1} ganglioside as does the B subunit of enterotoxin of V. cholerae (Sack, 1980).

Like cholera toxin, heat-labile E. coli enterotoxin stimulates the activity of adenylate cyclase in the mucosa of the small intestine (Field, 1971) causing a delayed secretion of isotonic fluid which persists for many hours after the binding of the toxin to the mucosal cell (Sack et al., 1971). Immunological cross reactivity between cholera enterotoxin and LT is well-established (Smith and Sack, 1973). In spite of the similarities, the enterotoxins are not identical. Differences in the amino acid composition together with similarities have been described for the A units of both toxins (Finkelstein and Clements, 1979). Moss and Richardson (1978) found different requirements necessary to achieve the optimal adenylate cyclase stimulation in vitro. While choleragen activity was enhanced by increasing the potassium phosphate or sodium acetate concentration, both salts inhibited LT activity.

The activation of adenylate cyclase by E. coli LT leads to an increase of cAMP in the mucosal cells of the small intestine (Evans et al., 1973). This increase alters the intestinal transport in two ways. It inhibits a coupled influx for Na^+ and Cl^- at the luminal border, so the absorption of NaCl and water is reduced. This effect is probably on the villus cells. The second mechanism causes active secretion of anion and Na^+ into the lumen; this probably takes place in the crypt cells. It might be concluded that LT, like cholera toxin, induces net ion fluxes to the luminal side, hypersecretion, and diarrhea (Moon, 1974; Rašková and Raška, 1980; Field, 1980).

The available information on ST suggests more heterogeneity than for LT. Purified preparations from different laboratories have given variable

results (Alderete and Robertson, 1978; Lallier et al., 1980; Lathe et al., 1980; Takeda et al., 1979) and ST preparations from human and animal strains react differently in assays of mouse, pig and rabbit intestine (Gyles, 1979; Kapitany et al., 1979). Alderete and Robertson (1978) have purified the supernatant of an E. coli strain of porcine origin. They found that the molecular weight was 4420-4425 daltons. Amino acid analysis yielded 47 residues with a calculated molecular weight of 5100 daltons. No trace of lipids or nucleic acids was present but there was a positive reaction for carbohydrates. The purified ST has a characteristic UV absorption spectrum at 270 nm. The biological activity remains intact after heating to 100°C for 30 min. In contrast, Kapitany et al. (1979) found that the crude products of E. coli enterotoxin of bovine origin were heat stable while the purified toxin was not. Burgess et al. (1978) concluded that there might be two different ST toxins. One is methanol-soluble, partly heat-stable, and active in neonatal piglets. The other is methanol-insoluble, active in weaned pigs and rabbit intestinal loops but inactive in the suckling mouse test. However, further research is needed before it can be firmly established whether only one or more E. coli ST are produced and if and how much they differ between species (Rašková and Raška, 1980).

There is general agreement that the ST is different from the E. coli LT product (Alderete and Robertson, 1978). Unlike LT, ST does not stimulate adenylate cyclase with subsequent increase in cAMP in the intestinal mucosa (Kantor, 1975; Hamilton et al., 1978). Cyclic guanylate cyclase stimulation with resulting accumulation of cyclic 3',5'-guanosine mono-

phosphate (cGMP) may be responsible for E. coli ST effects. The increase in cGMP is rapid and precedes the increase of intestinal secretion. In vitro studies of purified ST effects on rabbit ileum revealed abolition of net chloride absorption accompanied by large and persistent increases in cGMP concentration (Field et al., 1978). A central role of cGMP mediated action of ST is strongly supported by observations with other cGMP-related stimuli. Field et al. (1978) found a good correlation between the effects of the enterotoxin and those of the 8-bromo analogue of cGMP. Unlike cholera toxin, heat-stable enterotoxin appears to stimulate a nucleotide cyclase only in small and large intestine and does not have the effect on other tissues (Rao et al., 1979). Apparently, the binding of ST to the intestinal mucosa is reversible (Field et al., 1978; Hughes et al., 1978; Newsome et al., 1978). Alderete and Robertson (1978) found that it is possible to antagonize the biological activity of ST by antisera. However, ST is a poor antigen. Although ST-mediated intestinal secretion is associated with elevated mucosal cGMP, the precise role of this nucleotide in epithelial transport process is not understood.

Bioassay Methods for ST

The assay methods for ST are limited to animal models since toxin activity is specific for intestinal epithelium. The bioassays for ST may be divided into three major methods as follows:

1. Infant mouse assay--This test is used by most investigators with some modifications (Dean et al., 1972). The toxin is injected into the stomach of 2-4 day old mice. After 3-4 hours, the animals are killed and

the entire intestine is removed, weighed and compared to the remaining body weight. The natural log of the reciprocal of toxin dilution is plotted against the gut weight to body weight (GW/BW) ratio and linear regression determined. One mouse unit is defined as the dilution that gives a GW/BW ratio of 0.09 and normalized to mouse unit per ml of undiluted toxin. Moon et al. (1978) found that the ratio of GW/BW can change depending on ambient temperature and recommended that mice be maintained at 37°C following the administration of the toxin.

2. Ileal loop assay--Originally ST was discovered using the ligated homologous small intestine loop (Smith and Halls, 1967a). Later, measurement of fluid accumulation induced by ST in ileal loops was used in rabbits (Moon et al., 1970; Moon and Whipp, 1971; Evans et al., 1973), dog (Nalin et al., 1974), and swine (Moon et al., 1971). The method consisted of the introduction of toxin into ligated segments of the ileum of the animal. Fluid secretion in response to ST was immediate and reached a maximum within 6 hours irrespective of dose (Evans et al., 1973). After an appropriate time interval, the segments were excised and both the volume of fluid and the length of the emptied segments were determined. The volume per length ratios (ml/cm) were compared. This ligated loop model has been used in a variety of species to test the enteropathogenicity of different strains of *E. coli*. The accumulation of fluid in the loops is considered a manifestation of enteropathogenicity since nonpathogens do not cause the accumulation of fluid.

3. In situ perfusion technique--Toxin was tested in anesthetized rats by perfusing 20 cm jejunal segments at a rate of 0.5 ml/min with a

peristaltic pump. Net transport of water was calculated from changes in polyethylene glycol 4000 concentrations by the marker technique formula (Powell and Malawer, 1968). This method is useful for identifying toxin-producing strains for both heat-labile and heat-stable toxins (Klipstein et al., 1976).

Everted Gut Sac Preparation

The everted sac technique for studying intestinal absorption was introduced in 1954 by Wilson and Wiseman. A small piece of rat or golden hamster gut, 2-3 cm long, was everted, filled with fluid to distend the wall, and tied off at both ends. The distension increased the surface area of the sac and reduced the thickness of the sac wall. The everted sac was suspended in chamber containing physiological salt solution which was oxygenated and maintained at 37°C. Eversion of the intestine provided better oxygenation of the mucosal epithelium and facilitated analysis of solute absorption into the relatively small volume inside the sac. At the end of the experimental period, the ligature was cut and fluid inside the sac drained, measured, and analyzed for solute. In 1958, Crane and Wilson modified the method to permit serial sampling of the fluid on the serosal side of the intestine by inserting a cannula into one end of the sac. The stoppered tube was used and the hydrostatic pressure adjusted allowing serosal fluid to rise into the upper portion of the cannula when the pipetting was desired. Benson and Rampone reviewed gastrointestinal absorption in 1966 and named that period as an era of the everted sac.

Some of the problems of this in vitro method are (a) maintenance of biological viability, (b) maintenance of structural integrity, and (c) artifactual influences due to unnatural absorption barriers (Wolfe et al., 1973).

A number of workers have investigated the extent of viability of excised gut preparations. There is strong evidence that the intestinal preparations are "viable" with respect to certain metabolic processes and active transport mechanisms for several hours after removal from an intact blood supply (Gibaldi and Grundhofer, 1972). Bamford (1966) found that the rate of oxygen consumption by isolated ileal and jejunal segments was constant over 3 hour period. Robinson and Felber (1966) showed that the active uptake of L-methionine and L-phenylalanine by rat intestine at 37°C was maintained for 2-3 hours after isolation. Duration of viability is decreased by drugs that interfere with metabolic mechanisms and/or the use of buffered solutions that are not of optimum physiological composition (Wolfe et al., 1973).

The problem of structural integrity has been considered by Gibaldi and Grundhofer (1972) to be even more crucial to the absorption processes than viability, especially where passive mechanisms predominate. In 1970, Levine et al. observed that intestinal sacs of rats are morphologically intact after eversion but progressively lose structural integrity. In one hour, there is total disruption of the epithelial border. Tissue damage is slower at 23°C or in tissues of animals sacrificed under anesthesia. However, Benet et al. (1971) suggested that the determination of the constancy of transfer rate as a function of time might be a valid approach

to assessing the functional integrity (i.e., both biological and structural) of the everted gut preparations. Taraszka (1971) observed that the addition of 10 mM glucose in the buffer appeared to enhance the structural durability of cannulated rat gut preparations examined grossly.

One difficulty in extrapolating _in vitro_ flux data to _in vivo_ transport of drugs is that the _in vitro_ fluxes are measured through the columnar epithelium plus the underlying connective and muscle tissue (Wolfe et al., 1973). Therefore, the rate-limiting step for _in vitro_ transport might be the intestinal musculature, a barrier that would not be encountered during _in vivo_ transport. Parsons and Paterson (1960) attempted to correct this problem by removing the muscular coats of the intestinal segment to reduce hindrances to fluid and solute movements across the submucosal tissues. McDougal et al. (1960) reported that the selective and absorptive elements in the absorption of various drugs are the intestinal epithelia and not the intestinal musculature. Nayak and Benet (1971) attempted to measure the transport rate through rat intestinal muscle layer after the columnar epithelium was removed by EDTA treatment and found high transfer rates with no difference in directional transport. Field et al. (1971) observed that the transmural potential and sodium transport capacity of the intestinal mucosa are better maintained _in vitro_ if the muscularis is removed. A possible explanation for this may be that the unstripped intestine is relatively hypoxic due to an interference by the muscularis externa with oxygen diffusion from the serosal bathing solution to the basal surface of the epithelium. In support of this, Wolfe et al. (1973) found that aerating the serosal side

compartment, after the muscle has been stripped off, had no advantage over the method in which only the mucosal side was oxygenated. There was no damage to the epithelium or to the integrity of the submucosa of the stripped segment. They concluded that the use of stripped intestinal segments for in vitro drug transport studies would provide a well-controlled, reproducible technique for elucidating the mechanism involved in the in vivo absorption of drugs from the intestinal lumen.

Isolated Epithelial Cell Preparation

The use of isolated epithelial cell preparation which can be handled as homogeneous suspension is a more recent technique which allows a more direct assessment of epithelial cell transport process (Kimmich, 1970). Since cells can be collected in gradient from villus tip to crypt, this technique has permitted characterization of enzyme content, membrane permeability, and metabolic activity of cells as they mature along the villus. For example: The differentiated villus cells contain high activities of alkaline phosphatase and sucrase while crypt cells contain thymidine kinase which is the marker enzyme for the mitotically active cells (Fortin-Magana et al., 1970; Weiser, 1973).

One of the earliest attempts to isolate viable epithelial cells was reported in 1941 by Dickens and Weil-Malherbe using the edge of a glass microscope slide to scrape the mucosal layer of the intestine. The mucosal scrapings contained epithelial cell sheets as well as individual cells, small cell clumps, fragmented villi, and cell organelles including free nuclei. Studies of the biochemical activity of these cells revealed

a high aerobic glycolytic capability which declined rapidly in 40 min. With this technique, Schultz et al. (1966) demonstrated that the epithelial sheets could accumulate both alanine and 3-0-methylglucose against concentration gradients. However, this technique is limited by the cell aggregation induced by the large quantities of mucus released at the time of scraping (Sjostrand, 1968).

In recent years, three major methods of isolation of the enterocytes have been used: 1) mechanical vibration, 2) chemical and enzymatic dissociation, and 3) combinations of 1 and 2. The enzymes used are collagenase (Yousef and Kuksis, 1972) or hyaluronidase (Kimmich, 1970). The chemicals used are citrate (Stern, 1966) or EDTA (Weiser, 1973).

The mechanical method of isolation of the enterocytes has been described by many investigators (Sjostrand, 1968; Harrison and Webster, 1969). The intestine was everted over a rod which was then fastened to a motor drive and vibrated at high frequency. An initial 5 minute vibration was employed to remove mucus and loose cells near the villus tips prior to cell collection. Further vibrations yielded a gradient of cells from villus tips to crypts. The villus cells were harvested by vibrating at a speed of 100 cps with an amplitude of 2 mm for 30 min. The crypt cells could then be removed by distending the gut segment with air pressure and continuing vibration for 10-20 min. Cells could be removed from relatively specific areas of the villus by adjusting the vibration interval (Webster and Harrison, 1969). Cells near the villus tips are removed most readily, while longer vibration periods remove progressively those

cells close to the crypt region. Crypt cells are not removed unless the gut wall is distended.

Marsh et al. (1971) examined the isolated epithelial cells and their subcellular organelles using transmission and scanning electron microscopy. They reported that the morphology of the cell is well-preserved even after incubation for at least 1 hour. Kimmich (1975) estimated that approximately 80% of cells prepared by hyaluronidase treatment remain viable using trypan blue exclusion as an index of viability. This is in agreement with values obtained by other investigators for other preparative techniques (Stern and Reilly, 1965; Reiser and Christensen, 1971). However, the question of the usefulness of dye exclusion methods as indicators of cell integrity was raised (Barrett and Coleman, 1973; Barrett, 1974) due to the observation that even after osmium tetroxide fixation, cells of various kinds exclude trypan blue for several hours. This led Kimmich (1975) to place more emphasis on metabolic and transport capability as indices of cell integrity and function. He found that the metabolic activities of intestinal epithelial cells prepared from chickens exhibit linearity for period of up to 2 hours.

Potential Antidiarrheal Drugs

For many years, diarrhea was considered to result from hypermotility of the intestine and antidiarrheal drugs were designed to inhibit propulsive activity. However, for the diarrhea induced by bacterial enterotoxins less attention has been paid to changes in motility of the small intestine as an important factor in diarrhea. Instead, interest has

centered on biochemical events of enterotoxin activity (Rašková and Raška, 1980). Moon (1978) considered that even if propulsive muscular contractions occur in colibacillosis, it is probable that their contribution to diarrhea is much less significant than hypersecretion. Burns et al. (1978) found that CT, ST, and LT increased myoelectric activity in distal rabbit ileal loops. Pesti and Gordon (1978) tested ST filtrate on various smooth muscle preparations and found that the filtrates per se were not especially active but they did antagonize alpha-adrenergic mediated relaxation. They stated that the ST filtrates behaved like the alpha blocker phentolamine.

Field and McColl (1973) found that epinephrine and norepinephrine produced a marked and sustained drop in electric potential difference and short circuit current when added to isolated rabbit ileal mucosa. This decrease was greater in rabbit ileal mucosa incubated in HCO_3-Ringer solution than in HCO_3-free solution. The effect of epinephrine and norepinephrine on ion transport was also studied and found to increase absorption of Na (increase in mucosa to serosa unidirectional flux) and Cl (increase in mucosa to serosa and decrease serosa to mucosa unidirectional fluxes). These effects were not seen in tissue incubated in HCO_3-free Ringer solution. However, addition of isoproterenol or propranolol did not produce these changes. They concluded that stimulation of alpha-adrenergic receptors in the ileal mucosa by epinephrine and norepinephrine enhanced active absorption of Na and Cl and reduced short-circuit current, probably by inhibiting net HCO_3 secretion. Cyclic AMP and agents which increased its intracellular concentration such as theophylline have been

shown to increase Cl secretion (Field, 1974). Field et al. (1975) found that epinephrine significantly decreased theophylline-induced but not cAMP or cholera toxin-induced Cl secretion in isolated rabbit ileal mucosa. The base-line cAMP level was not affected by epinephrine, norepinephrine and isoproterenol. These results suggested that there might be factors other than cAMP that produced ion secretion from the intestine and that the effects of alpha-adrenergic stimuli on ion transport were not due to inhibition of cAMP accumulation. The possible role of cGMP in the action of epinephrine on intestinal ion transport was examined by Brasitus et al. (1976). They found that epinephrine increased cGMP in isolated rabbit ileal mucosa and this effect was blocked by atropine at a 100 μM but not at 1 μM concentration. The stimulating effect of epinephrine on cGMP was transient, reached a maximal value within 2 min and disappeared within 30 min.

Two classical systems, cholinergic and adrenergic, have long been known to be involved in controlling the secretion of the intestine (Wright et al., 1940; Florey et al., 1941). It is possible that the nonadrenergic, noncholinergic inhibitory (purinergic) neurons and the peptidergic neurons may also be involved; their precise roles are still not clear (Powell and Tapper, 1979). While the adrenergic agonists have been shown to increase water and electrolytes absorption (Field and McColl, 1973; Field et al., 1975; Brasitus et al., 1976), cholinergic drugs have been shown to have the opposite effect. Tidball (1961) found that bethanechol, a cholinergic agonist, increased Cl and water secretion from the dog jejunum. Hubel (1976) reported that pilocarpine increased transmural potential differ-

ence, reduced absorption of Na, K, HCO_3 and water and increased secretion of Cl in rat jejunum. Tapper et al. (1978) observed different results from various doses of carbachol using rabbit ileum. Low-dose carbachol caused a transient increase in the potential difference and short-circuit current and stimulated Cl secretion. These effects were inhibited by atropine (10^{-6} M). High-dose carbachol reduced the electrical parameters and stimulated Na and Cl absorption. These effects were inhibited by hexamethonium (10^{-5} M). They proposed that high-dose carbachol stimulated nicotinic receptors on postganglionic sympathetic fibers causing a release of catecholamine and a resulting alpha-adrenergic response by the intestinal epithelium. However, the physiological significance of this response in the gut remains to be determined.

Isaacs et al. (1976) studied the _in vitro_ effect of acetylcholine on ion transport in human intestinal mucosa and found that addition of acetylcholine caused a short-lived elevation in cAMP 20-30 seconds after drug addition. Acetylcholine also caused Cl secretion while unidirectional and net Na transport was unaffected. Cholinergic drugs have been shown to increase cGMP in the intestinal mucosa (Brasitus et al., 1976; Tapper et al., 1976). The role of cGMP in ion transport is still in question since the dose of carbachol necessary to increase cGMP levels in intestinal mucosa was a dose that also had an alpha-adrenergic effect. In addition, cholinergic and adrenergic drugs have opposing effects on electrolyte transport, yet they both increase mucosal cGMP (Tapper et al., 1978). Since the intestine has multiple cell types, it has been suggested

that more than one cyclic nucleotide pool may exist and each can be stimulated by different drugs (Powell and Tapper, 1979).

Opiate alkaloids have long been used in the treatment of diarrhea. Morphine and related drugs are known to increase nonpropulsive muscle activity by inducing rhythmic contractile activity of the circular muscle and inhibiting motility of the longitudinal muscle of the gut (Bass, 1968). The effects of these compounds on ion transport in rabbit ileum in vitro was studied by Racusen et al. (1978). They found that codeine reduced potential difference and short circuit current and increased Na absorption in rabbit ileal mucosa. These effects could be blocked by naloxone. Codeine reversed net Na secretion to net absorption in tissue pretreated with theophylline. McKay et al. (1981) found similar electrical responses to those reported by Racusen et al. (1978) but reported that morphine increased Cl absorption without any change in Na transport. Enkephalin has been shown to increase net Na and Cl absorption in rabbit ileum (Dobbins et al., 1980). Valiulis and Long (1973) demonstrated that morphine inhibited small intestinal fluid secretion stimulated by cholera toxin. This raised the possibility that the mechanism of antidiarrheal action of opiate compounds involved the mucosa and not the muscle (Powell, 1981). Although the opiate effect on ion transport is opposite that of cAMP, these compounds have no effect on basal cAMP levels or on VIP and theophylline-stimulated cAMP levels (Racusen et al., 1978; Dobbins et al., 1980). The precise mechanism of action of opiate alkaloids on ion transport remains to be elucidated.

Vasoactive intestinal peptide (VIP) occurs naturally within the intestinal wall (Said and Mutt, 1972). It has been shown to increase canine jejunal and ileal secretion (Barbezat and Grossman, 1971). Intravenous infusion of VIP (13 μg/min) for 15 min increased net secretion fourfold in both segments. The potential involvement of VIP in watery diarrheal syndromes has been suspected. Schwartz et al. (1974) investigated the possible role of VIP in the pathogenesis of "pancreatic cholera" syndrome since VIP has been identified in the plasma and tumors of patients with this disease. They found that VIP, 2 μg/ml, significantly increased cAMP levels in rabbit ileal mucosa, increased short circuit current and caused net secretion of Cl and Na. These effects are similar to those observed with cholera toxin (Krejs et al., 1978). Mailman (1978) injected VIP intravenously into the dog and observed mucosal blood flow in order to determine the contribution of vascular changes to intestinal absorption. He found that net Na and water absorption was reversed to secretion by VIP due to a significant decrease in unidirectional absorptive fluxes and smaller decreases in secretory fluxes. Arterial pressure and absorptive site blood flow were reduced in proportion to the changes in Na and water fluxes. Prior treatment with atropine inhibited most VIP effects while guanethidine pretreatment did not significantly alter the response to VIP. He concluded that VIP reduced gut absorption through a general cardiovascular effect in addition to cholinergic stimulation that released acetylcholine by the gut. However, VIP and PGE_1-stimulated fluid secretion in rat jejunum was inhibited by morphine and naloxone antagonized this inhibitory action of morphine (Lee and Coupar, 1980). A common

pathway or a final mediator of fluid secretion induced by these two secretagogues was suggested to be cAMP. Evidence supporting this view is that PGE_1-induced cAMP was blocked by morphine (Collier and Roy, 1974). Thus, the action of VIP is similar to the effects of cholera toxin and *E. coli* LT.

Research Objectives

The primary objective of the present study is to determine the effects of *E. coli* heat-stable enterotoxin on chloride transport in the jejunum of swine, a species which is naturally susceptible to enterotoxigenic colibacillosis. The study will be divided into two parts: everted gut sac studies and isolated enterocyte studies. In everted gut sac studies, the effect of ST on ^{36}Cl transport will be determined, and the ability of drugs such as alpha-adrenergics, muscarinics and antimuscarinics, morphine and VIP to modify the effects of ST will be assessed. In isolated enterocyte studies, basal and ST-exposed ^{36}Cl and ^{45}Ca efflux rates will be studied in villus and crypt cells. Clonidine, atropine and morphine will be used to modify ST effects on ^{36}Cl efflux rate constants. In addition, studies of metabolic activity of isolated villus cells will be performed using ^{14}C-glucose and ^{14}C-glutamic acid as substrates. The metabolic studies will indicate whether the isolated enterocytes are viable and suitable for use in efflux studies and whether ST modifies metabolic activity as part of its secretagogue effect. Information derived from these studies should add to our understanding of enterotoxin action and assist in the development of more rational antidiarrheal therapy.

MATERIALS AND METHODS

Enterotoxin

Heat-stable enterotoxin was produced and assayed by scientists at the National Animal Disease Center, Ames, Iowa, by previously described methods (Moon et al., 1978). Briefly, 1 ml of still culture of *E. coli* from agar slants was inoculated into 400 ml Trypticase soy broth in a 2 liter Erlenmeyer flask plugged with cotton and incubated overnight on a shaker. One drop of antifoam agent was added to prevent foaming. After the overnight incubation, the broth culture was centrifuged at 10,000 x g and filtered through a series of millipore filters under positive pressure. The millipore filters used were 1 micron, 0.45 micron, and 0.20 micron, respectively. The ST producing strain was 1261 (class 1, serotype 0138:k81) and the nonenterotoxigenic strain was 123 (serotype 043:k-:H28). The filtered broths were cultured to insure sterility and an aliquot was removed from the ST broth for the infant mouse bioassay. The filtrates were stored at 4°C until used.

ST enterotoxin activity was assayed by injection of infant mice. The mice were removed from the dams on the second day of life and were used within 3 hours. Diluted toxin, 0.1 ml, with Evan's Blue as a marker for proper inoculation was injected into the stomach. The mice were then maintained for 4 hours at 30°C and euthanized with chloroform. The intestinal tract from the pyloric sphincter to the rectal sphincter was removed and weighed. Gut weight (GW) and the remaining body weight (BW) were measured. The mean GW/BW ratio of three runs of four mice each was used to calculate a linear regression. The natural log of the reciprocal

of the toxin dilution was plotted against the GW/BW ratio. The dilution that gave a GW/BW ratio of 0.09 was calculated and defined as 1 mouse unit. The undiluted broth of Strain 1261 contained 90-130 mouse units/ml.

For isolated enterocytes, cell-free supernatant fluids from the broth culture were diluted 1:8 with incubation buffer solution. This dilution produced a maximal secretory response when used in _in vivo_ perfusion technique (Ahrens and Zhu, 1982a). For everted gut sacs, a dilution of 1:5 with balanced salt solution was used. Preliminary studies with everted sacs showed that this dilution produced a consistent secretory response.

Everted Gut Sac Studies

Six to eight unweaned male piglets from a closed herd, 17 to 23 days old, were used for each drug studied. The pig was anesthetized with halothane and a laparotomy was performed on the left side, posterior to the last rib. The pylorus was isolated and a Silastic® Foley catheter size 14 Fr (Dow Corning Corp.) was inserted into the proximal duodenum until the tip extended into the proximal jejunum. The jejunum was ligated 40 cm distal to this point and 20 ml of a 1:2 dilution of ST with saline (37°C) was injected at the proximal end which was then ligated. The wound was then closed and the pig was maintained on a heating pad. After a 45-minute-incubation period, the loop was removed and kept in oxygenated balanced salt solution at 37°C. The solution contained sodium, 148 mM; chloride, 120 mM; potassium, 6.5 mM; bicarbonate, 26 mM; calcium, 1 mM; phosphate, 1.4 mM; osmolality, 300 mOsm/kg. The serosa and muscularis externa were stripped off by the method described by Parsons and Paterson

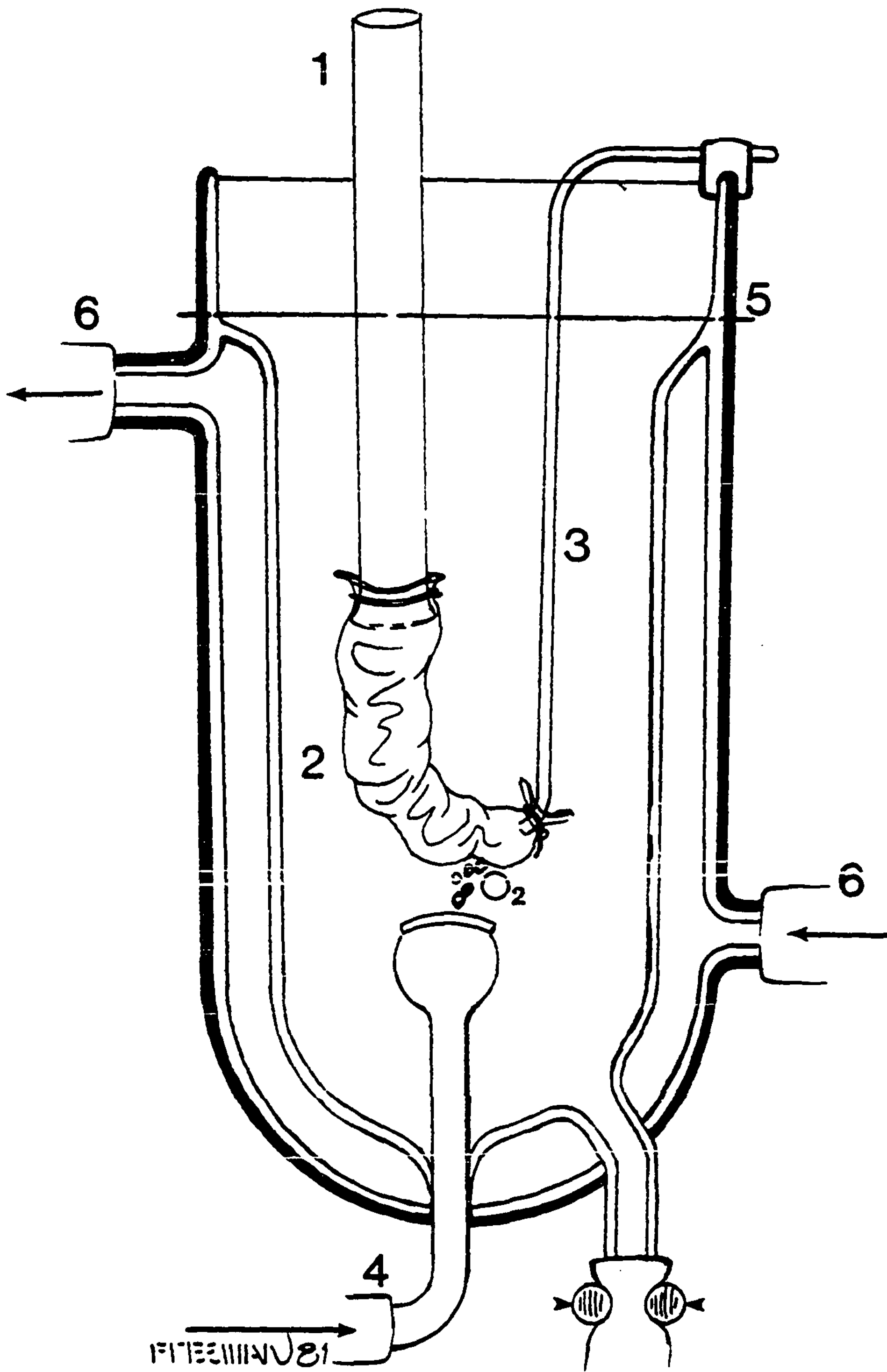
(1960) with slight modification. A glass tube, 7.5 mm in diameter, was inserted into the lumen of the intestine and the intestine was stripped by making a longitudinal incision with a blunt scalpel. The divided muscle layer retracted and was peeled off leaving the mucosa supported by a thin layer of muscularis mucosae. The intestine was then everted by means of a glass rod as described by Wilson and Wiseman (1954).

The procedure for mounting everted sacs in tissue baths and sampling of fluids was a modification of methods described by Wilson and Wiseman (1954) and Crane and Wilson (1958). Pieces of everted intestine about 7 cm long were cut. The lip end of the hollow glass tube, 5 mm in diameter and 10 cm long, was inserted into one end of the everted intestine and tied with a surgical suture. The other end of the everted intestine was ligated as a blind sac and tied tightly to a metal rod which then was placed vertically in 50 ml tissue bath (Metro Scientific, Farmingdale, NY). The bath contained 40 ml of a balanced salt solution and 10 ml 1261 (ST) or 123 (control) broth oxygenated at 37°C. The blind sac was fixed at the lower end of the bath and the upper end of the intestinal sac was tied to the hollow glass tube. This permitted serial sampling of the fluid on the serosal side (inside) of the intestine (Figure 8).

Four everted sacs were prepared from one animal for each experiment. The following series of experiments were done.

Figure 8. Schematic drawing of everted gut sac preparation

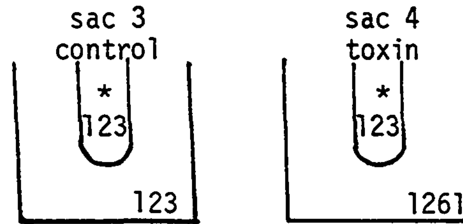
Hollow glass tube (1), connected to everted small intestine (2) which is held tightly to the metal rod (3), allowed serial sampling of the fluid on the serosal side of the intestinal blind sac. The balanced salt solution was placed in a 50 ml tissue bath (5) oxygenated with O_2 (4) and kept at 37°C by a circulating pump (6).


1
6
5
3
2
6
O_2
4

1. Control vs. ST, 8 experiments

Insorption
^{36}Cl transport outside to inside

Exsorption
^{36}Cl inside to outside



Asterisk indicates where ^{36}Cl was initially added.

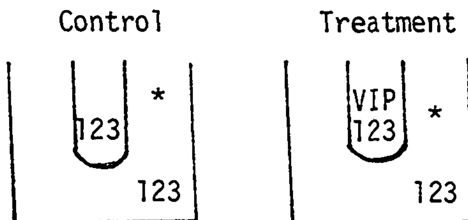
The volumes of solution added at the start of each experiment were 3.0 ml inside and 50 ml outside the sacs. Radioactive chloride was added at time zero, 5 μCi outside for insorption experiments, and 1.5 μCi inside for exsorption experiments. Mixing was accomplished by oxygenation of the solution. Samples of 0.1 ml were taken from both inside and outside the sac at 5, 10, 15, 20, 25, and 30 min and added to 10 ml of scintillation cocktail (Aquasol-2, New England Nuclear, Boston, MA). The counting was performed in a Packard model 2425 scintillation counter. All counts were corrected for background and counting efficiency.

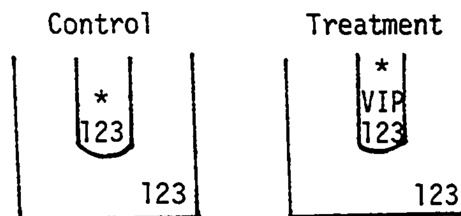
The sac was emptied at the end of the experimental period. A tissue sample about 2 x 5 mm was fixed in neutral buffered formalin for histological examination. The sac then was gently blotted with Whatman No. 40 filter paper and the wet weight was determined.

2. Control vs. VIP (2 μg/ml), 6 experiments

Insorption

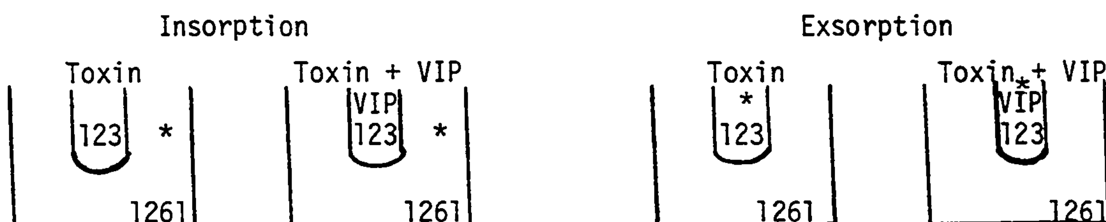
Exsorption



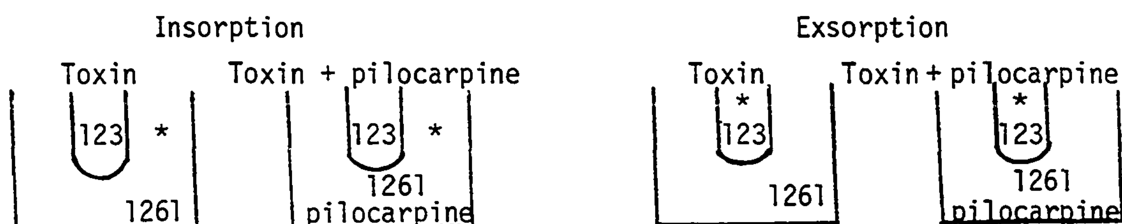


VIP was put in the sac because VIP has been shown to exert its effect on the serosal side.

3. Toxin vs. toxin plus VIP (2 μg/ml), 6 experiments



4. Toxin vs. toxin plus pilocarpine (10^{-5} M), 5 experiments



Experiments 5-8 followed the same protocol as Experiment 4.

5. Toxin vs. toxin plus atropine (2×10^{-5} M), 8 experiments

6. Toxin vs. toxin plus clonidine (10^{-6} M), 6 experiments

7. Toxin vs. toxin plus phenylephrine (10^{-5} M), 6 experiments

8. Toxin vs. toxin plus morphine (1.2 μg/ml), 5 experiments

Calculation and analyses

Exsorption The ratio of activity of ^{36}Cl detected outside to inside per gram of tissue at 5, 10, 15, 20, 25, and 30 minutes was determined. The average values from 5-8 experiments for each drug were calculated. Linear curves were constructed against time and slopes determined. Student paired t-test was used to assess the effects of toxin vs. control, VIP vs. control and drug (VIP, pilocarpine, atropine, clonidine, phenylephrine, or morphine) vs. toxin.

Insorption The ratio of activity of ^{36}Cl detected inside to outside per gram tissue at 5, 10, 15, 20, 25, and 30 minutes was determined. Calculation and analytical methods were the same as described above for exsorption rates.

Isolated Enterocytes Studies

Two types of studies were performed using isolated enterocytes: determination of ion efflux rates and determination of metabolic activity.

Buffer solutions for enterocyte studies

Isolation medium contained 154 mM NaCl, 2 mM Tris-HCl, 3 mM K_2HPO_4, 10 mM sucrose, 0.1% albumin, osmolality 310 mOsm/kg, pH 7.4, 37°C.

Incubation medium I contained 120 mM NaCl, 20 mM Tris-HCl, 3 mM K_2HPO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% albumin, osmolality 285 mOsm/kg, pH 7.4, 37°C.

Incubation medium II contained 60 mM NaCl, 120 mM mannitol, 20 mM Tris-HCl, 3 mM K_2HPO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% albumin, osmolality 290 mOsm/kg, pH 7.4, 37°C.

$MgCl_2$-Tris wash contained 110 mM $MgCl_2$, 2 mM Tris-HCl, osmolality 285 mOsm/kg, pH 7.4, 4°C.

Isolation of the enterocytes

The vibration method was modified from that described by Harrison and Webster (1964) and Harrison and Webster (1969). The jejunum of the piglet, 60 cm long, was taken out, as in the method described above, rinsed with isolation medium, 37°C, and everted onto a spiral steel rod over which wet cellulose dialysis tubing (Spectrapor® membrane tubing, dia

20.4 mm, Spectrum Medical Industries, Inc., Los Angeles, CA) had been placed. The upper and lower ends of the gut length were firmly tied with cable ties (Dannison Manufacturing Co., Framingham, MA). The diameter of the steel rod was 8 mm, coiled to have a spiral of 4.2 cm in width and 15 cm in height with the upper end of the spiral attached to the vibrator. There were holes along the coil for air inflation. The vibration apparatus is shown in Figure 9.

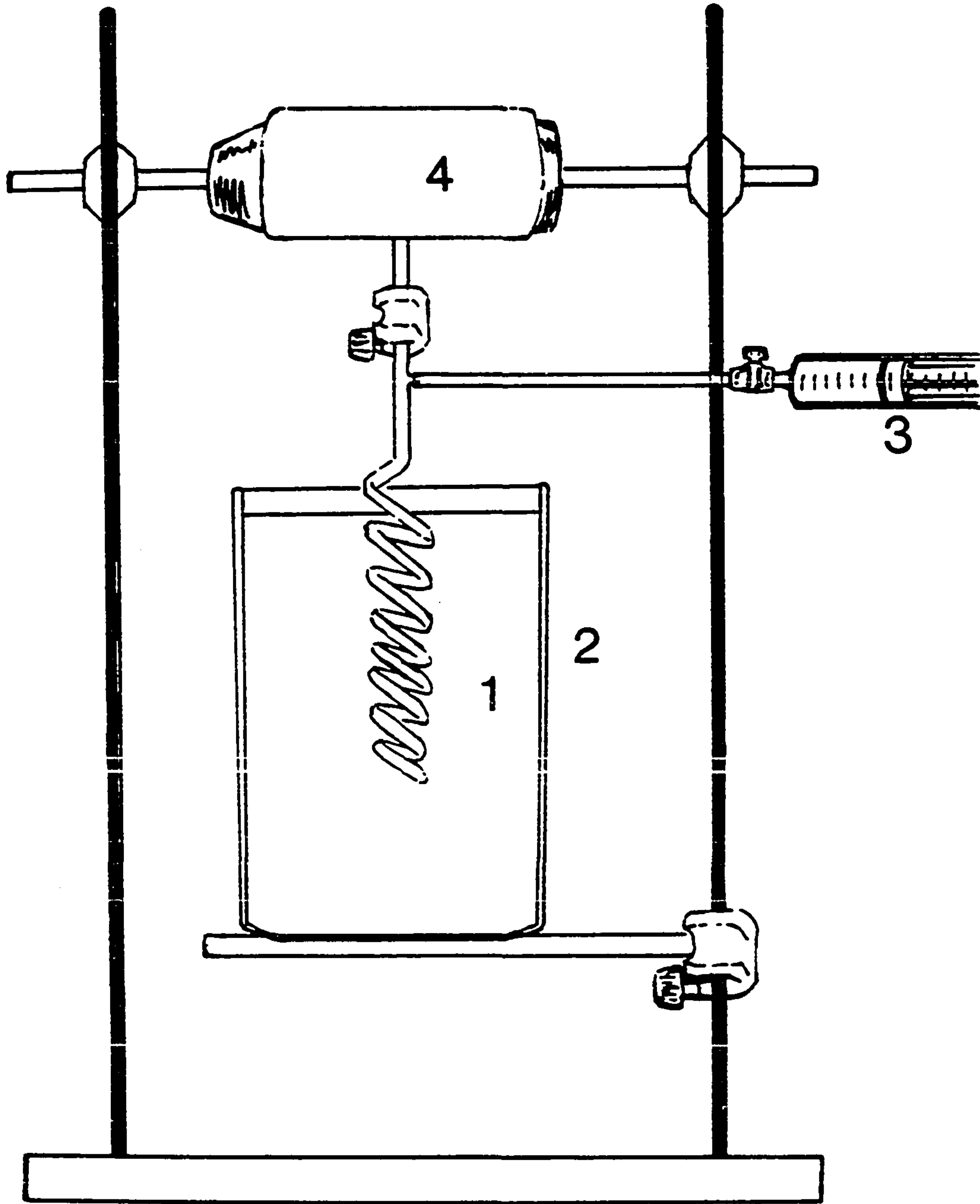
After the gut was everted on the spiral steel rod, it was incubated in oxygenated, 37°C isolation medium for 30 minutes. Then the rod was attached to the vibrator (Chemapec Inc., Woodbury, NY) and vibrated for a few minutes to get rid of the loose cells and mucus. For the determination of ion efflux rates, three fractions of enterocytes were collected. The first fraction consisted of mature enterocytes from the villus. The second fraction was a mixture of mature and immature cells. The third fraction was immature villus cells and crypt cells. The time for vibration varied and depended on the cloudiness of the isolation medium as an index of cell yield. Usually, about 30-45 minutes were required for each fraction. To collect the last fraction, which were mainly crypt cells, EDTA (1 M) was added to the medium and the intestine was inflated to a fully-distended state by injecting air into the steel rod.

After vibration, each fraction of cells was treated as follows:

a) The cells were centrifuged at 2,000 x g for 10 min, the supernatant was decanted, and the cells were washed with 15 ml of isolation medium. At this stage, a 2 ml sample of cell suspension was obtained for sucrase and protein assays.

Figure 9. Schematic drawing of vibration apparatus

The intestine was everted over a spiral rod (1) which was connected to a vibrator (4). Cells were collected in a plastic beaker (2) containing 800 ml isolation medium. Crypt cells were collected by inflation of the intestine via a syringe (3) attached to the spiral rod.


4
3
2
1

b) The cells were centrifuged at 1,000 x g for 3 min, decanted, and cells were washed with 7.5 ml isolation medium.

c) The washed cells were centrifuged at 1,000 x g for 3 min and decanted. The cells were then ready for incubation with toxin and/or drug. All centrifugations were performed at 4°C.

The cells from each fraction were subdivided into four groups, each consisting of 1 ml of cells: control, control plus drug, toxin, and toxin plus drug. Five ml of incubation medium containing either ^{36}Cl or ^{45}Ca were added to each of the four tubes, mixed and placed in a Dubnoff shaking bath at 37°C for 20 minutes.

Determination of ^{36}Cl and ^{45}Ca effluxes

The following procedure was described by Gall et al. (1974). After a 20 minute incubation with isotope, the cell suspension was centrifuged and the cells washed twice with $MgCl_2$-Tris buffer solution at 4°C. The cells were returned to fresh tracer-free medium at a dilution of 1:30 by volume and ^{36}Cl or ^{45}Ca efflux was determined by measuring the rate of appearance of radioactive material in the medium. Samples of cell suspension (0.5 ml) were obtained at the beginning and completion of the efflux period. Samples of supernatant (0.5 ml) were obtained at 0, 2, 4, 6, 8, 10, and 12 min by centrifugation of 1 ml aliquots of total suspension for 1 min. Ten ml of scintillation cocktail was used and counting was performed in a Packard model 2425 scintillation counter. At the end of the experiment, a cell suspension was collected from each fraction and viability was measured by Trypan Blue exclusion.

Calculation and analyses The outflux rate constant (that portion of the intracellular isotope extruded per unit of time) was calculated by Hoffman's method (Hoffman, 1962). The basic presumption was that the outflux occurred as a first order process. The values of (1-supernatant/suspension) x 100, the percent of radioactive material remaining within the cell at a given time, were plotted against time on a semilogarithmic scale. The rate constant was determined from the straight line obtained and was reported as the amount of chloride or calcium exchanged per hour. The values of the efflux rate constant then were compared between treatments and analyzed using analysis of variance.

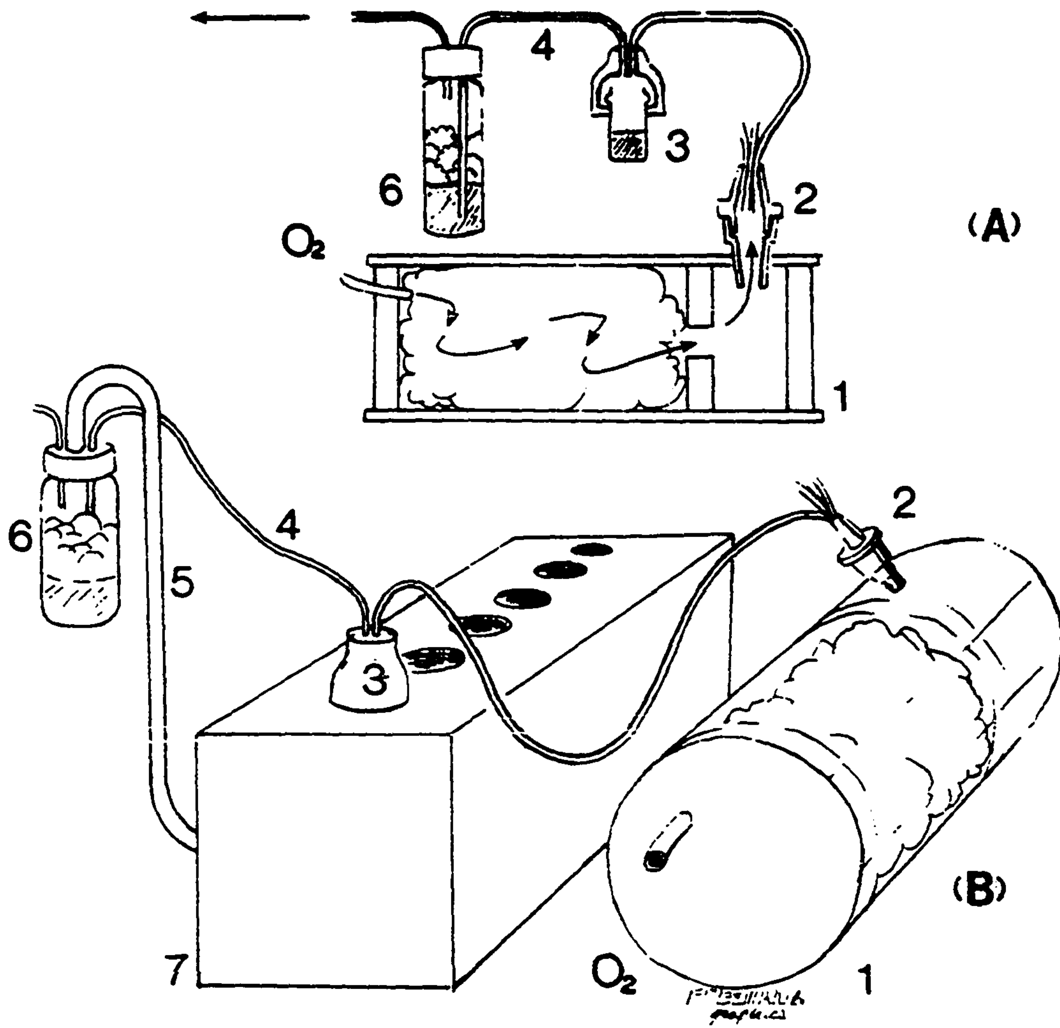
Determination of ^{14}C-glucose and ^{14}C-glutamic acid metabolic activity by isolated enterocytes

Only the first fraction of the cells from the upper villus were used to determine the metabolic activity. After 20 min incubation, 0.5 ml of dispersed cells were put into 2 ml incubation vials. ^{14}C-glucose or ^{14}C-glutamic acid, 2 μCi, was incubated with the cells and the incubation vials were attached to the apparatus used for the continuous trapping of $^{14}CO_2$ as described by Brendel and Meezan (1974). The apparatus is illustrated in Figure 10. Reaction vials were 2-ml bottles stoppered with rubber serum stoppers. PE 20 polyethylene tubing carried oxygen to each reaction vial and PE 50 polyethylene tubing carried $^{14}CO_2$ from the reaction vials to the trapping vials which consisted of scintillation vials containing 1.5 ml of 0.3 N sodium hydroxide with 40 drops of 5% Triton X-100 as a trapping solution.

Figure 10. Schematic diagram of CO_2 collection apparatus

A. Gas flow through a single reaction vial and collection vial.

B. Relationship of apparatus components.
(1) O_2 moistening chamber; (2) gas distribution manifold; (3) incubation vial; (4) polyethylene line; (5) stainless steel arm; (6) CO_2 trapping vial; (7) aluminum incubation block (redrawn from Brendel and Meezan, 1974).


4
3
6
2
(A)
O2
1
6
4
2
5
3
(B)
7
O2
1

The $^{14}CO_2$ collection apparatus permitted the continuous monitoring of $^{14}CO_2$ production from ten separate reaction mixtures simultaneously as shown in Figure 11. After the reaction vials and the trapping vials were connected, the whole apparatus was placed in a Dubnoff shaking bath at 37°C. At an interval of 30 min the shaker was stopped, and each scintillation vial containing trapping solution unscrewed and replaced with a fresh vial in turn. The rates of $^{14}CO_2$ production could be followed over a period of 3 hours and $^{14}CO_2$ collection was interrupted for only an insignificant 10-15 sec period every 30 min. Ten ml of scintillation cocktail were simply added in the collected $^{14}CO_2$ and counting was performed in a Packard model 2425 scintillation counter.

Calculation and analyses The metabolic activity was calculated as cumulative specific activity per mg protein. The maximal activity produced by the control at time 180 min was set as 100%. The activity of the other three groups (control plus ouabain, toxin and toxin plus ouabain) was then calculated according to and compared with this 100% maximal value. The means were averaged from four experiments for glucose and six for glutamic acid. The analysis of variance was used to determine the significant difference between each treatment.

Assays

Sucrase determination Sucrase determination was performed by the method of Dahlqvist (1968). The method is based upon the hydrolysis of sucrose to glucose and fructose by sucrase. Glucose oxidase-O-dianisidine was used to develop color with free glucose. The color developed was read on a Beckman Spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) at 420 mu against the reagent blank and compared to a standard curve of glucose.

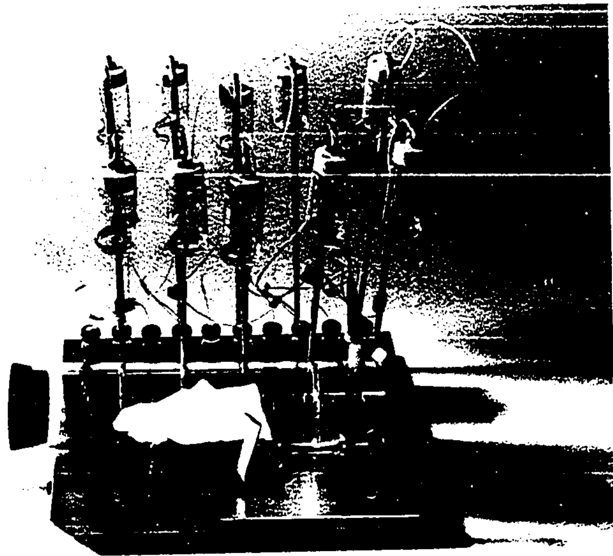


Figure 11. Apparatus for CO_2 collection from ten small incubation vials

Disaccharidase activity was calculated as units per gram protein. One disaccharidase unit is defined as the activity hydrolyzing 1 μmole of disaccharide per minute.

Protein determination Concentration of protein was performed by the method of Lowry et al. (1951). Briefly, two steps lead to the final color with protein: a) reaction with copper in alkali and b) reduction of the phosphomolybdic-phosphotungstic reagent (Folin phenol reagent) by the copper-treated protein. The color was measured spectrophotometrically at 660 mu against the reagent blank. Bovine albumin standard solution was used to construct a standard curve.

Viability assay Cell viability was determined by a method modified from that described by Phillips (1973). A 0.1% aqueous solution of Trypan Blue was used. The dilution of dye solution and cell suspension ranged from 1:20 to 1:5 depended on the amount of cells harvested. A drop of cell suspension was placed on a hemocytometer and a viability count was made immediately under a light microscope. The number of stained cells and nonstained cells in a given area on the hemocytometer was counted. The percentage of viable cells was calculated as the ratio of nonstained cells/(stained + nonstained cells) x 100.

Drug sources

1. Atropine sulfate (Sigma®, St. Louis, MO).
2. Clonidine HCl (Boehringer Ingelheim, Elmsford, NY).
3. Morphine sulfate (Lilly, Indianapolis, IN).
4. Ouabain (Sigma®, St. Louis, MO).
5. Phenylephrine (Sigma®, St. Louis, MO).

6. Pilocarpine HCl (Sigma®, St. Louis, MO).
7. Vasoactive Intestinal Peptide (VIP) (Sigma®, St. Louis, MO).

Radioisotope sources

All radioisotopes used were purchased from New England Nuclear (Boston, MA).

1. D-[14C(U)]Glucose, 1-5 mCi/mmol.
2. L-[14C(U)]Glutamic acid, 250 mCi/mmol.
3. Calcium-45, calcium chloride in water, 4-30 Ci/g calcium.
4. Chloride-36, 0.2-3.0 M HCl solution, 1-5 mCi/g chlorine.

RESULTS

Everted Gut Sac Studies

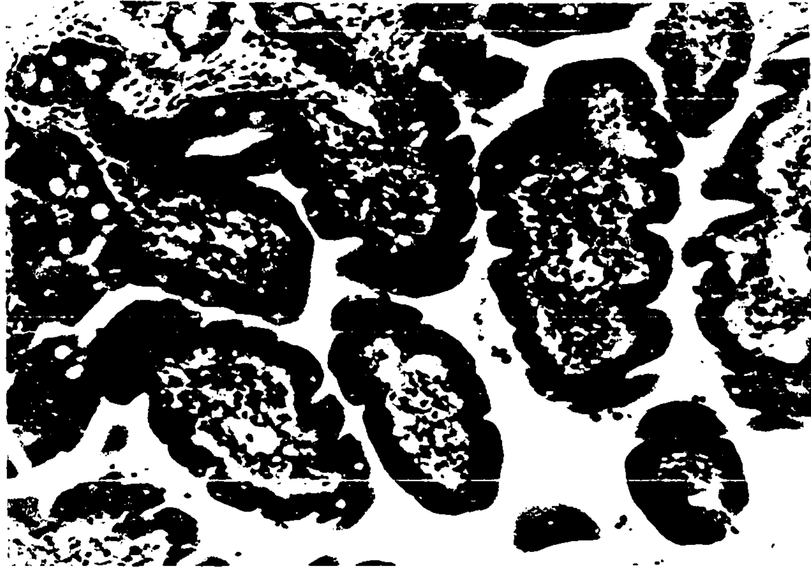
Microscopic examination

Histological examination of intestinal mucosa taken immediately prior to the suspension of the everted sacs in the chamber revealed no significant changes in the morphology of the cells (Figures 12 and 13). This indicated that the *in vivo* incubation for 45 min with the toxin and the mechanical manipulation did not damage the cells. Light microscopic examination at low power showed a normal pattern of villi, lamina propria and crypts. The external and internal muscle layers were stripped off so only the muscularis mucosae and submucosa were left intact. The striated border formed by microvilli was clearly visible in the villus tips (Figures 12 and 13). The columnar epithelial cells showed a normal pattern with the frequent occurrence of goblet cells. The lamina propria contained mononuclear cells, connective tissues and capillaries.

After the experimental period of at least 45-60 min, most of the villi appeared intact with a striated border of microvilli (Figure 14), although some preparations showed denudation of the epithelial cells and infiltration of mononuclear cells into the connective tissues of the lamina propria. Edematous spaces were occasionally found in the lamina propria of the villus and crypt regions (Figure 15). However, detailed examination of the intact villus and crypt cells revealed no significant changes in the architecture of the cells.

Figure 12. Jejunum of 3-week-old pig incubated *in vivo* with filtrate of nonenterotoxigenic strain of *E. coli* for 45 minutes (Eosin, 250X)

Figure 13. Jejunum of 3-week-old pig incubated *in vivo* with filtrate of ST producing strain of *E. coli* for 45 minutes (Eosin, 250X)



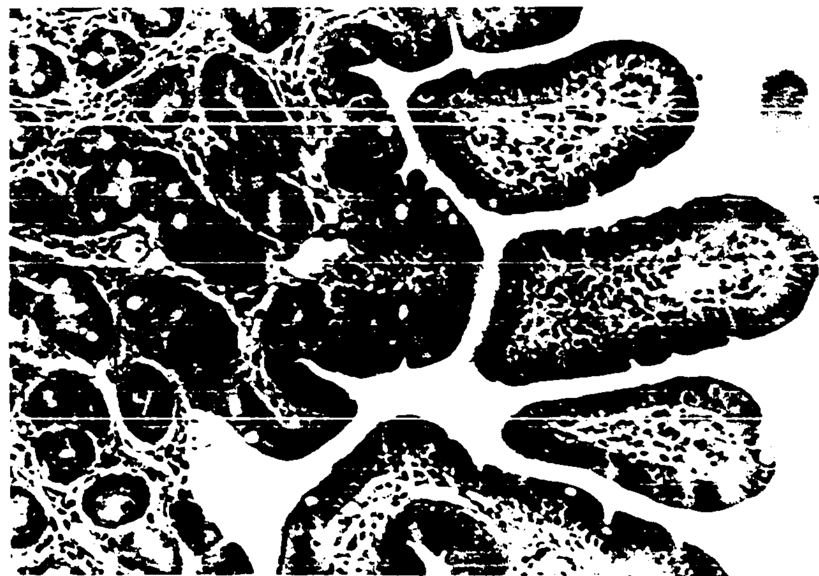
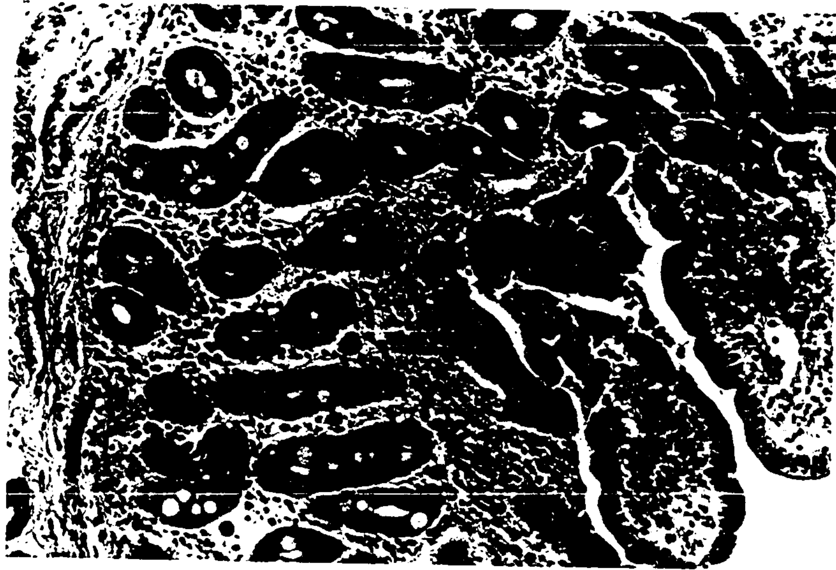


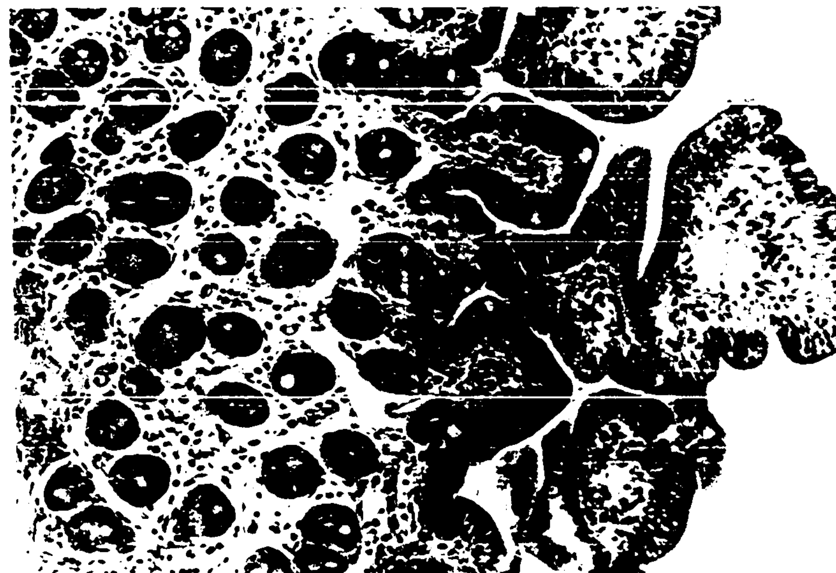
Figure 14. Specimen of everted gut sac after 60 minute-incubation at 37°C (Eosin, 250X)

Epithelial lining appeared intact with a striated border of microvilli.

Figure 15. Specimen of everted gut sac after 60 minute-incubation at 37°C (Eosin, 250X)

Edematous spaces were found in lamina propria of the villus and crypt regions.





Fluid volume

There was no significant change in volume of solution inside the sac during the 30-min experimental period. The initial volume put in the sac was 3.0 ml, the total volume taken out was 0.6 ml (0.1 ml at 5, 10, 15, 20, 25, and 30 min) and the volume left ranged from 2.3-2.5 ml. This revealed no gross change of water transport to the sac interior during the experimental period. The wet weight of the sacs from experiment to experiment ranged from 0.70 g to 1.15 g. However, the variation of sac weight within an experiment was less than 0.20 g.

Exsorption of ^{36}Cl

Chloride exsorption was determined as the ratio of ^{36}Cl activity outside the sac to inside per gram of tissue. Toxin increased chloride exsorption significantly ($p < 0.005$) compared to control (Table 1 and Figure 16). Slopes of activity ratio per unit time were compared. The exsorption slopes of control and toxin were 5.2×10^{-4} and 6.9×10^{-4}, respectively (Table 2).

Vasoactive intestinal peptide (VIP) significantly increased chloride exsorption from everted gut sacs (Table 3 and Figure 17). The exsorption slope of VIP treated sac was 7.1×10^{-4} compared to 5.6×10^{-4} of control (Table 2).

Addition of VIP to ST-treated everted sacs did not change the exsorption rate of chloride compared to toxin alone (Table 4 and Figure 18). The slopes were 6.8×10^{-4} for toxin alone and 6.7×10^{-4} for toxin plus VIP (Table 2). Thus, there was no additive or antagonizing effect of VIP on enterotoxin-stimulated efflux.

Table 1. Effects of 1261 ST toxin on the insorption and exsorption of ^{36}Cl from porcine everted jejunal sacs

Time (min)	Insorption[a] x 10^2		Exsorption[b] x 10^3	
	Control	Toxin	Control	Toxin
5	1.97±0.44	1.89±0.35	1.55±0.29	2.71±0.31
10	4.19±0.73	4.98±1.01	3.83±0.58	6.35±0.69
15	7.25±1.35	9.48±1.26	6.30±0.88	9.61±0.82
20	11.66±2.04	13.10±1.48	8.79±1.24	13.03±1.29
25	15.10±1.96	20.84±2.46	11.75±1.85	16.55±1.52
30	19.07±2.52	28.40±3.04	14.35±1.86	20.08±1.65

[a]Ratio of ^{36}Cl activity inside to outside per gram tissue. Mean (±SEM) from 8 animals.

[b]Ratio of ^{36}Cl activity outside to inside per gram tissue. Mean (±SEM) from 8 animals.

Pilocarpine, a muscarinic drug, added to ST-treated everted sacs did not augment chloride exsorption compared to toxin alone (Table 5 and Figure 19). The exsorption slope of toxin was 7.9×10^{-4} and of toxin plus pilocarpine was 7.5×10^{-4} (Table 2).

Atropine, an anticholinergic drug, added to toxin-treated everted sacs did not have any significant effect on chloride exsorption (Table 6 and Figure 20) compared to toxin alone. The slopes of chloride exsorption from toxin-treated sacs were 6.9×10^{-4} and 7.3×10^{-4}, respectively (Table 2).

Addition of clonidine, an alpha-2 adrenergic agonist, to toxin-treated sacs tended to reduce chloride exsorption rate though the reduction was not significantly different when compared to toxin alone (Table 7

Figure 16. Effects of 1261 ST toxin on the exsorption and insorption of ^{36}Cl from porcine everted jejunal sacs

^{36}Cl cpm $\frac{out}{in} \times 10^{3}$ g^{-1} = exsorption

^{36}Cl cpm $\frac{in}{out} \times 10^{2}$ g^{-1} = insorption

Each point represents the mean (±SEM) from 8 animals.

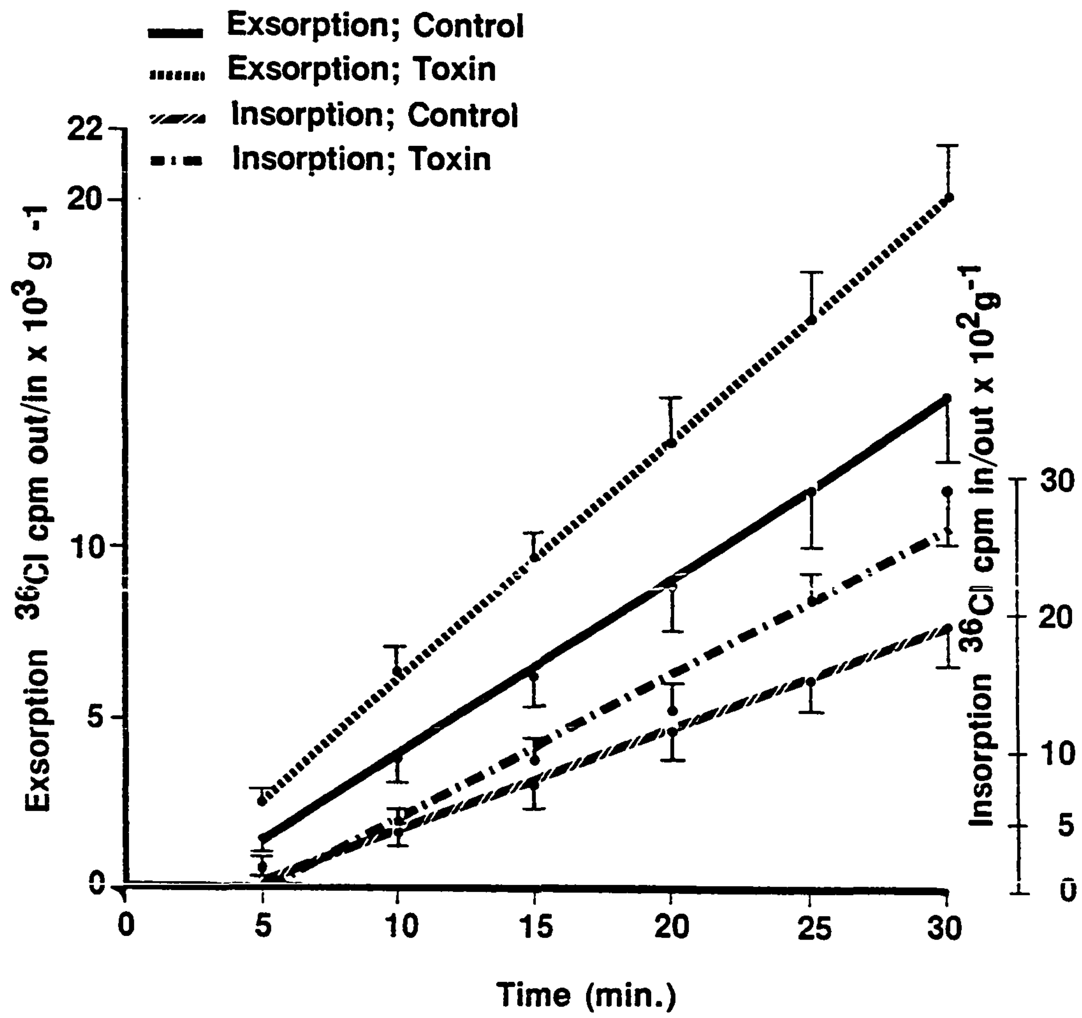

Exsorption; Control
Exsorption; Toxin
Insorption; Control
Insorption; Toxin
Exsorption 36Cl cpm out/in x 10³ g -1
Insorption 36Cl cpm in/out x 10²g-1
Time (min.)
0
5
10
15
20
22
25
30

Table 2. Comparison of the rate of ^{36}Cl insorption and exsorption in everted sacs exposed to ST and various drugs

		Insorption[a]		Exsorption[b]	
Treatment	n	Slope ($x10^3$)	p	Slope ($x10^4$)	p
Control	8	7.0	<0.01	5.2	<0.005
ST	8	10.5		6.9	
Control	6	9.8	N.S.	5.6	<0.005
VIP 2 μg/ml	6	9.8		7.1	
ST	6	12.6	N.S.	6.8	N.S.
ST + VIP 2 μg/ml	6	12.6		6.7	
ST	5	12.5	N.S.	7.9	N.S.
ST + pilocarpine 10^{-5} M	5	12.3		7.5	
ST	8	8.8	N.S.	6.9	N.S.
ST + atropine $2x10^{-5}$ M	8	10.6		7.3	
ST	6	10.4	N.S.	6.1	N.S.
ST + clonidine 10^{-6} M	6	10.9		5.8	
ST	6	12.7	<0.01	7.2	<0.005
ST + phenylephrine 10^{-5} M	6	10.2		8.1	
ST	5	13.2	N.S.	7.3	N.S.
ST + morphine 1.2 μg/ml	5	12.0		6.8	

[a]Ratio of ^{36}Cl activity inside to outside per gram tissue.

[b]Ratio of ^{36}Cl activity outside to inside per gram tissue.

Table 3. Effects of VIP on the insorption and exsorption of ^{36}Cl from porcine everted jejunal sacs

Time (min)	Insorption[a] x 10^2		Exsorption[b] x 10^3	
	Control	VIP[c]	Control	VIP
5	1.61±0.28	2.11±0.27	1.80±0.24	2.24±0.40
10	6.44±1.26	6.99±1.16	4.30±0.53	5.56±0.83
15	11.28±1.81	11.23±1.56	7.03±0.92	9.28±1.20
20	16.43±2.45	16.73±1.89	9.92±1.37	12.74±1.61
25	21.17±2.86	22.38±2.89	12.72±1.79	16.44±2.08
30	25.88±3.70	26.15±3.13	15.88±2.17	19.94±2.43

[a]Ratio of ^{36}Cl activity inside to outside per gram tissue. Mean (±SEM) from 6 animals.

[b]Ratio of ^{36}Cl activity outside to inside per gram tissue. Mean (±SEM) from 6 animals.

[c]Concentration of VIP in serosal solution was 2 µg/ml.

Table 4. Effects of VIP on the insorption and exsorption of ^{36}Cl from ST-exposed porcine everted jejunal sacs

Time (min)	Insorption[a] x 10^2		Exsorption[b] x 10^3	
	Toxin	Toxin+VIP[c]	Toxin	Toxin+VIP[c]
5	2.41±0.22	2.74±0.90	1.98±0.27	1.98±0.19
10	7.83±0.78	9.72±1.83	4.88±0.60	4.80±0.46
15	14.34±1.57	15.47±2.03	8.03±0.91	8.13±0.69
20	20.91±2.20	21.56±3.64	11.68±1.43	11.37±0.91
25	27.46±2.27	28.48±4.12	15.27±1.95	14.75±1.28
30	33.32±3.04	34.41±4.60	18.92±2.41	18.78±1.74

[a]Ratio of ^{36}Cl activity inside to outside per gram tissue. Mean (±SEM) from 6 animals).

[b]Ratio of ^{36}Cl activity outside to inside per gram tissue. Mean (±SEM) from 6 animals).

[c]Concentration of VIP in serosal solution was 2 µg/ml.

Figure 17. Effects of VIP on the exsorption and insorption of ^{36}Cl from porcine everted jejunal sacs

Concentration of VIP in serosal solution was 2 μg/ml.

^{36}Cl cpm $\frac{out}{in} \times 10^3\ g^{-1}$ = exsorption

^{36}Cl cpm $\frac{in}{out} \times 10^2\ g^{-1}$ = insorption

Each point represents the mean (±SEM) from 6 animals.

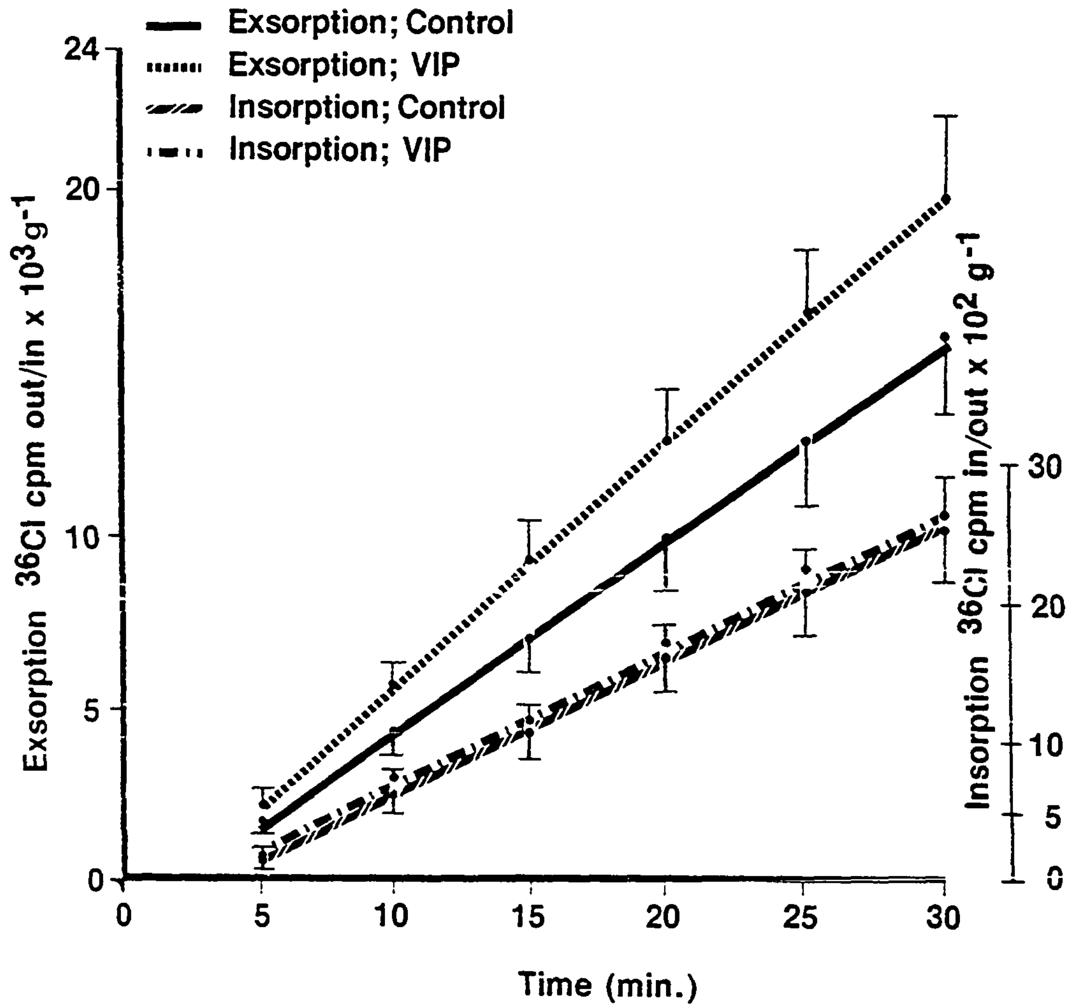

Exsorption; Control
Exsorption; VIP
Insorption; Control
Insorption; VIP
Exsorption 36Cl cpm out/in x 10^3 g^-1
Insorption 36Cl cpm in/out x 10^2 g^-1
Time (min.)
24
20
10
5
0
0
5
10
15
20
25
30
30
20
10
5
0

Figure 18. Effects of VIP on the exsorption and insorption of ^{36}Cl from ST-exposed porcine everted jejunal sacs

Concentration of VIP in serosal solution was 2 μg/ml.

^{36}Cl cpm $\frac{out}{in} \times 10^3 \ g^{-1}$ = exsorption

^{36}Cl cpm $\frac{in}{out} \times 10^2 \ g^{-1}$ = insorption

Each point represents the mean (±SEM) from 6 animals.

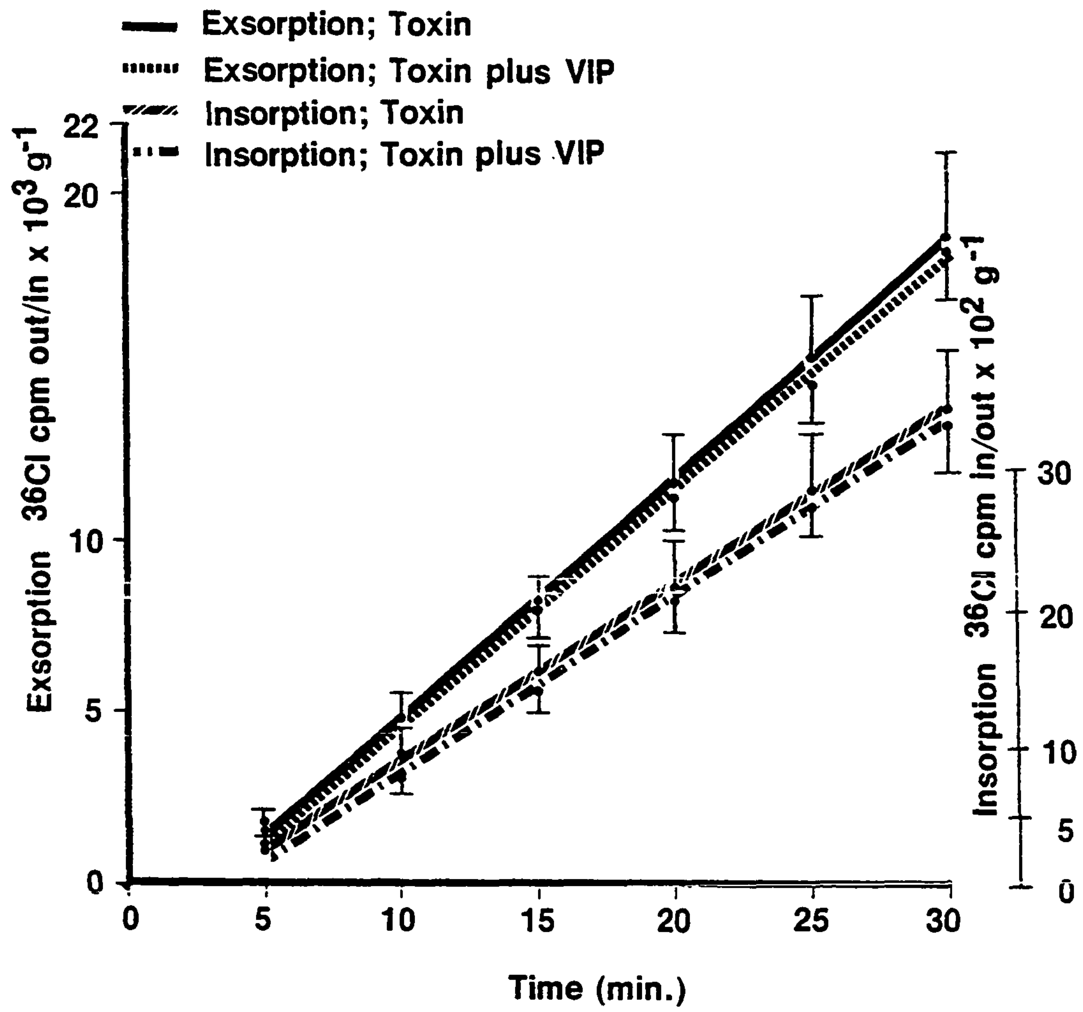

Exsorption; Toxin
Exsorption; Toxin plus VIP
Insorption; Toxin
Insorption; Toxin plus VIP
Exsorption 36Cl cpm out/in x 10³ g⁻¹
Insorption 36Cl cpm in/out x 10² g⁻¹
Time (min.)
0
5
10
15
20
25
30
22

Table 5. Effects of pilocarpine on the insorption and exsorption of ^{36}Cl from ST-exposed porcine everted jejunal sacs

Time (min)	Insorption[a] x 10^2		Exsorption[b] x 10^3	
	Toxin	Toxin+pilocarpine[c]	Toxin	Toxin+pilocarpine[c]
5	3.09±0.45	2.63±0.58	3.86±1.14	2.80±0.30
10	9.76±2.06	9.03±0.80	7.38±1.43	6.38±0.50
15	15.84±2.63	14.98±1.41	11.56±1.93	10.14±0.60
20	22.46±2.62	21.56±1.97	15.26±2.19	13.58±1.04
25	27.55±3.94	25.98±2.24	19.24±2.03	17.68±0.99
30	34.84±4.52	34.03±2.35	23.92±1.88	21.70±1.37

[a]Ratio of ^{36}Cl activity inside to outside per gram tissue. Mean (±SEM) from 5 animals.

[b]Ratio of ^{36}Cl activity outside to inside per gram tissue. Mean (±SEM) from 5 animals.

[c]Concentration of pilocarpine in mucosal solution was 10^{-5} M.

Table 6. Effects of atropine on the insorption and exsorption of ^{36}Cl from ST-exposed porcine everted jejunal sacs

Time (min)	Insorption[a] x 10^2		Exsorption[b] x 10^3	
	Toxin	Toxin+atropine[c]	Toxin	Toxin+atropine[c]
5	1.63±0.34	2.65±0.49	2.91±0.62	2.48±0.26
10	4.56±0.34	6.53±0.89	5.89±1.20	5.51±0.53
15	8.39±0.98	10.60±1.95	8.98±1.73	8.56±0.70
20	13.43±1.56	16.10±2.14	12.59±2.32	12.54±1.09
25	19.66±2.54	22.37±3.14	16.61±2.74	16.41±1.43
30	22.46±2.95	29.20±4.06	19.81±3.06	20.68±1.97

[a]Ratio of ^{36}Cl activity inside to outside per gram tissue. Mean (±SEM) from 8 animals.

[b]Ratio of ^{36}Cl activity outside to inside per gram tissue. Mean (±SEM) from 8 animals.

[c]Concentration of atropine in mucosal solution was 2 x 10^{-5} M.

Figure 19. Effects of pilocarpine on the exsorption and insorption of ^{36}Cl from ST-exposed everted jejunal sacs

Concentration of pilocarpine in mucosal solution was 10^{-5} M.

^{36}Cl cpm $\frac{out}{in} \times 10^{3}$ g^{-1} = exsorption

^{36}Cl cpm $\frac{in}{out} \times 10^{2}$ g^{-1} = insorption

Each point represents the mean (±SEM) from 5 animals.

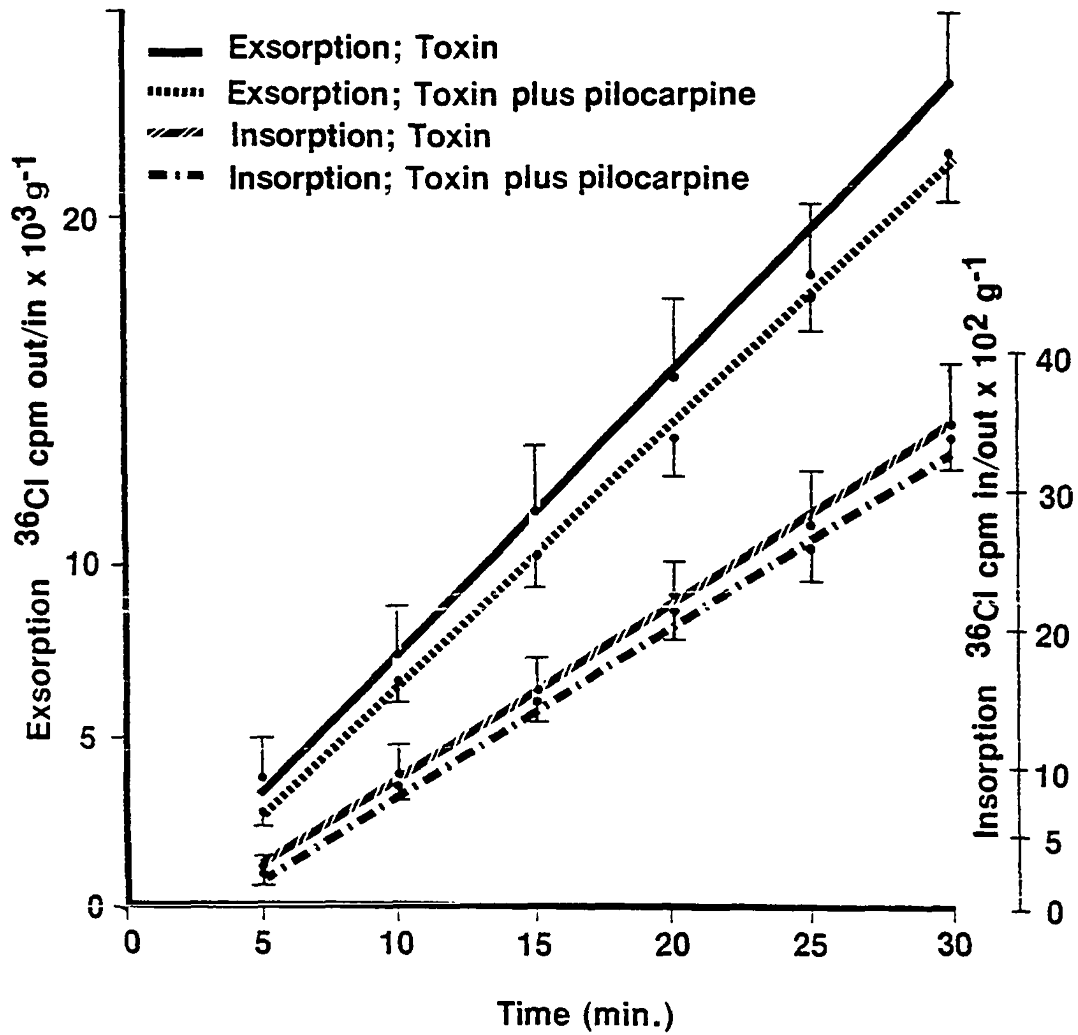

Exsorption; Toxin
Exsorption; Toxin plus pilocarpine
Insorption; Toxin
Insorption; Toxin plus pilocarpine
Exsorption 36Cl cpm out/in x 10³ g⁻¹
Insorption 36Cl cpm in/out x 10² g⁻¹
0
5
10
20
30
40
0
5
10
15
20
25
30
Time (min.)

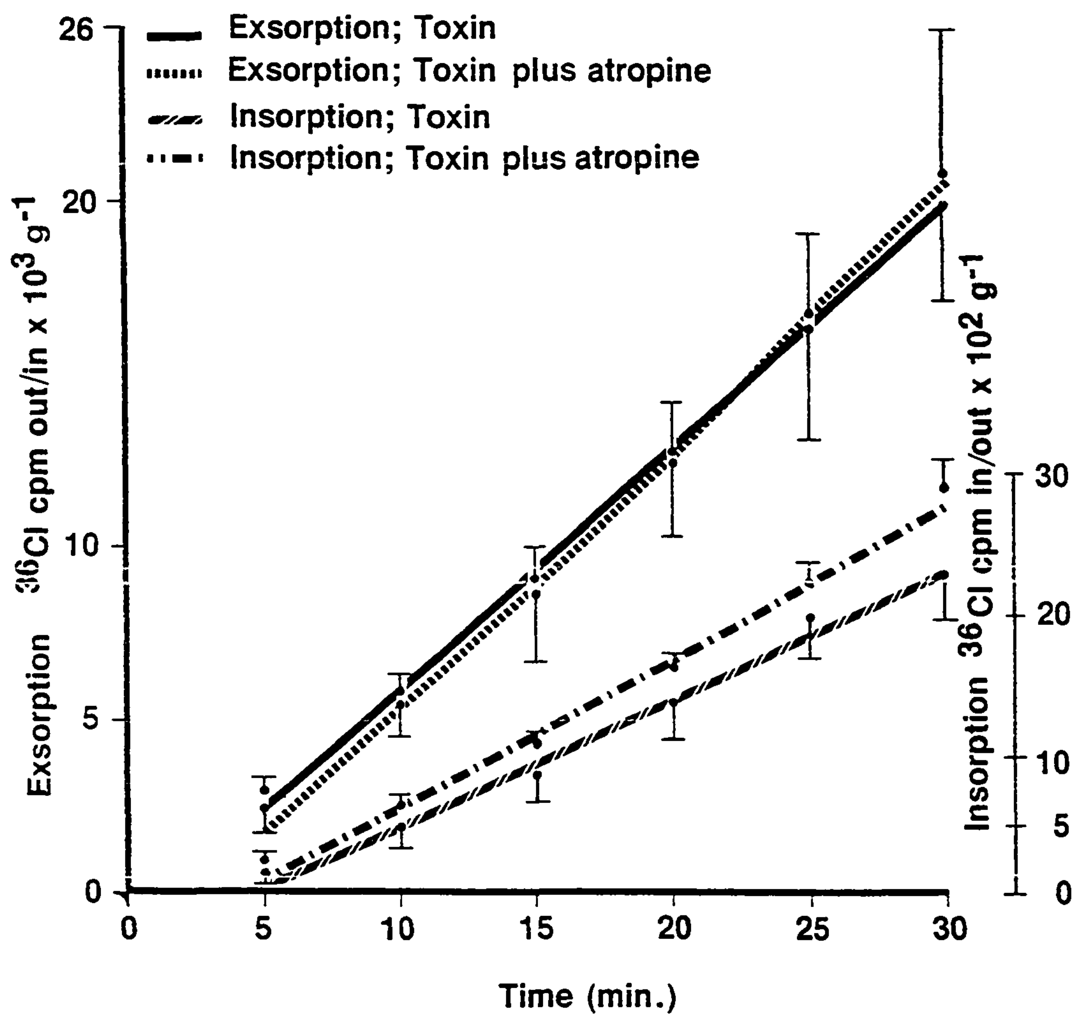
Figure 20. Effects of atropine on the exsorption and insorption of ^{36}Cl from ST-exposed porcine jejunal sacs

Concentration of atropine in mucosal solution was 2×10^{-5} M.

^{36}Cl cpm $\frac{out}{in} \times 10^{3}\ g^{-1}$ = exsorption

^{36}Cl cpm $\frac{in}{out} \times 10^{2}\ g^{-1}$ = insorption

Each point represents the mean (±SEM) from 8 animals.


Exsorption; Toxin
Exsorption; Toxin plus atropine
Insorption; Toxin
Insorption; Toxin plus atropine
Exsorption ^{36}Cl cpm out/in x 10^3 g^{-1}
Insorption ^{36}Cl cpm in/out x 10^2 g^{-1}
Time (min.)
26
20
10
5
0
30
20
10
5
0
0
5
10
15
20
25
30

and Figure 21). The exsorption slope was 6.1×10^{-4} for toxin and 5.8×10^{-4} for toxin plus clonidine (Table 2).

Phenylephrine, an alpha-1 adrenergic agonist, increased chloride exsorption from the sac treated with ST toxin (Table 8 and Figure 22). Table 2 shows the slopes of chloride exsorption from toxin-treated sacs to be 7.2×10^{-4} compared to 8.1×10^{-4} from toxin plus phenylephrine-treated sacs. These values were significantly different at the level of $p < 0.005$.

Addition of morphine to the toxin-treated sacs reduced chloride exsorption rate compared to toxin alone (Table 9 and Figure 23). However, there was no statistically significant difference. The exsorption slopes for toxin-treated was 7.3×10^{-4} and 6.8×10^{-4} for toxin plus morphine (Table 2).

<u>Insorption of ^{36}Cl</u>

Chloride insorption was determined as the ratio of ^{36}Cl activity inside the sac to outside per gram tissue. Toxin increased chloride insorption significantly compared to control (Table 1 and Figure 16). The mean insorption rate constants from control and toxin-treated everted sacs were 7.0×10^{-3} and 10.5×10^{-3}, respectively (Table 2).

VIP significantly increased chloride exsorption from the everted sacs but had no effect on chloride insorption compared to control (Table 3 and Figure 17). The mean rate of chloride insorption was 9.8×10^{-3} for both control and VIP-treated sacs (Table 2).

Addition of VIP to toxin-treated sacs did not change chloride insorption rate compared to toxin alone as shown in Table 4 and Figure 18.

Table 7. Effects of clonidine on the insorption and exsorption of ^{36}Cl from ST-exposed porcine everted jejunal sacs

Time (min)	Insorption[a] x 10^2 Toxin	Insorption[a] x 10^2 Toxin+ clonidine[c]	Exsorption[b] x 10^3 Toxin	Exsorption[b] x 10^3 Toxin+ clonidine[c]
5	2.17±0.21	2.54±0.43	1.90±0.15	3.12±0.14
10	5.63±1.07	7.03±1.01	4.60±0.35	5.95±1.45
15	10.94±1.22	13.13±2.00	7.43±0.62	8.61±1.40
20	17.04±1.66	17.73±2.08	10.48±0.85	11.50±1.75
25	21.98±1.87	23.97±2.56	13.48±1.12	14.57±1.99
30	27.37±2.29	29.50±2.95	17.35±1.57	17.78±2.18

[a]Ratio of ^{36}Cl activity inside to outside per gram tissue. Mean (±SEM) from 6 animals.

[b]Ratio of ^{36}Cl activity outside to inside per gram tissue. Mean (±SEM) from 6 animals.

[c]Concentration of clonidine in mucosal solution was 10^{-6} M.

Table 8. Effects of phenylephrine on the insorption and exsorption of ^{36}Cl from ST-exposed porcine everted jejunal sacs

Time (min)	Insorption[a] x 10^2 Toxin	Insorption[a] x 10^2 Toxin+phenyl- ephrine[c]	Exsorption[b] x 10^3 Toxin	Exsorption[b] x 10^3 Toxin+phenyl- ephrine[c]
5	1.24±0.30	2.76±0.69	2.82±0.63	3.78±1.48
10	4.48±1.17	6.52±1.31	6.15±1.28	7.67±2.46
15	11.71±2.67	11.32±2.42	9.72±1.94	11.73±3.71
20	19.06±4.28	17.39±3.95	13.35±2.61	15.40±4.13
25	25.09±5.70	23.08±5.56	16.58±3.04	19.92±5.70
30	31.78±7.05	27.31±5.67	21.05±4.06	24.17±6.47

[a]Ratio of ^{36}Cl activity inside to outside per gram tissue. Mean (±SEM) from 6 animals.

[b]Ratio of ^{36}Cl activity outside to inside per gram tissue. Mean (±SEM) from 6 animals.

[c]Concentration of phenylephrine in mucosal solution was 10^{-5} M.

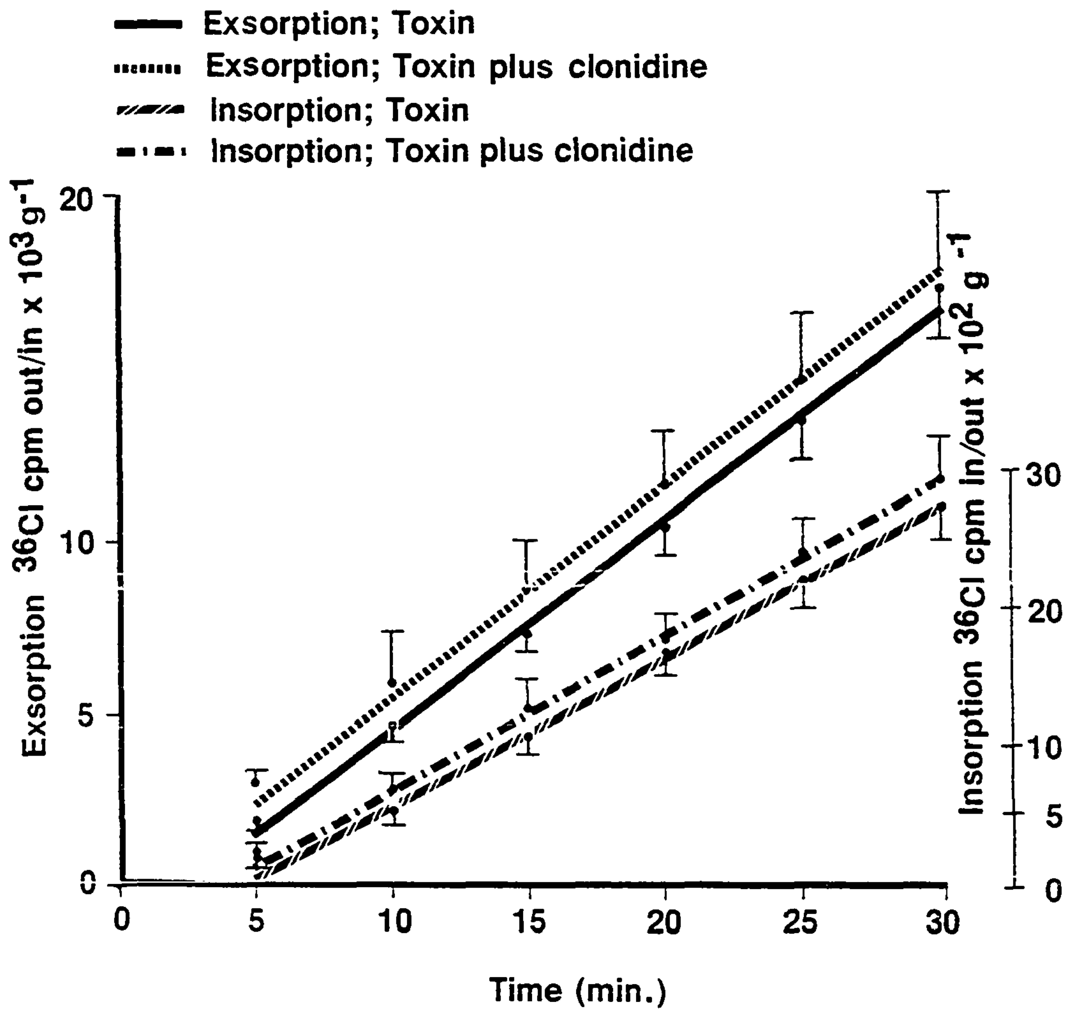
Figure 21. Effects of clonidine on the exsorption and insorption of ^{36}Cl from ST-exposed porcine everted sacs

Concentration of clonidine in mucosal solution was 10^{-6} M.

^{36}Cl cpm $\frac{out}{in} \times 10^{3}\ g^{-1}$ = exsorption

^{36}Cl cpm $\frac{in}{out} \times 10^{2}\ g^{-1}$ = insorption

Each point represents the mean (±SEM) from 6 animals.



Exsorption; Toxin
Exsorption; Toxin plus clonidine
Insorption; Toxin
Insorption; Toxin plus clonidine
Exsorption 36Cl cpm out/in x 10^{3}g^{-1}
Insorption 36Cl cpm in/out x 10^2 g^{-1}
20
10
5
0
30
20
10
5
0
0
5
10
15
20
25
30
Time (min.)

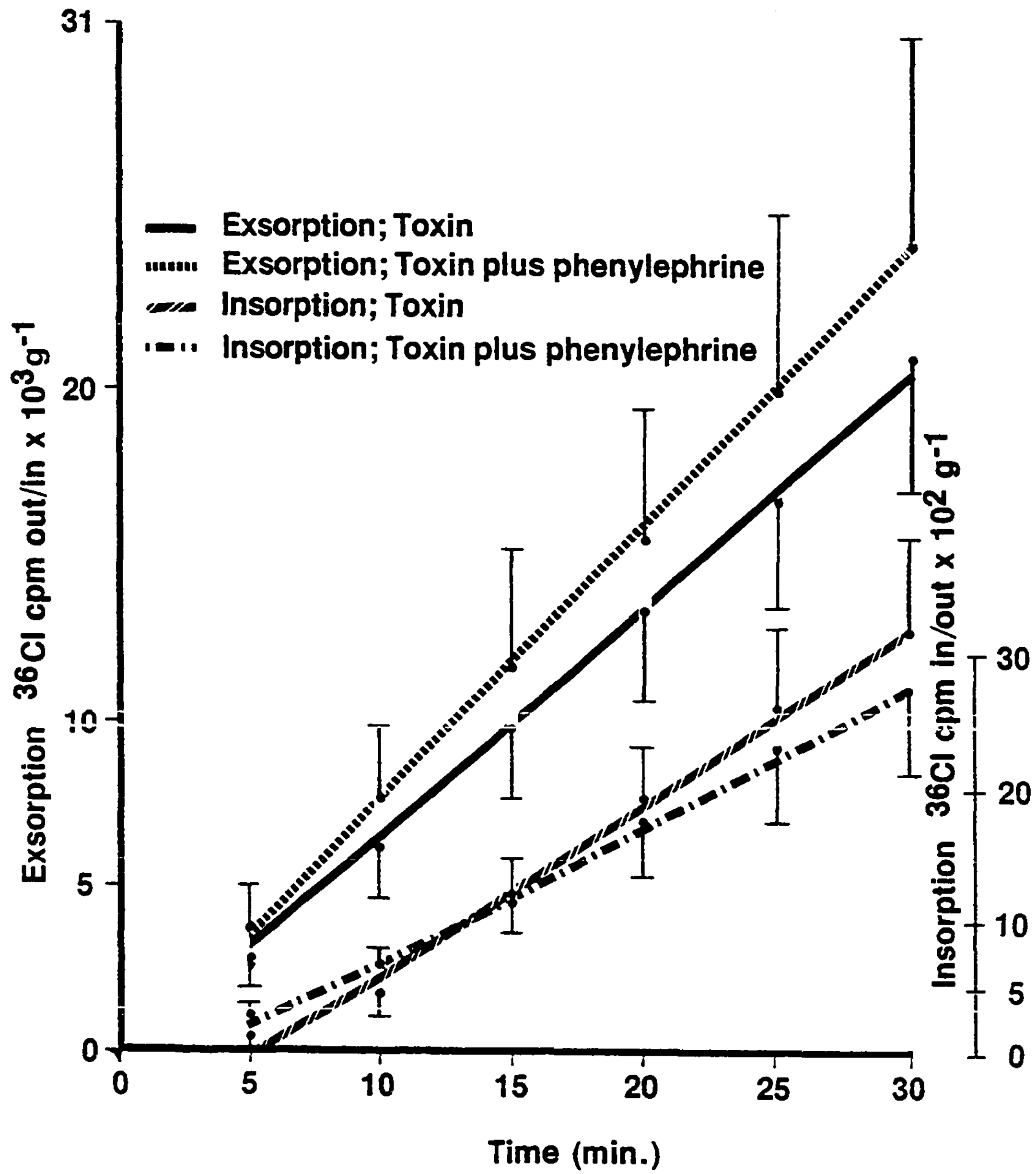
Figure 22. Effects of phenylephrine on the exsorption and insorption of ^{36}Cl from ST-exposed porcine everted sacs

Concentration of phenylephrine in mucosal solution was 10^{-5} M.

^{36}Cl cpm $\frac{out}{in} \times 10^{3}\ g^{-1}$ = exsorption

^{36}Cl cpm $\frac{in}{out} \times 10^{2}\ g^{-1}$ = insorption

Each point represents the mean (±SEM) from 6 animals.



Exsorption; Toxin
Exsorption; Toxin plus phenylephrine
Insorption; Toxin
Insorption; Toxin plus phenylephrine
Exsorption 36Cl cpm out/in x 10^3 g^-1
Insorption 36Cl cpm in/out x 10^2 g^-1
Time (min.)
31
20
10
5
0
0
5
10
15
20
25
30
30
20
10
5
0

Table 9. Effects of morphine on the insorption and exsorption of ^{36}Cl from ST-exposed porcine everted jejunal sacs

Time (min)	Insorption[a] x 10^2		Exsorption[b] x 10^3	
	Toxin	Toxin+ morphine[c]	Toxin	Toxin+ morphine[c]
5	2.75±1.03	2.68±0.74	2.24±0.47	2.52±0.37
10	7.67±1.76	7.42±1.24	5.44±0.91	5.54±0.69
15	13.51±2.88	13.61±2.29	8.60±1.31	8.64±1.02
20	21.45±3.95	19.62±3.08	12.50±1.93	12.24±1.34
25	28.66±5.56	25.78±4.08	16.56±2.57	15.48±1.38
30	34.67±7.23	32.58±5.80	20.22±3.03	19.68±1.62

[a]Ratio of ^{36}Cl activity inside to outside per gram tissue. Mean (±SEM) from 5 animals.

[b]Ratio of ^{36}Cl activity outside to inside per gram tissue. Mean (±SEM) from 5 animals.

[c]Concentration of morphine in mucosal solution was 1.2 μg/ml.

Toxin-treated sacs showed a mean chloride insorption rate of 12.6 x 10^{-3}. The same rate was observed in the presence of toxin plus VIP (Table 2).

Pilocarpine did not change chloride insorption rate of toxin-treated sacs (Table 5 and Figure 19). The rate of chloride insorption was 12.5 x 10^{-3} for toxin alone compared to 12.3 x 10^{-3} for toxin plus pilocarpine (Table 2). Thus, pilocarpine did not exhibit an additive effect to toxin either on chloride exsorption or chloride insorption from the everted sacs.

Addition of atropine increased chloride insorption rate from 8.8 x 10^{-3} in toxin-treated sacs to 10.6 x 10^{-3} (Table 2). However, the difference was not significant (Table 6 and Figure 20).

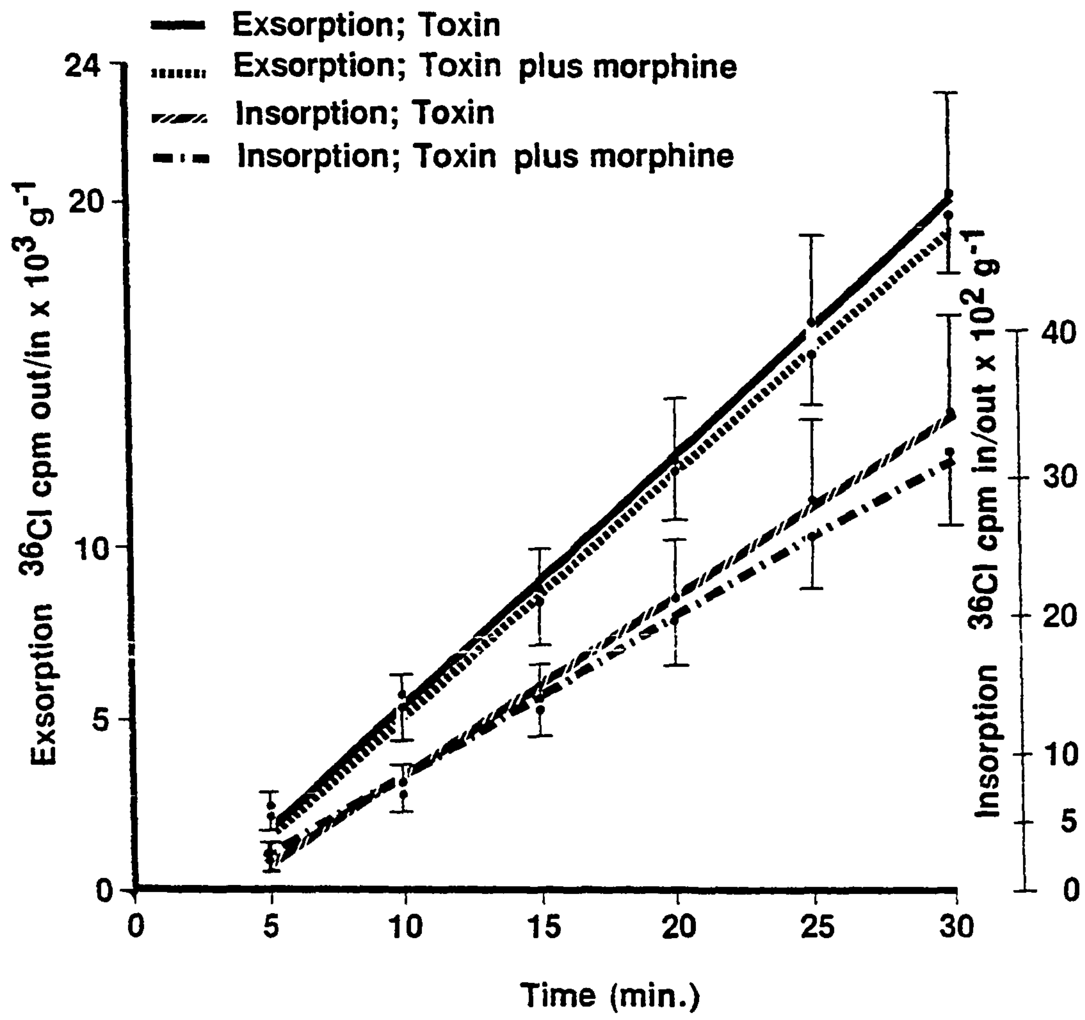
Figure 23. Effects of morphine on the exsorption and insorption of ^{36}Cl from ST-exposed porcine everted jejunal sacs

Concentration of morphine in mucosal solution was 1.2 μg/ml.

^{36}Cl cpm $\frac{out}{in} \times 10^3\ g^{-1}$ = exsorption

^{36}Cl cpm $\frac{in}{out} \times 10^2\ g^{-1}$ = insorption

Each point represents the mean (±SEM) from 5 animals.


Exsorption; Toxin
Exsorption; Toxin plus morphine
Insorption; Toxin
Insorption; Toxin plus morphine
Exsorption 36Cl cpm out/in x 10^3 g^-1
Insorption 36Cl cpm in/out x 10^2 g^-1
24
20
10
5
0
40
30
20
10
5
0
0
5
10
15
20
25
30
Time (min.)

Clonidine did not have any significant effect on chloride insorption when added to toxin-treated sacs (Table 7 and Figure 21). The rate of chloride insorption was 10.4×10^{-3} for toxin-treated sacs and 10.9×10^{-3} for toxin plus clonidine (Table 2).

Addition of phenylephrine to everted sacs treated with toxin significantly decreased chloride insorption (Table 8 and Figure 22). Chloride insorption rate produced by the toxin was 12.7×10^{-3} compared to 10.2×10^{-3} when phenylephrine was added (Table 2). Thus, phenylephrine significantly increased chloride exsorption and decreased chloride insorption from everted sacs.

Morphine reduced chloride insorption rate from 13.2×10^{-3} in toxin-treated sacs to 12.0×10^{-3} (Table 2). This decrease was not statistically significant (Table 9 and Figure 23).

Isolated Enterocyte Studies

Microscopic examination

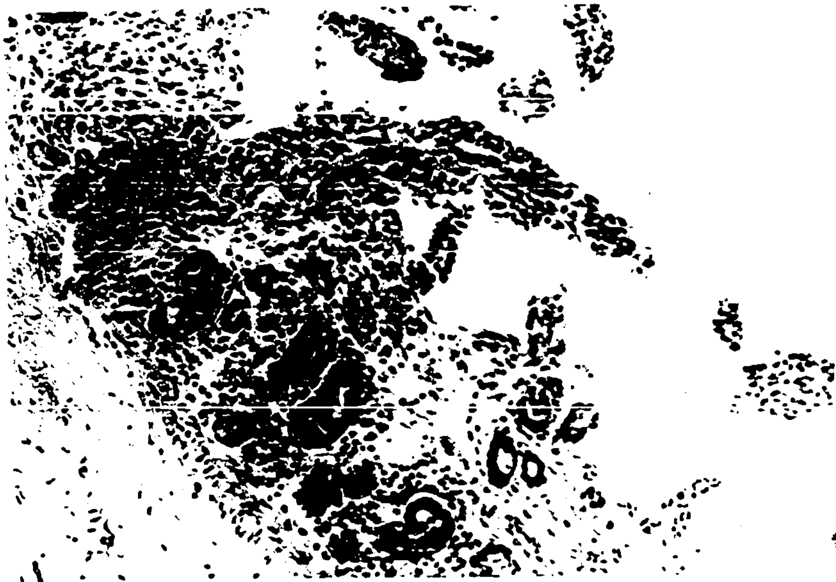
After three fractions of cells were isolated, a section of intestine was taken for histological examination. There was complete denudation of the villi and absence of villus epithelial cells. Many but not all of the mucosal crypts were devoid of cells (Figures 24 and 25). There was infiltration of mononuclear cells in the lamina propria. The results indicated that mechanical vibration effectively removed enterocytes from the intestinal mucosa.

Figure 24. Jejunum of 3-week-old pig after vibration (Eosin, 250X)

Note complete denudation of the villi and absence of villus and crypt cells.

Figure 25. Jejunum of 3-week-old pig after vibration (Eosin, 250X)

There was complete denudation of the villi and absence of villus cells. Some of the crypt cells are intact.



Trypan Blue exclusion test

More than 70% of the isolated enterocytes excluded Trypan Blue when examined at the end of each experiment. This suggested that the majority of cells remained viable throughout the experimental period.

Sucrase activity assay

To determine the origin of the cells isolated in each fraction, sucrase activity was determined. The results are summarized in Table 10. Cells in fraction one contained the highest activity of sucrase. The average value was 103.34±7.71 units/g protein which indicated that cells in the first fraction were mature villus cells. Cells in the fraction two and fraction three contained sucrase activity of 64.05±5.69 and 32.25±3.75 units/g protein, respectively. The low value obtained for sucrase activity in the third fraction indicates that this fraction was composed of immature cells from the lower villus and crypt regions.

Chloride efflux rate determination

Chloride efflux rate constants for control and toxin treated enterocytes obtained from fractions one, two, and three are shown in Figures 26, 27, and 28, respectively. The curves were plotted as logarithmic values of $\%^{36}Cl$ remaining in the cells versus time. At time zero, the percent of radioactive chloride remaining in the cells was set at 100%. The radioactive chloride remaining in the cells declined linearly with time as chloride moved out of the cells. The slopes of the lines were determined as chloride efflux rate constants (amount of chloride exchanged per hour). In most experiments, about 10% of the initial chloride radioactivity was left in the cells at 12 min.

Table 10. Sucrase activity in isolated enterocytes (units/g protein; values are mean (±SEM) from 19 animals)

Fraction I = 103.34±7.71
Fraction II = 64.05±5.69
Fraction III = 32.25±3.75

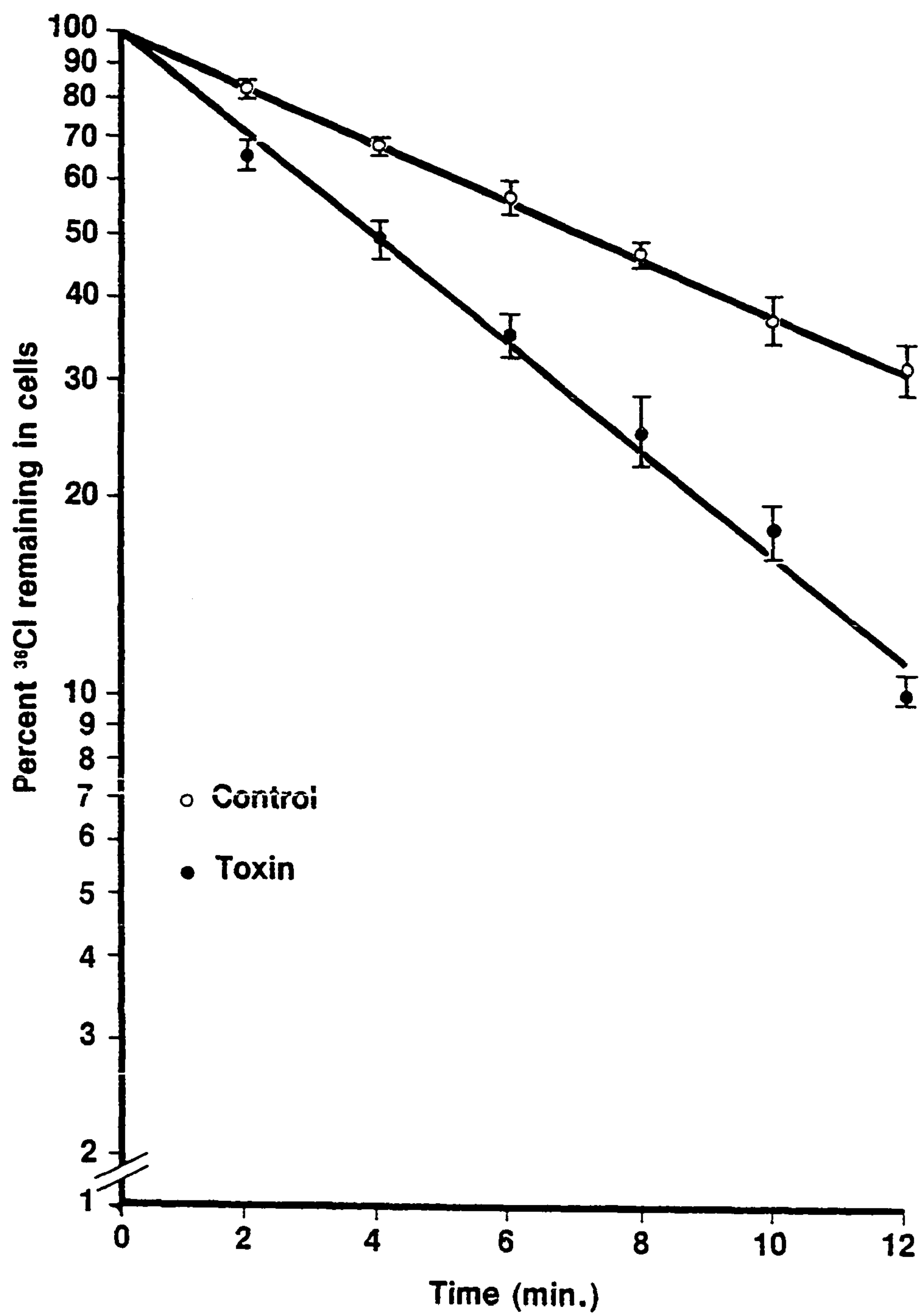
Table 11 summarizes the values of chloride efflux rate constants from control and toxin-treated cells in fractions one, two, and three. There was no difference in chloride efflux rate constants in cells obtained from fraction one compared with those obtained from fractions two or three. Thus, chloride efflux rate constants were the same in villus and crypt cells. The efflux rates of chloride were -6.32±0.44 from upper villus, -6.73±0.45 from lower villus and -7.02±0.46 from crypt cells. Addition of toxin significantly increased chloride efflux rate to levels twice those of control. There was no difference in the effect of toxin on villus compared to crypt cells. The values of efflux rates of chloride from toxin-treated enterocytes isolated from upper villus, lower villus, and crypt cells were -12.91±1.19, -12.14±0.77, and -12.34±1.60, respectively.

Effect of drugs on chloride efflux rate constants from isolated enterocytes

Atropine Table 12 summarizes the effects of atropine on chloride efflux rates in control and ST-exposed cells. In fraction one, the chloride efflux rate constant in control enterocytes was -6.02±0.76. Atropine, when added to control enterocytes, did not significantly change

Figure 26. Comparison of chloride efflux rate constants in control and ST-exposed upper villus cells

Each point represents mean (±SEM) from 19 animals.



100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
Percent 36Cl remaining in cells
Control
Toxin
0
2
4
6
8
10
12
Time (min.)

Figure 27. Comparison of chloride efflux rate constants in control and ST-exposed lower villus cells

Each point represents mean (±SEM) from 19 animals.

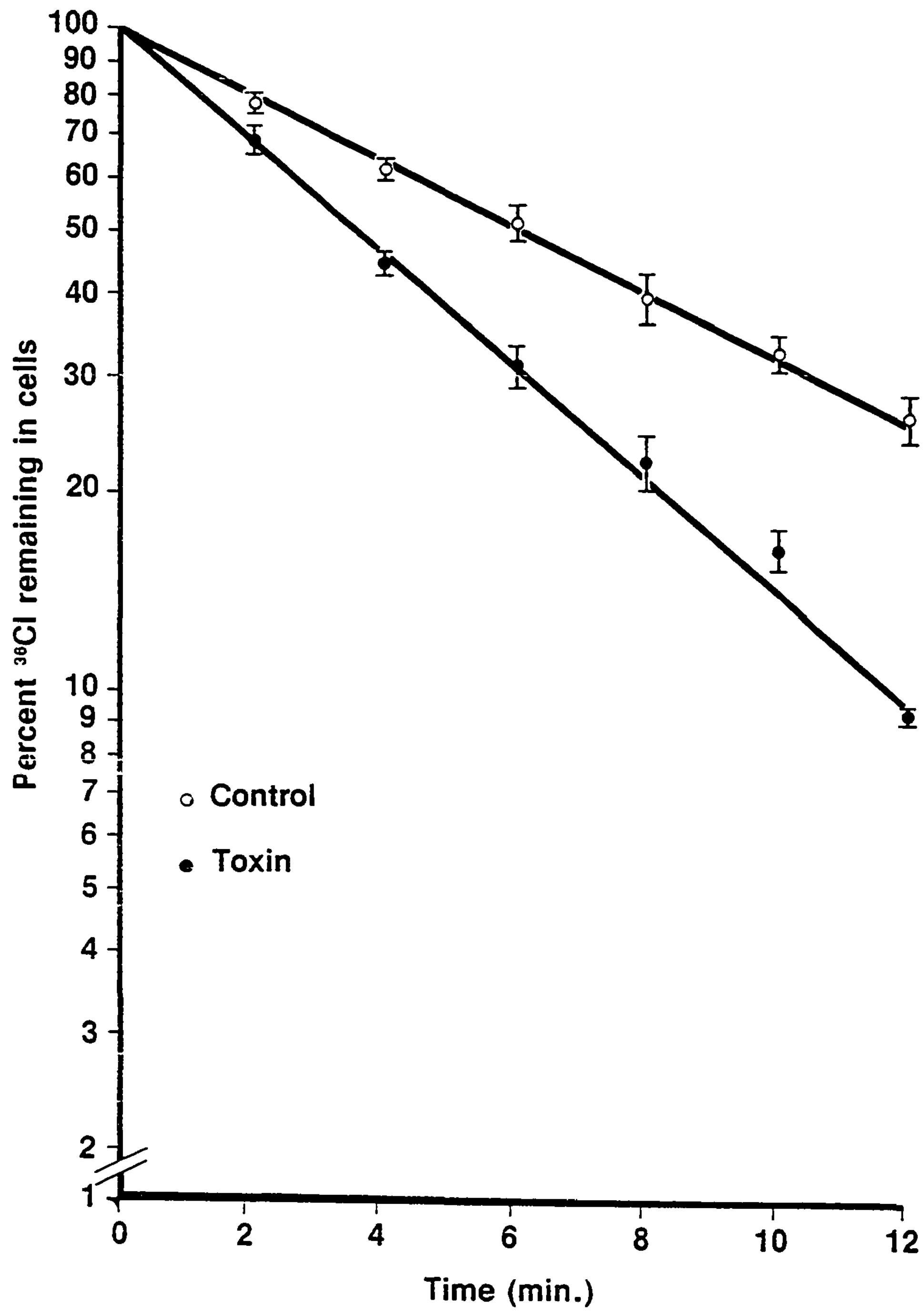
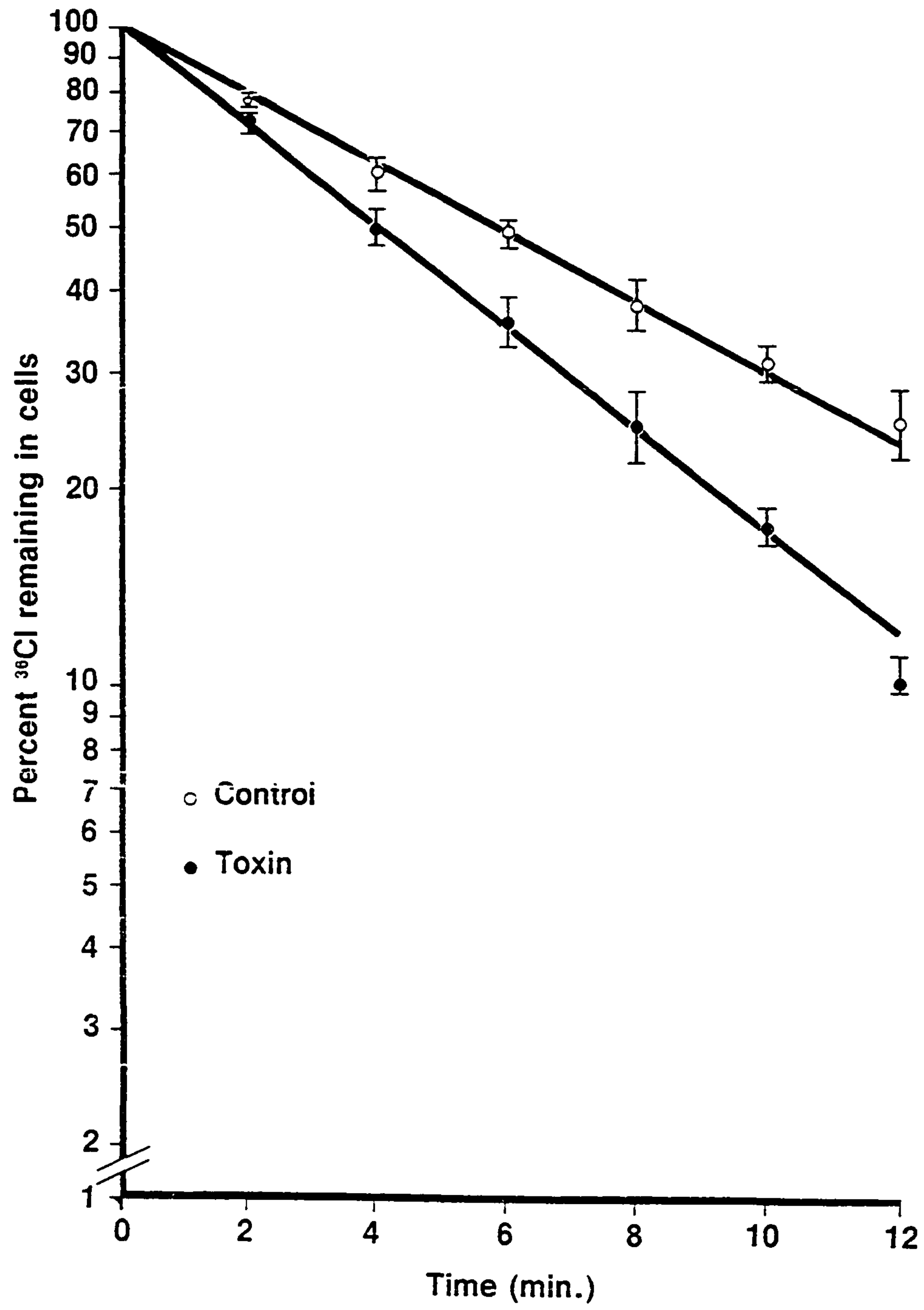

100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
Percent ³⁶Cl remaining in cells
○ Control
● Toxin
0
2
4
6
8
10
12
Time (min.)

Figure 28. Comparison of chloride efflux rate constant in control and ST-exposed crypt cells

Each point represents mean (±SEM) from 19 animals.



100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
Percent ^{36}Cl remaining in cells
○ Control
● Toxin
0
2
4
6
8
10
12
Time (min.)

Table 11. Effects of ST on ^{36}Cl efflux rate constants from enterocytes[a]

Fraction	Control	Toxin	p <
I	-6.32±0.44	-12.91±1.19	0.001
II	-6.73±0.45	-12.14±0.77	0.001
III	-7.02±0.46	-12.34±1.60	0.01

[a]Values are mean (±SEM) from 19 animals.

Table 12. Effect of atropine on ^{36}Cl efflux rate from control and ST-exposed enterocytes[a]

Fraction	Control	Control +atropine[b]	Toxin	Toxin +atropine[b]
I	-6.02±0.76	-5.60±0.39	-12.12±1.36*	-11.24±1.01*
II	-7.81±0.81	-8.21±1.05	-13.47±1.44*	-9.81±1.18
III	-6.66±0.70	-6.12±0.48	-13.28±1.90*	-11.71±0.78*

[a]Values are given as mean (±SEM) of efflux rate constants from 7 animals.

[b]Concentration of atropine in incubation medium was 2×10^{-5} M.

*$p < 0.05$ compared to control.

chloride efflux rate. Toxin increased chloride efflux rate significantly to -12.12±1.36 compared to control. When atropine was added to toxin-treated enterocytes, the efflux rate was reduced, though not significantly, compared to toxin alone.

In fraction two, the chloride efflux rate constant was -7.81±0.81 in control enterocytes. Addition of atropine to control did not significantly change the efflux rate of chloride. Addition of toxin significantly increased the chloride efflux rate to -13.47±1.44 compared to control.

Addition of atropine to toxin-treated enterocytes decreased the efflux rate to -9.81±1.18, but the decrease was not significant.

In fraction three, the chloride efflux rate constant was -6.66±0.70 in control enterocytes. Addition of atropine to control did not change the efflux rate of chloride. Toxin increased chloride efflux rate significantly from -6.66±0.70 in control to -13.28±1.90. Atropine addition to toxin-treated enterocytes did not significantly change chloride efflux rate compared to toxin alone.

In summary, the addition of atropine, a muscarinic blocker, did not alter basal chloride efflux rates in enterocytes. Although ST-augmented secretion of chloride was reduced by atropine in all fractions, the decrease was not significant.

Clonidine The effect of clonidine, an alpha-2 adrenergic agonist, on chloride efflux rate is summarized in Table 13. In fraction one, the chloride efflux rate constant in control enterocytes was -5.56±0.90. Clonidine did not have any effect on chloride efflux rate in control cells. Toxin doubled the chloride efflux rate to -10.77±1.84 and this effect was not altered by clonidine.

In fraction two, the chloride efflux rate constant was -6.18±0.38 for control and -10.62±0.89 for ST-exposed entercytes. Clonidine reduced toxin-augmented efflux by approximately 10% but the reduction was not significant. Similar results were obtained in fraction three except that clonidine inhibited ST-stimulated chloride by more than 20%. This produced a chloride efflux rate which was intermediate between control and ST and not significantly different from either.

Table 13. Effect of clonidine on ^{36}Cl efflux rate from control and ST-exposed enterocytes[a]

Fraction	Control	Control+ clonidine[b]	Toxin	Toxin+ clonidine[b]
I	-5.56±0.90	-5.93±0.84	-10.77±1.84*	-10.27±1.82*
II	-6.18±0.38	-7.32±0.85	-10.62±0.89*	-9.15±0.83*
III	-6.62±0.73	-6.66±0.80	-10.94±1.28*	-8.49±2.05

[a]Values are given as mean (±SEM) of efflux rate constants from 5 animals.

[b]Concentration of clonidine in incubation medium was 10^{-6} M.

*$p < 0.05$ compared to control.

<u>Morphine</u> Table 14 summarizes the effects of morphine on chloride efflux rates in control and toxin-exposed enterocytes. The value of control chloride efflux rate in cells from fraction one was -6.79±0.86. Morphine did not significantly change chloride efflux rate in control enterocytes. Addition of toxin to control enterocytes significantly increased the chloride efflux rate to -16.89±2.01. When morphine was added to toxin-treated enterocytes, chloride efflux rate was significantly reduced to -10.62±1.60.

The control chloride efflux rate in cells from fraction two was -6.43±0.65. Addition of toxin significantly increased chloride efflux rate to -12.70±0.96 compared to control. Morphine did not have any effect on control or toxin-exposed enterocytes.

Cells from fraction three had a chloride efflux rate of -7.16±0.85. Morphine did not alter this basal rate of transport. Toxin significantly increased chloride efflux rate to -14.68±2.39 compared to control. The

Table 14. Effect of morphine on ^{36}Cl efflux rate from control and ST-exposed enterocytes[a]

Fraction	Control	Control+ morphine[b]	Toxin	Toxin+ morphine[b]
I	-6.79±0.86	-4.82±2.04	-16.89±2.01*	-10.62±1.60*#
II	-6.43±0.65	-8.45±1.00	-12.70±0.96*	-13.69±1.84*
III	-7.16±0.85	-7.42±1.19	-14.68±2.39*	-9.75±1.59

[a]Values are given as mean (±SEM) from 7 animals.

[b]Concentration of morphine in incubation medium was 1.2 μg/ml.

*$p < 0.05$ compared to control.

#$p < 0.05$ compared to toxin.

effect of morphine on ST-stimulated efflux was similar to that observed for clonidine in immature enterocytes. Chloride efflux was reduced to a point between basal and maximal secretion and was not significantly different from either.

Calcium efflux rate determination

The effects of ouabain and ST toxin on calcium efflux rates are shown in Table 15. Results are the average from two experiments. The efflux rate of calcium was low compared to chloride efflux rate from enterocytes. Although there were some variations in the efflux rate of calcium among fraction one, two, and three, there was no significant difference. When ouabain was added to control enterocytes, the efflux rate tended to decrease, especially in cells from fraction one, but the decrease was not significant. Toxin-exposed enterocytes had the same rate of calcium efflux as control. Addition of ouabain to toxin-exposed enterocytes did

Table 15. Effect of ouabain on calcium-45 efflux rate from control and ST-exposed enterocytes[a]

Fraction	Control	Control+ ouabain[b]	Toxin	Toxin+ ouabain[b]
I	-1.13±0.07	-0.74±0.09	-0.94±0.20	-0.80±0.16
II	-0.95±0.12	-1.28±0.15	-1.31±0.36	-1.96±0.75
III	-1.22±0.43	-0.71±0.54	-1.82±0.42	-1.77±0.47

[a]Values are given as mean (±SEM) from 2 animals.

[b]Concentration of ouabain in incubation medium was 1 mM.

not change the efflux rate of calcium. The results indicated that there was no difference in calcium efflux rates from villus cells compared to crypt cells and neither ouabain nor toxin had an effect on calcium efflux rate.

Determination of metabolic activity

Metabolic activity determination was performed in villus cells using ^{14}C-glucose or ^{14}C-glutamic acid as a substrate. In each experiment, the treatments were divided into control, control plus ouabain, toxin and toxin plus ouabain. $^{14}CO_2$ was collected for 30 min intervals up to 3 hours. The maximum value produced from control was set as 100% and the activity produced by cells in the other three treatments was compared to this maximum control value.

Glucose Table 16 and Figure 29 summarize the effects of toxin and ouabain on $^{14}CO_2$ produced from glucose. When glucose was used as substrate for the cells, CO_2 production was linear up to 3 hours. Addition of ouabain significantly decreased the rate of CO_2 production from glucose

Table 16. ^{14}C-glucose metabolism in isolated enterocytes[a]

Time (min)	% Metabolic activity			
	Control	Control+ ouabain[b]	Toxin	Toxin+ ouabain[b]
30	15.93±3.91	10.08±2.15	15.65±3.68	15.45±3.43
60	47.13±6.46	25.73±3.33*	42.43±6.48	35.08±5.99*
90	67.20±6.01	36.70±5.40*	62.00±8.77	46.55±4.47*
120	82.25±4.76	45.25±7.65*	76.33±10.66	55.18±4.32*
150	91.98±2.62	51.80±9.04*	86.78±11.82	61.78±5.03*
180	100.00	56.75±10.24*	93.88±11.94	68.45±7.17*

[a]Values are given as mean of percent metabolic activity compared to maximum value of control (±SEM) from 4 animals.

[b]Concentration of ouabain in incubation medium was 1 mM.

*$p < 0.05$ compared to control.

in control cells. Total ^{14}C-glucose metabolized to $^{14}CO_2$ by 3 hours was reduced by approximately one-half when ouabain was added to control enterocytes.

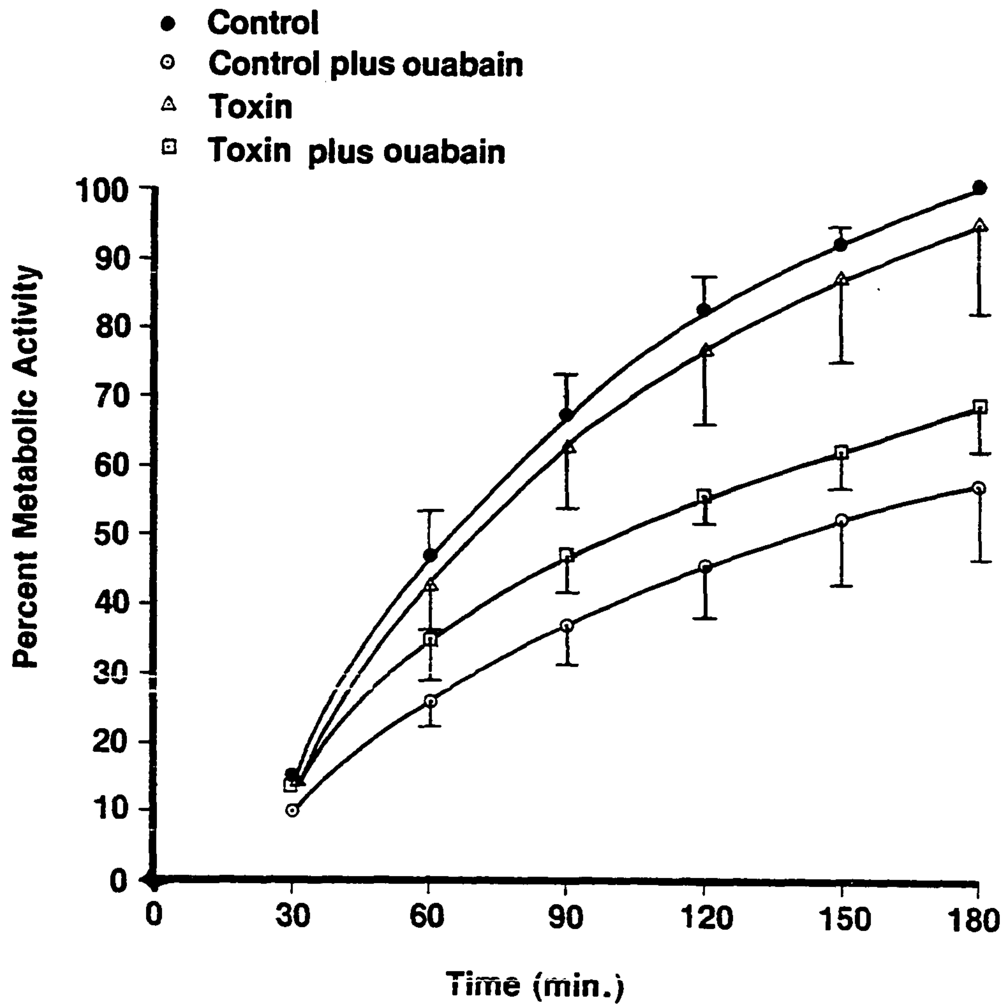
Addition of toxin did not significantly change the CO_2 production rate from glucose compared to control. When ouabain was added to toxin-exposed enterocytes, the rate of CO_2 production was significantly reduced compared to control. Thus, ouabain inhibited glucose metabolism in both control and ST-exposed enterocytes.

Glutamic acid The effects of ouabain and toxin on $^{14}CO_2$ production from glutamic acid are summarized in Table 17 and Figure 30. The cells metabolized glutamic acid linearly during the 3-hour experimental period. Incubation of cells with ST did not alter the rate of glutamic

Figure 29. Effects of ouabain on ^{14}C-glucose metabolism in isolated villus cells from porcine jejunum

The maximal value of $^{14}CO_2$ produced from control was set as 100%. The activity produced by cells in control plus ouabain, toxin and toxin plus ouabain experiments was compared to the maximal control value.

Each point represents mean (±SEM) from 4 animals. Concentration of ouabain in incubation medium was 1 mM.


Control
Control plus ouabain
Toxin
Toxin plus ouabain
Percent Metabolic Activity
100
90
80
70
60
50
40
30
20
10
0
0
30
60
90
120
150
180
Time (min.)

acid metabolism. Ouabain had no effect on $^{14}CO_2$ production from glutamic acid in control or ST-treated enterocytes.

Table 17. ^{14}C-glutamic acid metabolism in isolated enterocytes[a]

Time (min)	% Metabolic activity			
	Control	Control+ ouabain[b]	Toxin	Toxin+ ouabain[b]
30	31.37±2.34	9.23±1.78	12.37±2.46	9.98±1.69
60	31.37±4.89	28.32±4.98	35.57±5.25	28.47±3.57
90	53.20±7.30	49.55±7.39	48.90±8.09	48.87±5.74
120	71.10±8.13	66.10±9.08	76.85±8.00	64.78±5.92
150	85.45±6.30	78.93±7.73	91.10±5.34	77.48±3.12
180	100.00	93.65±6.84	105.20±6.32	89.65±2.57

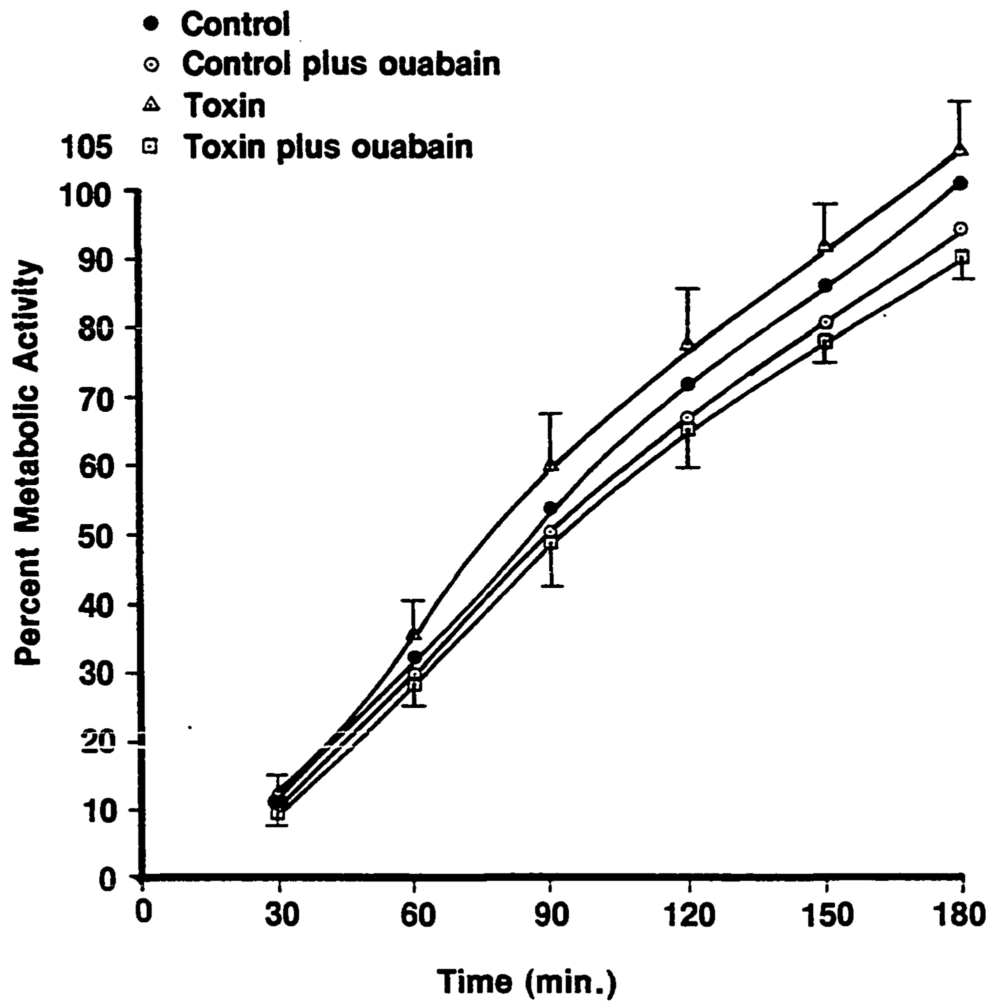
[a]Values are given as mean of percent metabolic activity compared to maximum value of control (±SEM) from 6 animals.

[b]Concentration of ouabain in incubation medium was 1 mM.

Figure 30. Effects of ouabain on ^{14}C-glutamic acid metabolism in isolated villus cells from porcine jejunum

The maximal value of $^{14}CO_2$ produced from control was set as 100%. The activity produced by cells in control plus ouabain, toxin and toxin plus ouabain experiments was compared to the maximal control value.

Each point represents mean (±SEM) from 6 animals. Concentration of ouabain in incubation medium was 1 mM.



Control
Control plus ouabain
Toxin
Toxin plus ouabain
Percent Metabolic Activity
105
100
90
80
70
60
50
40
30
20
10
0
0
30
60
90
120
150
180
Time (min.)

DISCUSSION

Everted Gut Sac Studies

Histological examination of everted sacs prepared after 45-60 minute experimental periods revealed a generally intact epithelium. In some preparations, small amounts of edema were observed in the lamina propria but the accumulation was minimal since removal of the serosal and muscular coats of the intestine reduces the hindrance to fluid and solute movements (Parsons and Paterson, 1960). Field et al. (1971) determined Na and Cl transport across short-circuited rabbit ileum and found out that the stripped preparation had a higher and more stable short-circuit current than the unstripped preparation. A possible explanation for this observation might be that the unstripped intestine was relatively hypoxic due to an interference by the muscularis externa with oxygen diffusion. Wolfe et al. (1973) found that removal of the serosa and muscularis externa increased salicylate transport rates up to 50-85% compared to the intact one. The majority of the capillaries in the small intestine are found in the lamina propria at the base of the epithelial cells. Surgical removal of the serosa and muscularis externa may allow *in vitro* transport processes to approximate those observed *in vivo*. The serosa and muscularis externa could be removed from an intestinal segment in one intact sheet with no damage to the absorptive tissue. Evidence that barriers to diffusion must be removed in *in vitro* systems is supported by studies of the role of the mesentery in water absorption in rat intestine. Removal of mesentery reduced absorption by 40% (Lee, 1963). Thus, the accumula-

tion of water in tissue spaces *in vitro* may result from the absence of the circulatory dynamics for removal of absorbed fluid (Crane, 1965). These artifacts were minimized by stripping intestinal mucosa, oxygenating tissues, and controlling hydrostatic pressures across the epithelium. In the present study, the observation of morphologically intact mucosa and linear ion transport over the experimental period indicate retention of viability of the everted gut sacs.

ST toxin significantly increased chloride insorption rate and exsorption rate across jejunal mucosa. The increase was greater for the exsorption rate than the insorption and there was no lag period. The insorption rate from ST-exposed gut sacs was comparable to control during the first 20 minute experimental period but was greater than control at 25 min and after. In contrast, the exsorption rate from ST-exposed sacs was increased during the first 5 minutes and continued to increase for the entire experimental period. Thus, the primary effect of ST toxin was to increase the secretion of chloride. ST may cause secretory diarrhea by increasing chloride conductance from epithelial cells and inhibition of the neutral NaCl coupled transport may not be the primary cause of ST effects. This is not consistent with results reported by Field et al. (1978) who showed that partially purified ST caused a rapid and persistent increase in electrical potential difference and short circuit current and abolished net chloride absorption with no effect on net chloride secretion. The change in ion transport was half that produced by theophylline which stimulated net chloride secretion. Theophylline has been shown to increase intestinal electrolyte secretion by increasing cAMP concentration in intestinal cells (Field et al., 1972), in the same manner as cholera

toxin. Theophylline, CT and E. coli LT have been shown to inhibit a NaCl coupled uptake in the brush border membrane of the villus cells and stimulate active chloride secretion from crypt cells (Field et al., 1972; Field, 1979). Supporting evidence for this is that selective destruction of the villus cells by osmotic shock did not significantly diminish cholera toxin-induced fluid production in rabbit small intestine in vivo (Roggin et al., 1972). Furthermore, in the founder intestine (Frizzell et al., 1979b) and rabbit gall bladder (Frizzell et al., 1975), organs which contain no crypts, cAMP inhibited coupled NaCl absorption without any effect on ion secretion. Na coupled Cl transport is absent in rabbit colon where crypts are prominent and cAMP stimulated active chloride secretion, with no effect on sodium fluxes (Frizzell et al., 1976). There has been no attempt to determine the effect of ST on these organs. However, ST exerted its secretory effects via increasing guanylate cyclase with subsequent increase in cGMP levels (Hughes et al., 1978; Guerrant et al., 1980; Scoot et al., 1980) and some evidence exists that most guanylate cyclase in the small intestine is located in the villus cells in association with brush border membranes (De Jonge, 1975; Quill and Weiser, 1975). Guandalini and Field (1979) demonstrated that ST and cAMP inhibited Na coupled Cl influx equally in in vitro rabbit ileal mucosa. In addition, ^{36}Cl uptake across the brush border membrane indicated that ST inhibited Na coupled Cl uptake into the villus cells (Field, 1979). Even though theophylline, ST, and 8-bromo cyclic GMP all inhibited cotransport of NaCl across brush border membrane maximally, ST and 8-bromo cyclic GMP had a smaller effect on the electrogenic component of in-

testinal secretion suggesting they were less potent than cAMP in activating secretion by crypt cells. Field (1979) concluded that the action of ST should be viewed as primary antiabsorptive, whereas that of V. cholerae and heat labile E. coli are both antiabsorptive and secretory. In the present study, however, the increase of chloride flux indicated that ST exerts its effect primarily on increasing chloride secretion from epithelial cells, and far from inhibiting absorption, actually stimulates mucosa to serosa fluxes of chloride.

In the present study, VIP added to the serosal side, increased chloride secretion from everted sacs. There was no change in chloride absorption. When added to ST-exposed gut sacs, VIP did not have any additive effect. Krejs et al. (1978) reported that low doses of VIP (0.02 ug/kg/min) infused into the superior mesenteric artery in dogs decreased sodium absorption from jejunum while high doses (0.24 ug/kg/min) increased secretion of sodium and chloride in addition to decreased sodium absorption. These effects of VIP were similar to those of cholera toxin (Field, 1979). Schwartz et al. (1974) reported that VIP increased adenylate cyclase with a subsequent increase in cAMP and electrolyte secretion in in vitro rabbit ileal mucosa. Net Cl secretion exceeded net Na secretion. However, VIP may have systemic effects in addition to local effects in causing intestinal secretion. Mailman (1978) found that intravenous infusion of VIP in anesthetized dogs reversed net sodium and water absorption to secretion due to a significant decrease in unidirectional absorptive fluxes and smaller increases in secretory fluxes in association with a decrease in absorptive site blood flow and a decrease in arterial pressure. Pretreatment with atropine inhibited most of VIP effects while

pretreatment with guanethidine, an adrenergic neuron blocking agent, did not. He concluded that VIP reduced gut absorption by decreased blood pressure and by acting locally upon acetylcholine release from intestinal tissue. In addition, VIP has been shown to increase contraction of guinea pig ileum and rabbit jejunum (Cohen and Landry, 1980). This effect was partially blocked by atropine indicating that one component of the contractile response was due to the release of acetylcholine. Histamine did not contribute to the ileal contractile response of VIP since pyrilamine, an H_1 receptor antagonist, did not alter VIP-induced increase in intestinal tone. However, they concluded that the role of VIP in producing intestinal pathologies might be to increase cAMP more than intestinal motility. The failure of VIP to enhance ST secretory effect in this study may be due to a final common pathway between these two agents in increasing ion secretion after cyclic nucleotide stimulation.

Pilocarpine added to ST-exposed gut sacs showed no additive effect on ion secretion. Cholinergic drugs have been shown to increase intestinal secretion both *in vivo* and *in vitro* (Tidball, 1961; Isaacs et al., 1976). Morris and Turnberg (1980) observed that a cholinergic drug, neostigmine, caused a significant change from absorption to secretion of sodium, chloride and water in human jejunum. These effects were blocked by atropine. Isaacs et al. (1976) demonstrated the distribution of cholinergic fibers around the crypt and villi using a cholinesterase staining technique. Thus, the parasympathetic nervous system may play a role in the control of intestinal transport which is independent of its effects on motor activity (Morris and Turnberg, 1980). The mechanism of action of cholinergic drugs in increasing electrolyte secretion has been

shown to be via cyclic guanylate cyclase with subsequence increase in cGMP (Brasitus et al., 1976; Tapper et al., 1976) similar to the ST secretory effect (Field et al., 1978; Guerrant et al., 1980). Field et al. (1978) demonstrated that ST had similar secretory effects to theophylline, though smaller in magnitude. The combination of the two did not produce a greater effect than did theophylline alone. Field (1979) suggested that ST action was not limited by the availability of external cell membrane receptors but rather by an intrinsic limitation in the action of cGMP itself. This may explain why, in the present study, ST and pilocarpine which exerted their secretory effects via cGMP did not have an additive effect on chloride secretion from everted gut sacs. However, the results of the present study also indicated that membrane receptors for ST appeared to be different from muscarinic receptors as will be discussed below.

Atropine added to ST-exposed gut sacs did not antagonize the effects of toxin either on chloride secretion or chloride absorption. This suggested that ST receptors were not blocked by muscarinic antagonism. This is consistent with results reported by Guerrant et al. (1980) who showed that atropine, 10^{-5} M, given intragastrically or subcutaneously to the rat, had no effect on ST-induced secretion. However, Ahrens and Zhu (1982a) reported that atropine, 2×10^{-5} M, augmented net chloride and sodium absorption from control pig jejunum *in vivo*. They suggested that atropine blocked a cholinergically mediated secretory component of normal small intestine in which chloride transport might play a significant role. They also showed that intraluminal atropine significantly reduced net loss of water and electrolytes produced by ST from perfused swine jejunum.

The difference between _in vivo_ and _in vitro_ experiments may be that in the _in vitro_ system, the gut is not under continuous cholinergic tone and thus, a secretory component is absent. Also, in the present study, the gut was incubated with the toxin _in vivo_ for 45 minutes before isolation and the secretory process caused by ST was well developed prior to the addition of atropine. The results are consistent with a model in which ST and cholinergic drugs stimulate secretion by a final common pathway but via different receptors. There is evidence that this pathway is the guanylate cyclase system since the secretory effects of both ST and cholinergic drugs are associated with elevated cGMP (Brasitus et al., 1976; Tapper et al., 1976; Field et al., 1978). Elevations in guanylate cyclase activity induced by ST in particulate preparations of rat intestine were not affected by atropine (Guerrant et al., 1980).

In the present study, clonidine, an alpha-2 adrenergic agonist, did not alter chloride transport from ST-exposed gut sacs. It has been well established that the sympathetic system plays a role in intestinal transport (Wright et al., 1940; Field and McColl, 1973; Munday et al., 1980). Stimulation of alpha-adrenergic receptors in rabbit ileal mucosa enhanced active absorption of Na and Cl probably by increasing cGMP levels (Field and McColl, 1973; Brasitus et al., 1976). The precise mechanism of action is not clear but it is believed that catecholamines exert their effects in regulation of intestinal transport via alpha-adrenergic receptors (Field and McColl, 1973; Hubel, 1976). Ahrens and Zhu (1982b) reported that epinephrine reversed chloride flux from net absorption to net secretion in the perfused jejunum of pigs. In addition, epinephrine reduced net loss

of water and electrolytes in the presence of ST. When phenylephrine or clonidine was added to perfusates, absorption was enhanced in control segments and secretion was reduced in the presence of ST.

The alpha-2 adrenoreceptors have been suggested to be involved in regulating acetylcholine release at postganglionic cholinergic nerve terminals (Paton and Vizi, 1969; Kosterlitz et al., 1970). The myenteric (Auerbach's) plexus of guinea pig ileum contain synapses between preganglionic and postganglionic parasympathetic nerves. Stimulation of these nerves released acetylcholine which caused contraction of the longitudinal muscle fibers (Paton and Vizi, 1969). Furness and Costa (1974) reviewed adrenergic innervation of the intestine and showed by histochemical techniques that there was sparse adrenergic innervation to longitudinal muscle of the small intestine. However, the adrenergic nerve terminals formed a network around the intramural neurones. Stimulation of the adrenergic nerves inhibited cholinergic nerve activity and reduced responses to cholinergic nerve stimulation. This adrenergic-inhibitory effect was reduced by phentolamine (an α-1 and α-2 adrenergic blocker). Drew (1978) reported that electrical stimulation of the cholinergic nerves supplying the longitudinal muscle of the guinea-pig ileum caused a twitch response which could be inhibited by clonidine. The inhibitory effect of clonidine was clearly mediated presynaptically because it did not alter the responsiveness of the ileum to exogenous acetylcholine. In perfused swine jejunum where presynaptic terminals are intact, clonidine has been shown to reduce ST effects (Ahrens and Zhu, 1982b). However, in the present study where serosal and muscularis external layers were stripped

off, the antagonizing effects of clonidine could not be seen. It may be concluded that stimulation of alpha-2 adrenoreceptors *in vivo* inhibits neuronal acetylcholine release and decreases the net secretion induced by ST. These effects were not observed *in vitro* because the preparation is not under cholinergic tone. The results do not support a role for alpha-2 adrenoreceptors localized on enterocytes and directly modulating epithelial ion transport.

Phenylephrine, an alpha-1 adrenoreceptor agonist, significantly increased chloride serosal to mucosal flux and decreased mucosal to serosal flux in ST-exposed gut sacs. This augmentation of the secretory action of ST is in contrast to its reported effect in the perfused jejunum of the pig in which phenylephrine reduced the net loss of water and electrolytes caused by ST (Ahrens and Zhu, 1982b). Their results were opposite to those which might be predicted from studies on intestinal smooth muscle. Bauer (1981) demonstrated alpha-1 adrenoreceptors in ileal smooth muscle and considered them to be stimulatory postjunctional modulators of cholinergic nerves. Thus, it might be predicted that phenylephrine would augment ST *in vivo* but not *in vitro*, especially since pilocarpine, a muscarinic drug, did not enhance the secretory action of ST in everted sacs. The present study suggests that phenylephrine has a direct effect on gut epithelium to stimulate chloride secretion and that this stimulus-secretion coupling pathway is independent of that stimulated by ST. Phenylephrine may increase calcium concentration in the cells and calcium may act as a direct activator of a transport process responsible for chloride secretion. This is supported by evidence that A-23187, a calcium

ionophore, acted in transferring calcium across peripheral adrenergic neurones by increasing catecholamine output (Ito et al., 1978) and calcium has been shown to stimulate secretory processes in various cells including the intestinal epithelium (Foreman et al., 1973; Prince et al., 1973; Garcia et al., 1975; Frizzell, 1976). It is clear, however, that further studies are needed to define the site of adrenergic agonist action on ion transport.

Morphine did not show any significant effects on chloride exsorption and insorption produced by ST in everted sacs. These results are in contrast to those of Ahrens and Zhu (1982b) who showed that morphine reduced electrolyte secretion in perfused porcine jejunum exposed to ST. They also showed that morphine stimulated net water and electrolyte absorption in normal jejunum. In addition to its effect on intestinal motility, morphine has been shown to have direct effect on ion transport by increasing chloride absorption in *in vitro* rabbit ileal mucosa (McKay et al., 1981). Coupar (1978) and Beubler and Lembeck (1979) reported that morphine inhibited the secretory response to VIP, prostaglandin and carbachol in rat jejunum *in vivo*. Valiulis and Long (1973) demonstrated that oral administration of morphine inhibited spontaneous and cholera toxin-induced secretion in ligated ileal loops of guinea pigs and rabbits. These effects were blocked by naloxone. Dobbins et al. (1980) performed *in vitro* experiments with rabbit ileal mucosa and found that enkephalins increased sodium and chloride absorption. These effects were blocked by naloxone and tetrodotoxin but were not blocked by atropine, phentolamine, haloperidol or pretreatment with 6-hydroxydopamine. They

concluded that enkephalin may stimulate ion transport by altering the release of a nonadrenergic, noncholinergic neurotransmitter. Kachur et al. (1980) found that enkephalins, but not morphine, abolished net chloride secretion in *in vitro* experiments with guinea pig ileum. This suggested that the antisecretory action of endogenous opiates might involve different receptors than those for morphine. However, McKay et al. (1981) reported the presence of specific opiate receptors in rabbit ileal mucosa which could be activated by morphine or an enkephalin analogue and blocked by naloxone. A significant increase in chloride absorption was observed and was due primarily to a decrease in the serosa to mucosa flux. No change in sodium transport was detected. They concluded that opiate receptors exist in rabbit ileal mucosa and that these influence electrical and ion transport changes across mucosa. Furthermore, the electrical response to morphine was not blocked by atropine, propranolol, phentolamine, or haloperidol which suggested that cholinergic, adrenergic, or dopaminergic nerves were not involved in mediating the response (Dobbins et al., 1980). The results of the present study in which morphine failed to alter ST-stimulated chloride fluxes in everted sacs suggest that the opiate inhibition of secretion observed *in vivo* (Ahrens and Zhu, 1982b) may act via modulation of such undefined neurotransmitters and thus requires the presence of functioning enteric neurones. However, a direct action of opiates on mucosal cells cannot be ruled out considering the effects of morphine on rabbit ileal mucosa *in vitro* reported by McKay et al. (1981) and discussed above. It is possible that these differences in opiate activity on jejunal and ileal ion transport *in vitro* may be due to an in-

crease in opiate receptor density on gut epithelial cells from proximal to distal intestinal segments or the masking of opiate action in the presence of a maximal secretory response in our studies. Further studies using agonists and antagonists of opiates in the presence of varying doses of ST are required to clarify the site of opiate action.

Isolated Enterocyte Studies

Most *in vitro* experimental techniques in gastroenterology have focused on the function of the columnar epithelial cells of the small intestinal mucosa as the basis for intestinal absorptive phenomena (Kimmich, 1975). However, transport of substances across intestinal tissue is normally evaluated in tissue preparations with multiple cell types where accumulation and diffusion to underlying cell layers occur (Wilson and Wiseman, 1954; Crane and Wilson, 1958). A more direct evaluation of transport mechanisms has been attempted through the use of isolated epithelial cell preparations (Kimmich, 1970). The mechanical vibration method has been used by several investigators in order to isolate cells in a gradient from villus tips to crypts (Sjostrand, 1968; Harrison and Webster, 1969; Webster and Harrison, 1969). In this study, the vibration method was used to isolate cells from porcine jejunum. Histological examination of the gut at the end of the experiment revealed denudation of the villi with some crypts intact. Thus, some but not all crypt cells were removed in the third fraction. Harrison and Webster (1969) reported that low-power microscopic examination of the mucosal surface of the gut after a 20-min vibration period showed denuded villi

with crypts occupied in the deep clefts. However, after addition of EDTA, vibration of the inflated gut resulted in an explosive release of material. Phase microscopic examination showed these suspended particles to be crypt cells with complete absence of mature epithelial cells.

Scanning and electron microscopic examination of isolated enterocytes was performed by Marsh et al. (1971). They found that the morphology of the cells was well-preserved after incubation for at least one hour. Brush border and lateral basal membranes were intact. Similar findings were reported by Gall et al. (1974). Hoffman and Kuksis (1979) also reported that isolated cells contained well-defined mitochondria, smooth and rough endoplasmic reticulum and microfilaments. Pink et al. (1970) examined cells obtained by washing the human and rat small intestine and found that 80-85% were intestinal absorptive cells. Although the microvilli were fairly well-preserved, the cells showed marked vacuolation indicating severe cell damage. They suggested that these were aged cells cast from the villus tips at the end of their life span. They were destroyed rather rapidly because of high enzyme content of the cell itself and of the intestinal luminal fluid. In the present study, the aged cells were discarded at the beginning of the experiment by initial vibration prior to cell collection.

The epithelial cell begins as an undifferentiated, mitotically active cell at the base of the crypt and differentiates as it moves up the villus to reach full maturity. Enzymic studies have been made at various stages of development (Dahlqvist and Nordström, 1966; Nordström et al., 1967; Imondi et al., 1969; Webster and Harrison, 1969). The methods of isola-

tion of epithelial cells for enzyme assays performed by these investigators varied from horizontal sectioning of frozen gut to mechanical vibration and chemical treatments. The results, however, were the same. For example, a marker enzyme for the mitotically active cell, thymidine kinase, was found mostly in crypt cells. In contrast, alkaline phosphatase and sucrase were found in villus cells where digestive and absorptive functions occurred. In the present study, a descending gradient of sucrase activity was observed from fraction 1 to fraction 3. The highest activity was found in the first fraction of cells isolated indicating the presence of mature villus cells. Sucrase activity was lowest in the third fraction of cells, which indicated that this fraction was composed of immature villus and crypt cells.

Villus and crypt cells have been suggested to have a difference in function with the former serving as absorptive cells while crypt cells are secretory (Trier, 1964; Hendrix and Bayless, 1970). Cholera toxin and cAMP activators have been demonstrated to alter electrolyte transport by inhibiting coupled NaCl exchange across the brush border in villus cells and increasing anion conductance of the crypt luminal membrane (Field, 1980). ST, on the other hand, has been reported to increase guanylate cyclase with subsequent increase in cGMP (Field et al., 1978; Guerrant et al., 1980). Since theophylline, an inhibitor of phosphodiesterase, increased cGMP concentration in the presence as well as in the absence of ST, it was concluded that elevated cGMP produced by ST was due to increased synthesis and not decreased breakdown of the nucleotide. Phosphodiesterase was not affected by ST. Hughes et al. (1978) showed that the

8-bromo analogue of cGMP, which is not metabolized, evoked a secretory response without increasing the mucosal concentration of cAMP. Guerrant et al. (1980) concluded that ST stimulated fluid secretion by activating particular intestinal guanylate cyclase. Quill and Weiser (1975) found villus cells isolated from rat small intestine contained higher guanylate cyclase activity than the crypt cells. In contrast, adenylate cyclase activity was higher in crypts than in the villus cells. The subcellular localization of the cyclases was also evaluated by Quill and Weiser (1975). Guanylate cyclase was found to reside in the brush border membrane while adenylate cyclase in the lateral basal membrane. Field et al. (1978) reported that ST absolished chloride absorption from _in vitro_ rabbit ileal mucosa. Measurement of ^{36}Cl uptake across the brush border membrane indicated that ST and probably cGMP inhibited Na coupled Cl uptake into villus cells (Field, 1979). These data suggest that the mature villus cell should be more sensitive to the effects of ST than immature enterocytes.

In the present study, there was no difference between chloride efflux rate in control villus and crypt cells. ST significantly increased chloride efflux rates in villus and crypt cells to the same degree, indicating that the toxin exerted its effects on both mature and immature intestinal cells. These findings are not consistent with the results of Field et al. (1978) who reported that ST altered electrolyte transport by active Cl absorption, but not secretion, from rabbit ileum. Walling et al. (1978) studied subcellular distribution of nucleotide cyclases in rat intestinal cells. They demonstrated that while adenylate cyclase was

largely confined to the basolateral surface of the epithelial cells, guanylate cyclase was found on the brush-border and basolateral membrane fractions in the ratio 2.4:1. Thus, crypt cells, with a sparse brush border, contain guanylate cyclase in the basolateral membrane which may respond to ST. Schleuter (Iowa State University, Ames, Iowa, personal communication, 1982) found equal amounts of guanylate cyclase activity in the porcine jejunal villus and crypt cells and that ST had the same effect in increasing guanylate cyclase in both cell fractions. The difference in distribution of enzyme might be due to species difference. If ST toxin exerts its secretory effect via guanylate cyclase stimulation, then in porcine species, villus and crypt cells were equally affected because guanylate cyclase is present in both types of cells.

Atropine did not alter chloride efflux rate from control or ST-exposed isolated enterocytes. This was consistent with the results obtained with everted sacs. The secretory effect of cholinergic drugs can be blocked by atropine (Tidball, 1961; Hubel, 1976, 1977; Morris and Turnberg, 1980). Cholinergic drugs exert their secretory effects via increasing guanylate cyclase and cGMP (Brasitus et al., 1976; Tapper et al., 1976). However, short-lived elevations in cAMP after addition of acetylcholine have also been observed (Isaacs et al., 1976). Rimele et al. (1981) reported evidence for muscarinic receptors on rat colonic epithelial cells. Their study revealed one specific binding site in the jejunum, ileum and colon. The receptor density was higher in the colon than in the jejunum which contained the same amount as the ileum. These results suggest that the effects of muscarinic drugs on intestinal epi-

thelial cell function are mediated by interaction with receptors which stimulate activation of guanylate cyclase and secretion. Muscarinic and ST receptor activation may increase chloride secretion by increasing guanylate cyclase, but inhibition of muscarinic receptors by atropine in the present study did not interfere with secretory effects of ST. In addition, pilocarpine did not enhance ST effects in everted gut sac experiments. This supports the idea that ST and muscarinic drugs may have a final common pathway in increasing cGMP and that ST action is not limited by the availability of membrane receptors but rather by an intrinsic limitation in the maximal effect of cGMP.

Clonidine did not have any effect on chloride efflux rate either in control or ST-exposed enterocytes. Previous studies have suggested that antisecretory effects of adrenergic drugs are mediated via alpha-adrenergic receptors (Field and McColl, 1973; Hubel, 1976). Tapper et al. (1981) demonstrated that norepinephrine release induced by tyramine stimulated Na and Cl absorption from rabbit ileum. Phentolamine blocked these effects of tyramine, which indicated an alpha-adrenergic effect of endogenous norepinephrine release. There has been no direct evidence indicating alpha-adrenergic receptors are present in the epithelial cells but noradrenergic fibers extending from the submucosal plexus to the tip of intestinal villi have been demonstrated by Thomas and Templeton (cf. Tapper et al., 1981). This suggested a direct effect of endogenous catecholamine on intestinal ion absorption. However, results from the present study indicated that an alpha-2 adrenergic agonist did not alter basal or ST-stimulated chloride efflux rate from isolated enterocytes. It

is possible, as postulated by Tapper et al. (1981), that norepinephrine may not be the final mediator of Na and Cl absorption. Stimulating effects of norepinephrine on neurotransmitters such as somatostatin or enkephalin have been suggested.

In the present study, morphine reduced chloride efflux rate from ST-exposed mature villus cells isolated from pig jejunum. No effects on ST-exposed immature cells or control enterocytes have been found. The results suggest that ST exerts its effect of increased chloride efflux from all age enterocytes and that morphine diminishes this effect in mature enterocytes. Whether this antagonizing effect of morphine is via opiate receptors needs to be confirmed by using an opiate antagonist--naloxone. However, McKay et al. (1981) reported that morphine significantly increased chloride absorption in _in vitro_ rabbit ileal mucosa primarily by decreasing serosa to mucosa flux. These responses were blocked competitively by naloxone. They suggested the presence of opiate receptors in rabbit ileal mucosa. Other evidence of a direct effect of morphine on the mucosal cells was reported by Collier and Roy (1974) who observed that morphine inhibited prostaglandin E_1-induced cAMP formation in rat intestinal homogenate. Beubler and Lembeck (1979) demonstrated that morphine reduced the basal intestinal fluid volume (IFV) in rat jejunum and inhibited the increased IFV caused by PGE_1, VIP, and carbachol. The inhibitory effect of morphine on PGE_1-stimulated secretion was dose-dependent, inhibited by naloxone, and associated with decreased levels of cAMP (Coupar, 1978; Beubler and Lembeck, 1980). Valiulis and Long (1973) reported that morphine prevented intestinal fluid accumulation caused by cholera

toxin in rabbits and guinea pigs. In addition, morphine inhibited ST effects on ion and water loss in swine jejunum perfused in vivo (Ahrens and Zhu, 1982b). Thus, it has been clearly demonstrated that morphine exerts its antisecretory effect directly on the epithelial cells. This effect is nonspecific since morphine inhibits actions of different secretory agents whose mechanisms of action are not alike. For example, it is clear that adenylate cyclase inhibitory effect of morphine (Beubler and Lembeck, 1980) is not the sole basis for the antisecretory action of morphine since it inhibited the action of ST.

ST did not alter calcium efflux rate from isolated enterocytes. Ouabain did not have any effect either on basal or ST-exposed calcium effluxes. The results suggest that the ST effect in increasing chloride secretion does not involve calcium concentration in the epithelial cells. Calcium ion, however, has been shown to be involved in stimulus-secretion coupling in many epithelial cell types. By using A-23187, a calcium ionophore, the relationships between calcium and secretory processes could be demonstrated. Frizzell (1977) reported that, in the presence of calcium, A-23187 reversed active chloride absorption to secretion in isolated rabbit colon. Bolton and Field (1977) found that secretory actions of carbamylcholine and serotonin in stripped rabbit ileal mucosa depended on extracellular calcium. In those cases, the ionophore did not alter the level of cAMP suggesting the secretory capability of calcium was independent of cAMP. However, the effects of the ionophore in the intestine was smaller in magnitude than those seen with agents which increased the level of cAMP, theophylline for example (Bolton and Field,

1977). A number of secretory cells such as the insect salivary gland, the intestine, the parotid, the cornea and the sweat glands possess receptors for both agonists that gate calcium and for agonists that activate adenylate cyclase (Berridge, 1979). For most instances, though not all, the effects of calcium and cAMP are complementary, as in the stimulation of fluid secretion by fly salivary gland (Prince and Berridge, 1973), activation of renal gluconeogenesis (Kurokawa and Rasmussen, 1973), and stimulation of amylase secretion by exocrine pancreas (Williams and Lee, 1974). While cAMP has been shown to increase calcium from the intracellular site (Christophe et al., 1976; Frizzell, 1976), the ability of acetylcholine to stimulate secretion by intestine (Bolton and Field, 1977) or sweat gland (Sato, 1977) was totally dependent upon external calcium. Nevertheless, VIP, prostaglandin, and theophylline exerted their effects on intestinal secretion independent of calcium (Bolton and Field, 1977; Berridge, 1979). Field et al. (1978) reported that ST when added to rabbit ileal mucosa _in vitro_ evoked a rapid and persistent secretory action which was not quite as large as those produced by addition of theophylline or dibutyryl cAMP. The secretory action of ST, however, was reversible and occurred in the absence of extracellular calcium. The results in this study indicate that the chloride secretory effect of ST is not dependent on calcium.

In the present study isolated enterocytes maintained their metabolic activity during the experimental period of 3 hours. $^{14}CO_2$ liberated from ^{14}C-glucose or ^{14}C-glutamic acid was detected linearly according to time which indicated the viability of the cells throughout the experimental

period. ST did not have any effect on $^{14}CO_2$ production from either ^{14}C-glucose or ^{14}C-glutamic acid. Sugars and amino acids are transported across the small intestine by a two stage process (Hopfer et al., 1976; Weiss et al., 1978; Mircheff et al., 1980; Wright et al., 1980): The first is the entry of substances into the epithelium across the brush border membrane and the second is the efflux of substances out of the cell across the basolateral membrane into blood. In the brush border membrane, the major transport mechanism is sodium cotransport in which sugars and amino acids are transported against their chemical potential gradient through a coupling to the sodium electrochemical potential gradient across the membrane. Kimmich and Randles (1975, 1976) and Randles and Kimmich (1978) found that in the absence of sodium, the sodium cotransport system of glucose was inoperative in isolated chick enterocytes. This is also true for amino acid transport in rat isolated enterocytes (Hopfer et al., 1976). The potential gradient of sodium, however, is maintained by Na-K activated ATPase, an enzyme located on the basolateral membrane (Field, 1980). This enzyme is unaffected by ST toxin (Ahrens and Zhu, 1982a).

Ouabain significantly reduced $^{14}CO_2$ production from ^{14}C-glucose in both control and ST-exposed enterocytes but had no effect on $^{14}CO_2$ production from ^{14}C-glutamic acid. Ouabain, a cardiac glycoside, specifically blocks ATPase on the basolateral membrane of the epithelial cell leading to inhibition of the sodium pump (Lehninger, 1976). Thus, sodium-dependent sugar and amino acid transport into the cell via the brush border membrane are inhibited and in the presence of ouabain, both ^{14}C-

glucose and ^{14}C-glutamic acid transport through the brush border membrane should decrease. However, there is evidence that glucose transport is more sensitive to ATPase inhibition than is amino acid transport. For example, in the absence of sodium, glucose transport was inhibited more than transport of L-valine in brush border preparations (Hopfer et al., 1976). In addition, sodium-independent transport or simple diffusions of amino acids into the cell may occur across the basolateral membranes (Wright et al., 1980). Experiments with isolated basolateral membranes revealed L-valine transport was greater than glucose both in the presence and absence of sodium (Hopfer et al., 1976). The results of the present study are consistent with these observations since isolated enterocytes have exposed basolateral membranes and thus ^{14}C-glutamic acid transport was less affected by ouabain than ^{14}C-glucose. More importantly, the results indicate that ST does not alter the ability of enterocytes to metabolize glucose or glutamic acid either in the presence or absence of ouabain and support the current hypothesis that glucose coupled sodium transport is not impaired by enterotoxin.

SUMMARY

The *in vitro* effects of *E. coli* heat-stable enterotoxin (ST) on isolated jejunum of 3-week-old piglets were studied using everted gut sac and isolated enterocyte techniques. ST increased chloride secretion and chloride absorption in the everted gut sac studies. VIP (2 μg/ml) increased chloride secretion but had no effect on chloride absorption. Neither VIP nor pilocarpine (10^{-5} M) had any additive effect to ST. ST secretory effects were not blocked by atropine (2×10^{-5} M), clonidine (10^{-6} M), or morphine (1.2 μg/ml). Phenylephrine (10^{-5} M), an alpha-1 adrenergic agonist, enhanced ST secretory effects.

Three fractions of cells were isolated from pig jejunum: upper villus, lower villus, and crypt cells. ST increased chloride efflux rates from all three fractions. These effects were not blocked by atropine or clonidine. Morphine significantly reduced chloride efflux rate from mature villus cells with no significant effect on immature epithelial cells. ST did not have any effect on calcium efflux rate from isolated enterocytes.

Metabolic activity studies performed by using ^{14}C-glucose and ^{14}C-glutamic acid as substrates revealed no significant effect of ST on $^{14}CO_2$ production. Ouabain (1 mM) blocked $^{14}CO_2$ production from ^{14}C-glucose but not from ^{14}C-glutamic acid. The results indicated that isolated enterocytes maintained metabolic activity in the presence of enterotoxin.

The results suggest that ST produces loss of fluid and electrolytes primarily by stimulating secretion rather than impairing absorption. This action is exerted directly on both mature villus and immature crypt cells

and is not inhibited by alpha-adrenergic agonists or cholinergic antagonists. Opiate agonists may inhibit secretion, in part, by a direct action on mature enterocytes.

REFERENCES

Ahrens, F. A., and B. Zhu. 1982a. Effects of indomethacin, acetazolamide, ethacrynic sodium, and atropine on intestinal secretion mediated by Escherichia coli heat stable enterotoxin in pig jejunum. Can. J. Physiol. Pharmacol. (In press)

Ahrens, F. A., and B. Zhu. 1982b. Effects of epinephrine, clonidine, L-phenylephrine and morphine on intestinal secretion mediated by Escherichia coli heat stable enterotoxin in pig jejunum. Can. J. Physiol. Pharmacol. (In press)

Alderete, J. F., and D. C. Robertson. 1978. Purification and chemical characterization of the heat-stable enterotoxin produced by porcine strains of enterotoxigenic Escherichia coli. Infect. Immun. 19:1021-1030.

Allen, W. D., and P. Porter. 1970. The demonstration of immunoglobulins in porcine intestinal tissue by immunofluorescence with observations on the effect of fixation. Immunology 18:799-806.

Allen, W. D., C. G. Smith, and P. Porter. 1973. Localization of intracellular immunoglobulin A in porcine intestinal mucosa using enzyme-labelled antibody. Immunology 25:55-70.

Allweiss, B., J. Dostal, K. E. Carey, T. F. Edwards, and R. Freter. 1977. The role of chemotaxis in the ecology of bacterial pathogens of mucosal surfaces. Nature (London) 266:448-450.

Argenzio, R. A., and S. C. Whipp. 1980. Pathophysiology of diarrhea. Pages 220-232 in N. V. Anderson, ed. Veterinary gastroenterology. Lea and Febiger, Philadelphia.

Babcock, D. F., N. L. First, and H. A. Lardy. 1976. Action of ionophore A 23187 at the cellular level. Separation of effects at the plasma and mitochondrial membranes. J. Biol. Chem. 251:3881-3886.

Bamford, D. R. 1966. Studies in vitro of the passage of serum proteins across the intestinal wall of young rats. Proc. Roy. Soc. (London) Series B 166:30-45.

Barbezat, G. O., and M. Grossman. 1971. Intestinal secretion: Stimulation of peptides. Science 174:422-424.

Barrett, E. G. 1974. Ultrastructural and transport properties of isolated intestinal epithelial cells. Ph.D. Thesis. University of Rochester (Libr. Congr. Card No. 75-24, 450). 147 pp. University Microfilms, Ann Arbor.

Barrett, E. G., and J. R. Coleman. 1973. Sodium and potassium content of single cells: Effects of metabolic and structural changes. Page 60 in Proceedings of the 8th National Conference on Electron Probe Analysis, New Orleans.

Bass, P. 1968. In vivo electrical activity of the small bowel. Pages 2051-2074 in C. F. Code, ed. Handbook of physiology. Alimentary canal, Vol. 6. American Physiology Society, Washington, DC.

Bauer, V. 1981. Distribution and types of adrenoceptors in the guinea pig ileum: The action of alpha and beta-adrenoceptor agonists. Br. J. Pharmacol. 72:201-210.

Benet, L. Z., J. M. Orr, R. H. Turner, and H. S. Webb. 1971. Effect of buffer constituents and time on drug transfer across in vitro rat intestine. J. Pharmacol. Sci. 60:234-244.

Benson, J. A., Jr., and A. J. Rampone. 1966. Gastrointestinal absorption. Ann. Rev. Physiol. 28:201-226.

Berridge, M. J. 1979. Relationship between calcium and the cyclic nucleotides in ion secretion. Pages 65-81 in H. J. Binder, ed. Mechanisms of intestinal secretion. Kroc Foundation Series, Vol. 12. Alan R. Liss, Inc., New York.

Berridge, M. J., B. D. Lindley, and W. T. Prince. 1975. Membrane permeability changes during stimulation of isolated salivary glands of Calliphora by 5-hydroxytryptamine. J. Physiol. (London) 244: 549-567.

Bertschinger, H. U., H. W. Moon, and S. C. Whipp. 1972. Association of Escherichia coli with the small intestinal epithelium. I. Comparison of enteropathogenic and nonenteropathogenic porcine strains in pigs. Infect. Immun. 5:595-605.

Beubler, E., and F. Lembeck. 1979. Inhibition of stimulated fluid secretion in the rat small and large intestine by opiate agonists. Naunyn-Schmiedeberg's Arch. Pharmacol. 306:113-118.

Beubler, E., and F. Lembeck. 1980. Inhibition by morphine of prostaglandin E_1-stimulated secretion and cyclic adenosine 3',5'-monophosphate formation in the rat jejunum in vivo. Br. J. Pharmacol. 68:513-518.

Binder, H. J., and C. L. Rawlins. 1973. Electrolyte transport across isolated intestinal mucosa. Am. J. Physiol. 225:1232-1239.

Bolton, J. E., and M. Field. 1977. Ca ionophore-stimulated ion secretion in rabbit ileal mucosa: Relation to actions of cyclic 3',5'-AMP and carbamylcholine. J. Memb. Biol. 35:159-173.

Borle, A. B. 1974. Calcium metabolism at the cellular level. Fed. Proc. 32:1944-1950.

Brendel, K., and E. Meezan. 1974. A simple apparatus for the continuous monitoring of $^{14}CO_2$ production from several small reaction mixtures. Anal. Biochem. 60:88-101.

Brasitus, T. A., M. Field, and D. V. Kimberg. 1976. Intestinal mucosal cyclic GMP: Regulation and relation to ion transport. Am. J. Physiol. 231:275-282.

Brown, A. L., Jr. 1962. Microvilli of the human jejunum epithelial cells. J. Cell. Biol. 12:623-627.

Burgess, M. N., R. J. Bywater, C. M. Cowley, N. A. Mullan, and P. M. Newsome. 1978. Biological evaluation of a methanol-soluble, heat-stable Escherichia coli enterotoxin in infant mice, pigs, rabbits and calves. Infect. Immun. 21:526-531.

Burns, T. W., J. R. Mathias, G. M. Carlson, J. L. Martin, and R. P. Shields. 1978. Effect of toxigenic Escherichia coli on myoelectric activity of small intestine. Am. J. Physiol. 235:E311-E315.

Butcher, F. R. 1975. The role of calcium and cyclic nucleotides in α-amylase release from slices of rat parotid: Studies with the divalent cation ionophore A-23187. Metabolism 24:409-418.

Carpenter, C. C. J. 1980. Clinical and pathophysiologic features of diarrhea caused by Vibrio cholerae and Escherichia coli. Pages 67-83 in M. Field, J. S. Fordtran, and S. G. Schultz, eds. Secretory diarrhea. American Physiological Society, Bethesda, MD.

Carpenter, C. C. J., G. T. Curlin, and W. B. Greenough, III. 1969. Response of canine Thiry-Vella jejunal loops to cholera toxin and its modification by ethacrynic acid. J. Infect. Dis. 120:332-338.

Carpenter, C. C. J., W. B. Greenough, III, and R. C. Gordon. 1974. Pathogenesis and pathophysiology of cholera. Pages 129-141 in D. Barva and W. Burrows, eds. Cholera. W. B. Saunders Company, Philadelphia.

Chien, W., and C. E. Stevens. 1972. Coupled active transport of Na and Cl across forestomach epithelium. Am. J. Physiol. 223:997-1003.

Christophe, J. P., E. K. Frandson, T. P. Conlon, G. Krishna, and J. D. Gardner. 1976. Action of cholecystokinin, cholinergic agents and A-23187 on accumulation of guanosine 3',5'-monophosphate in dispersed guinea pig pancreatic acinar cells. J. Biol. Chem. 251:4640-4645.

Clements, J. D., and R. A. Finkelstein. 1979. Isolation and characterization of homogeneous heat-labile enterotoxins with high specific activity from Escherichia coli cultures. Infect. Immunity 24:760-769.

Cohen, M. L., and A. S. Landry. 1980. Vasoactive intestinal polypeptide: Increased tone, enhancement of acetylcholine release, and stimulation of adenylate cyclase in intestinal smooth muscle. Life Sciences 26: 811-822.

Collier, H. O. J., and A. C. Roy. 1974. Morphine-like drugs inhibit the stimulation by E-prostaglandins of cyclic AMP formation by rat brain homogenate. Nature (London) 248:24-27.

Coupar, I. M. 1978. Inhibition of morphine of prostaglandin-induced fluid secretion in rat jejunum. Br. J. Pharmacol. 63:57-63.

Crane, R. K. 1965. Na^+-dependent transport in the intestine and other animal tissues. Fed. Proc. 24:1000-1006.

Crane, R. K., and T. H. Wilson. 1958. In vitro method for the study of the rate of intestinal absorption of sugars. J. Appl. Physiol. 12: 145-146.

Curran, R. C., and B. Creamer. 1963. Ultrastructural changes in some disorders of the small intestine associated with malabsorption. J. Pathol. Bacteriol. 86:1-8.

Dahlqvist, A. 1968. Assay of intestinal disaccharidases. Anal. Biochem. 22:99-107.

Dahlqvist, A., and C. Nordström. 1966. The distribution of disaccharidase activities in the villi and crypts of the small-intestinal mucosa. Biochim. Biophys. Acta 113:624-626.

Dallas, W. S., and S. Falkow. 1979. The molecular nature of heat-labile enterotoxin CLT7 of Escherichia coli. Nature (London) 277:406-407.

De, S. N., K. Bhattacharza, and J. K. Sarkar. 1956. A study of the pathogenicity of strains of Bacterium coli from acute and chronic enteritis. J. Pathol. Bacteriol. 71:201-209.

Dean, A. G., A. C. Ching, R. G. Williams, and L. B. Harden. 1972. Test for Escherichia coli enterotoxin using infant mice: Application in a study of diarrhea in children in Honolulu. J. Infect. Dis. 125:407-411.

De Jonge, H. R. 1975. The localization of guanylate cyclase in rat small intestinal epithelium. FEBS Lett. 53:237-242.

Diamond, J. M. 1962. The mechanism of solute transport by the gall-
bladder. J. Physiol. (London) 161:474-502.

Diamond, J. M. 1964. Transport of salt and water in rabbit and guinea
pig gallbladder. J. Gen. Physiol. 48:1-14.

Dickens, F., and H. Weil-Malherbe. 1941. Metabolism of normal and tumor
tissue. 19. The metabolism of intestinal mucous membrane. Biochem.
J. 35:7-15.

Dietschy, J. M. 1964. Water and solute movement across the wall of the
everted rabbit gallbladder. Gastroenterology 47:395-408.

Dobbins, J., L. Racusen, and H. J. Binder. 1980. The effect of
enkephalin on ion transport in the rabbit ileum. J. Clin. Invest.
66:19-28.

Donta, S. T., M. King, and K. Sloper. 1973. Induction of steroidogenesis
in tissue culture by cholera enterotoxin. Nature (London) New Biol.
243:246-247.

Drew, G. M. 1978. Pharmacological characterization of the presynaptic
α-adrenoceptors regulating cholinergic activity in the guinea-pig
ileum. Br. J. Pharmacol. 64:293-300.

Elliott, H. L., C. C. J. Carpenter, R. B. Sack, and J. H. Yardley. 1970.
Small bowel morphology in experimental cholera. A light and electron
microscopic study. Lab. Invest. 22:112-120.

Erlandsen, S. L., and D. G. Chase. 1972a. Paneth cell function: Phago-
cytosis and intracellular digestion of intestinal microorganisms. I.
Hexamita muris. J. Ultrastruct. Res. 41:296-318.

Erlandsen, S. L., and D. G. Chase. 1972b. Paneth cell function: Phago-
cytosis and intracellular digestion of intestinal microorganisms.
II. Spiral microorganism. J. Ultrastruct. Res. 41:319-333.

Evans, D. G., D. J. Evans, and N. F. Pierce. 1973. Differences in the
response of rabbit small intestine to heat-labile and heat-stable
enterotoxins of Escherichia coli. Infect. Immun. 7:873-880.

Evans, D. G., D. J. Evans, Jr., W. S. Tjoa, and H. L. Dupont. 1978. De-
tection and characterization of colinization factor of enterotoxi-
genic Escherichia coli isolated from adults with diarrhea. Infect.
Immun. 19:727-736.

Farquhar, M. G., and G. E. Palade. 1963. Junctional complexes in various
epithelia. J. Cell. Biol. 17:375-412.

Field, M. 1971. Intestinal secretion: Effect of cyclic AMP and its role in cholera. New Engl. J. Med. 284:1137-1140.

Field, M. 1974. Intestinal secretion. Gastroenterology 66:1063-1084.

Field, M. 1979. Modes of action of enterotoxins from Vibrio cholera and Escherichia coli. Rev. Infect. Dis. 1:918-925.

Field, M. 1980. Regulation of intestinal ion transport by cyclic nucleotides and calcium. Pages 21-30 in M. Field, J. S. Fordtran, and S. G. Schultz, eds. Secretory diarrhea. American Physiological Society, Bethesda, MD.

Field, M., and I. McColl. 1973. Ion transport in rabbit ileal mucosa. III. Effects of catecholamines. Am. J. Physiol. 225:852-857.

Field, M., G. R. Plotkin, and W. Silen. 1968. Effects of vasopressin, theophylline and cyclic adenosine monophosphate on short-circuit current across isolated rabbit ileal mucosa. Nature (London) 217: 469-471.

Field, M., D. Fromm, and I. McColl. 1971. Ion transport in rabbit ileal mucosa. I. Na and Cl fluxes and short-circuit current. Am. J. Physiol. 220:1388-1396.

Field, M., D. Fromm. Q. Al-Awgati, and W. B. Greenough. 1972. Effects of cholera enterotoxin on ion transport across isolated ileal mucosa. J. Clin. Invest. 51:796-804.

Field, M., H. E. Sheerin, A. Henderson, and P. L. Smith. 1975. Catecholamine effects on cyclic AMP levels and ion secretion in rabbit ileal mucosa. Am. J. Physiol. 229:86-92.

Field, M., L. H. Graf, W. J. Laird, and P. L. Smith. 1978. Heat stable enterotoxin of Escherichia coli: In vitro effects of guanylate cyclase activity, cyclic GMP concentration, and ion transport in small intestine. Proc. Nat. Acad. Sci. USA 75:2800-2804.

Finkelstein, R. A., and J. D. Clements. 1979. Preparation and characterization of highly purified LT from Escherichia coli. Symposium on Animal, Plant and Bacterial Toxins, Uppsala. (Abstr. Suppl.)

Fishman, P., and R. Brady. 1976. Biosynthesis and junction of gangliosides. Science 194:906-915.

Florey, H. W., R. D. Wright, and M. A. Jennings. 1941. The secretion of the intestine. Physiol. Rev. 21:36-69.

Foreman, J. C., J. L. Mongar, and B. D. Gomperts. 1973. Calcium ionophore and movement of calcium ions following the physiological stimulus to a secretory process. Nature (London) 245:249-251.

Forssmann, W. G., L. Orci, R. Pictet, A. E. Reynold, and C. Rouiller. 1969. The endocrine cells in the epithelium of the gastrointestinal mucosa of the rat. J. Cell. Biol. 40:692-715.

Fortin-Magana, R., R. Hurwitz, J. J. Herbst, and N. Kretchmer. 1970. Intestinal enzymes: Indicators of proliferation and differentiation in the jejunum. Science 167:1627-1628.

Friedman, H. I., and R. R. Cardell, Jr. 1972. Effects of puromycin on the structure of rat intestinal epithelial cells during fat absorption. J. Cell. Biol. 52:15-40.

Frizzell, R. A. 1976. Ion transport by rabbit colon: Effects of cyclic AMP and calcium ionophore. Physiologist 19:200. (Abstr.)

Frizzell, R. A. 1977. Active chloride secretion by rabbit colon: Calcium dependent stimulation by ionophore A23187. J. Memb. Biol. 35:175-187.

Frizzell, R. A., M. Dugas, and S. G. Schultz. 1975. Sodium chloride transport by rabbit gallbladder: Direct evidence for a coupled NaCl influx process. J. Gen. Physiol. 65:769-795.

Frizzell, R. A., M. J. Koch, and S. G. Schultz, 1976. Ion transport by rabbit colon. I. Active and passive components. J. Memb. Biol. 27: 297-316.

Frizzell, R. A., M. Field, and S. G. Schultz. 1979a. Sodium-coupled chloride transport by epithelial tissue. Am. J. Physiol. 236:F1-F8.

Frizzell, R. A., P. L. Smith, E. Vosbuogh, and M. Field. 1979b. Coupled sodium-chloride influx across border of flounder intestine. J. Memb. Biol. 46:27-39.

Frizzell, R. A., K. Heintze, and C. P. Stewart. 1980. Mechanism of intestinal chloride secretion. Pages 11-19 in M. Field, J. S. Fordtran, and S. G. Schultz, eds. Secretory diarrhea. American Physiological Society, Bethesda, MD.

Furness, J. B., and M. Costa. 1974. The adrenergic innvervation of the gastrointestinal tract. Rev. Physiol. 69:1-51.

Gall, D. G., D. G. Butler, F. Tepperman, and R. Hamilton. 1974. Sodium ion transported in isolated intestinal epithelial cells: The effect of actively transported sugars on sodium ion efflux. Biochim. Biophys. Acta 339:291-302.

Garcia, A. G., S. M. Kirpeker, and J. C. Prat. 1975. A calcium ionophore stimulating the secretion of catecholamines from the cat adrenal. J. Physiol. (London) 244:253-262.

Gardner, J. D. 1979. Regulation of pancreatic exocrine function in vitro: Initial steps in the actions of secretogogues. Ann. Rev. Physiol. 41:55-66.

Gibaldi, M., and B. Grundhofer. 1972. Drug transport VI: Functional integrity of the rat everted small intestine with respect to passive transfer. J. Pharm. Sci. 61:116-119.

Guandalini, S., and M. Field. 1979. In vitro effects of heat stable Escherichia coli enterotoxin (ST) on intestinal ion transport. Gastroenterology 76:1146. (Abstr.)

Guentzel, M. N., L. H. Field, E. R. Eubanks, and L. J. Berry. 1977. Use of fluorescent antibody in studies of immunity to cholera in infant mice. Infect. Immun. 15:539-548.

Guerrant, R. L., J. M. Hughes, B. Chang, D. C. Robertson, and F. Murad. 1980. Activation of intestinal guanylate cyclase by heat-stable enterotoxin of Escherichia coli: Studies of tissue specificity, potential receptors, and intermediates. J. Infect. Dis. 142:220-228.

Gyles, C. L. 1979. Limitations of the infant mouse test for Escherichia coli heat-stable enterotoxin. Can. J. Comp. Med. 43:371-379.

Hamilton, D. L., M. R. Johnson, G. W. Forsyth, W. E. Roe, and N. O. Nielsen. 1978. The effect of cholera toxin and heat labile and heat stable Escherichia coli enterotoxin on cyclic AMP concentrations in small intestinal mucosa of pig and rabbit. Can. J. Comp. Med. 42: 327-331.

Hardcastle, P. T., and J. Eggenton. 1973. The effect of acetylcholine on the electrical activity of intestinal epithelial cells. Biochim. Biophys. Acta 298:95-100.

Harrison, D. D., and H. L. Webster. 1964. An improved method for the isolation of brush borders from the rat intestine. Biochim. Biophys. Acta 93:662-664.

Harrison, D. D., and H. L. Webster. 1969. The preparation of isolated intestinal crypt cells. Exp. Cell. Res. 55:257-260.

Hendrix, T. R., and T. M. Bayless. 1970. Digestion: Intestinal secretion. Ann. Rev. Physiol. 32:139-164.

Herbst, J. J., R. Fortin-Magana, and P. Sunshine. 1970. Relationship of pyrimidine biosynthetic enzymes to cellular proliferation in rat intestine during development. Gastroenterology 59:240-246.

Hoffman, A. G. D., and A. Kuksis. 1979. Improved isolation of villus and crypt cells from rat small intestinal mucosa. Can. J. Physiol. Pharmacol. 57:832-842.

Hoffman, J. F. 1962. The active transport of sodium by ghosts of human red blood cells. J. Gen. Physiol. 45:837-859.

Hopfer, U., K. Sigrist-Nelson, E. Ammann, and H. Murer. 1976. Differences in neutral amino acid and glucose transport between brush border and basolateral membrane of intestinal epithelial cells. J. Cell. Physiol. 89:805-810.

Hubel, K. A. 1976. Intestinal ion transport: Effect of norepinephrine, pilocarpine, and atropine. Am. J. Physiol. 231:252-257.

Hubel, K. A. 1977. Effects of bethanechol on intestinal ion transport in the rat. Proc. Soc. Exp. Biol. Med. 154:41-44.

Hughes, J. M., F. Murad, B. Chang, and R. L. Guerrant. 1978. Role of cyclic GMP in the action of heat-stable enterotoxin of *Escherichia coli*. Nature (London) 271:755-756.

Hull, B. E., and L. A. Staehelin. 1976. Functional significance of the variations in the geometrical organization of tight junction networks. J. Cell. Biol. 68:688-704.

Imondi, A. R., M. E. Balis, and M. Lipkin. 1969. Changes in enzyme levels accompanying differentiation of intestinal epithelial cells. Exp. Cell. Res. 58:323-330.

Isaacs, P. E. T., C. L. Corbett, A. K. Riley, P. C. Hawker, and C. A. Turnberg. 1976. *In vitro* behavior of human intestinal mucosa. The influence of acetylcholine on ion transport. J. Clin. Invest. 58: 535-542.

Ito, S. 1969. Structure and function of the glycocalyx. Fed. Proc. 28: 12-25.

Ito, S., and J. P. Revel. 1964. Incorporation of radioactive sulfate and glucose on the surface coat of enteric microvilli. J. Cell. Biol. 23:44a.

Ito, S., Y. Nakazato, and A. Ohga. 1978. The effect of the ionophores X-537A and A-23187 on the noradrenaline output from peripheral adrenergic neurones in the presence of various divalent cations. Br. J. Pharmacol. 62:91-98.

Johnson, C. F. 1969. Hamster intestinal brush-border surface particles and their function. Fed. Proc. 28:26-29.

Johnson, F. R., and J. H. Kugler. 1953. The distribution of alkaline phosphatase in the mucosal cells of the small intestine of the rat, cat and dog. J. Anat. 87:247-256.

Jones, G. W., and J. M. Rutter. 1972. Role of the K88 antigen in the pathogenesis of neonatal diarrhea caused by Escherichia coli piglets. Infect. Immun. 6:918-927.

Kachur, J. F., R. J. Miller, and M. Field. 1980. Control of guinea pig intestinal electrolyte secretion by a δ-opiate receptor. Proc. Nat. Acad. Sci. 77:2753-2756.

Kantor, H. S. 1975. Enterotoxins of Escherichia coli and Vibrio cholerae: Tools for the molecular biologist. J. Infect. Dis. 131:S22-32.

Kapitany, R. A., A. Scoot, G. W. Forsyth, S. F. McKenzie, R. W. Worthington. 1979. Evidence for two heat-stable enterotoxins produced by enterotoxigenic Escherichia coli. Infect. Immun. 24:965-966.

Kenworthy, R., and W. D. Allen. 1966. The significance of Escherichia coli to the young pig. J. Comp. Pathol. 76:31-44.

Kenworthy, R., J. M. Stubbs, and G. Syme. 1967. Ultrastructure of small-intestinal epithelium in weaned and unweaned pigs and pigs with post-weaning diarrhea. J. Pathol. Bacteriol. 93:493-498.

Kimberg, D. V., M. Field, J. Johnson, A. Henderson, and E. Gershon. 1971. Stimulation of intestinal mucosal adenyl cyclase by cholera enterotoxin and prostaglandin. J. Clin. Invest. 50:1218-1230.

Kimmich, G. A. 1970. Preparation and properties of mucosal epithelial cells isolated from small intestine of the chicken. Biochemistry 9: 3659-3668.

Kimmich, G. A. 1975. Preparation and characterization of isolated intestinal epithelial cells and their use in studying intestinal transport. Pages 51-115 in E. D. Korn, ed. Methods in membrane biology, Vol. 5. Transport. Plenum Press, New York.

Kimmich, G. A., and J. Randles. 1975. A Na^+-independent, phloretin-sensitive monosaccharide transport system in isolated intestinal epithelial cells. J. Memb. Biol. 23:57-76.

Kimmich, G. A., and J. Randles. 1976. 2-deoxyglucose transport by intestinal epithelial cells isolated from the chick. J. Memb. Biol. 27:363-379.

Klipstein, F. A., C. S. Lee, and R. F. Engert. 1976. Assay of Escherichia coli enterotoxins by in vivo perfusion in the rat jejunum. Infect. Immun. 14:1004-1010.

Klyce, S. D., and R. K. S. Wong. 1977. Site and mode of adrenaline action on chloride transport across the rabbit corneal epithelium. J. Physiol. (London) 266:777-799.

Kohler, E. M. 1968. Enterotoxic activity of filtrates of Escherichia coli in young pigs. Am. J. Vet. Res. 29:2263-2274.

Kosterlitz, H. W., R. J. Lydon, and A. J. Watt. 1970. The effect of adrenaline, noradrenaline and isoprenaline on inhibitory α- and β-adrenoceptors in the longitudinal muscle of the guinea-pig ileum. Br. J. Pharmacol. 39:398-413.

Krejs, G. J., R. M. Barkley, N. W. Reed, and J. S. Fordtran. 1978. Intestinal secretion induced by vasoactive intestinal polypeptide. A comparison with cholera toxin in the canine jejunum in vivo. J. Clin. Invest. 61:1337-1345.

Kunkel, S. L., and D. C. Robertson. 1979. Purification and chemical characterization of the heat-labile enterotoxin produced by enterotoxigenic Escherichia coli. Infect. Immun. 25:586-596.

Kurokawa, K., and H. Rasmussen. 1973. Ionic control of renal gluconeogenesis. IV. Effect of extracellular phosphate concentration. Biochim. Biophys. Acta 313:59-71.

Lallier, R., S. Lariviere, and S. St-Pierre. 1980. Escherichia coli heat-stable enterotoxin: Rapid method of purification and some characteristics of the toxin. Infect. Immun. 28:469-474.

Lathe, R., P. Hirth, M. Dewilde, N. Harford, and J. P. Lecoco. 1980. Cell-free synthesis of enterotoxin of Escherichia coli from a cloned gene. Nature 284:473-474.

Leblond, C. P., and P. Messier. 1958. Renewal of chief cells and goblet cells in the small intestine as shown by radioautography after injection of thymidine-H^3 into mice. Anat. Rec. 132:247-260.

Lee, J. S. 1963. Role of mesenteric lymphatic system in water absorption from rat intestine in vitro. Am. J. Physiol. 204:92-96.

Lee, M. K., and I. M. Coupar. 1980. Opiate receptor-mediated inhibition of rat jejunal fluid secretion. Life Sci. 27:2319-2325.

Lehninger, A. L. 1976. Active transport across membranes. Pages 779-805 in Biochemistry: The molecular basis of cell structure and function. 2d ed. Worth Publishers, Inc., New York.

Levine, R. R., W. F. McNary, P. J. Kornguth, and R. LeBlanc. 1970. Histological reevaluation of everted gut technique for studying intestinal absorption. European J. Pharmacol. 9:211-219.

Levitan, R., and D. E. Wilson. 1974. Absorption of water soluble substances. Pages 293-342 in E. D. Jacobson and L. L. Shanbour, eds. Physiology Series One, Vol. 4. Gastrointestinal physiology. University Park Press, Baltimore.

Lipkin, M., P. Sherlock, and B. Bell. 1963. Cell proliferation kinetics in the gastrointestinal tract of man. II. Cell renewal in stomach, ileum, colon and rectum. Gastroenterology 45:721-729.

Loeschke, K., C. J. Bentzel, and T. Z. Csáky. 1970. Asymmetry of osmotic flow in frog intestine: Functional and structural correlation. Am. J. Physiol. 218:1723-1731.

Lonnroth, I., and J. Holmgren. 1973. Subunit structure of cholera toxin. J. Gen. Microbiol. 76:417-427.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and J. R. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

MacDonald, W. C., J. S. Trier, and N. B. Everett. 1964. Cell proliferation and migration in the stomach, duodenum, and rectum of man: Radioautographic studies. Gastroenterology 46:405-417.

Mailman, D. 1978. Effects of vasoactive intestinal polypeptide on intestinal absorption and blood flow. J. Physiol. 279:121-132.

Marsh, M. N., T. J. Peters, and A. C. Brown. 1971. Observations of isolated enterocytes and of their subcellular components using transmission and scanning electron microscopy. Gut 12:499-508.

McDougal, D. B., Jr., L. D. Little, and R. K. Crane. 1960. Studies on the mechanism of intestinal absorption of sugars. IV. Localization of galactose concentrations within the intestinal wall during active transport, in vitro. Biochim. Biophys. Acta 45:483-489.

McKay, J. S., B. D. Linaker, and L. A. Turnberg. 1981. The influence of opiates on ion transport across rabbit ileal mucosa. Gastroenterology 80:279-284.

Miller, D., and R. K. Crane. 1961. A procedure for the isolation of the epithelial brush border membrane of hamster small intestine. Anal. Biochem. 2:284-286.

Mircheff, A. K., C. H. van Os, and E. M. Wright. 1980. Pathway for alanine transport in intestinal basal lateral membrane vesicles. J. Memb. Biol. 52:83-92.

Moon, H. W. 1974. Pathogenesis of enteric diseases caused by Escherichia coli. Adv. Vet. Sci. Comp. Med. 18:179-211.

Moon, H. W. 1976. Intestine. Pages 380-407 in N. F. Cheville, ed. Cell pathology. Iowa State University Press, Ames.

Moon, H. W. 1978. Mechanism in the pathogenesis of diarrhea. J.A.V.M.A. 17:443-448.

Moon, H. W., and S. C. Whipp. 1970. Development of resistance with age by swine intestine to effects of enteropathogenic Escherichia coli. J. Infect. Dis. 122:220-223.

Moon, H. W., and S. C. Whipp. 1971. Systems for testing the enteropathogenicity of Escherichia coli. Ann. N.Y. Acad. Sci. 176:197-211.

Moon, H. W., S. C. Whipp, G. W. Engstorm, and A. L. Baetz. 1970. Response of the rabbit ileal loop to cell free products from Escherichia coli enteropathogenic for swine. J. Infect. Dis. 121: 182-187.

Moon, H. W., S. C. Whipp, and A. L. Baetz. 1971. Comparative effects of enterotoxins from Escherichia coli and Vibrio cholerae on rabbit and swine small intestine. Lab. Invest. 25:133-140.

Moon, H. W., P. Y. Fung, S. C. Whipp, and R. E. Isaacson. 1978. Effects of age and ambient temperature on the response of infant mice to heat-stable enterotoxin of Escherichia coli: Assay modification. Infect. Immun. 20:36-39.

Morris, A. I., and L. A. Turnberg. 1980. The influence of a parasympathetic agonist and antagonist on human intestinal transport in vivo. Gastroenterology 79:861-866.

Moss, J., and S. H. Richardson. 1978. Activation of adenylate cyclase by heat-labile Escherichia coli enterotoxin. J. Clin. Invest. 21: 281-288.

Munday, K. A., B. J. Parsons, J. A. Poat, and M. E. Upsher. 1980. Control of intestinal fluid transport by endogenous noradrenaline. J. Physiol. (London) 307:73P-74P.

Nalin, D. R., A. K. Bhattacharjee, and S. H. Richardson. 1974. Cholera-like toxic effect of culture filtrates of an Escherichia coli. J. Infect. Dis. 130:595-601.

Nayak, R. K., and L. Z. Benet. 1971. Drug transfer across rat intestinal musculature after edetic acid treatment. J. Pharm. Sci. 60:1508-1511.

Nellans, H. N., R. A. Frizzell, and S. G. Schultz. 1973. Coupled sodium-chloride influx across the brush border of rabbit ileum. Am. J. Physiol. 225:467-475.

Newsome, P. M., M. N. Burgess, and N. A. Mullan. 1978. Effect of Escherichia coli heat-stable enterotoxin on cyclic GMP levels in mouse intestine. Infect. Immun. 22:290-291.

Nordström, C., A. Dalqvist, and L. Josefsson. 1967. Quantitative determination of enzymes in different parts of the villi and crypts of rat small intestine. Comparison of alkaline phosphatase, disaccharidases and dipeptidases. J. Histochem. Cytochem. 15:713-721.

Oates, J. A., K. Melmon, A. Sjoerdsma, L. Gillespie, and D. Mason. 1964. Release of kinin peptide in the carcinoid syndrome. Lancet 1:514-517.

O'Hanley, P. D., and J. R. Cantey. 1978. Surface structures of Escherichia coli that produce diarrhea by a variety of enteropathic mechanisms. Infect. Immun. 21:874-878.

Ohtomo, N., T. Muracka, A. Tashiro, Y. Zinnaka, and K. Amako. 1976. Size and structure of the cholera toxin molecule and its subunits. J. Infect. Dis. Suppl. 133:S31-S40.

Palay, S. L., and L. J. Karlin. 1959. An electron microscopic study of the intestinal villus. I. The fasting animal. J. Biophys. Biochem. Cytol. 5:363-372.

Parsons, D. S., and C. R. Paterson. 1960. Movements of fluid and glucose in an everted sac preparation of rat colonic mucosa. Biochim. Biophys. Acta 41:173-175.

Paton, W. D. M., and E. S. Vizi. 1969. The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. Br. J. Pharmacol. 35:10-28.

Pesti, L., and H. A. Gordon. 1978. Effects and bioassay of Escherichia coli enterotoxin (heat-stable fraction) on smooth muscle. Vet. Microbiol. 2:313-324.

Phillips, H. J. 1973. Dye exclusion tests for cell viability. Pages 406-408 in P. F. Kruse and M. K. Patterson, eds. Tissue cultures. Methods and applications. Academic Press, New York.

Pierce, N. F., J. R. Graybill, M. M. Kaplan, and D. L. Bouwman. 1972. Systemic effects of parenteral enterotoxin in dogs. J. Lab. Clin. Med. 79:145-156.

Pink, I. J., D. N. Croft, and B. Creamer. 1970. Cell loss from small intestinal mucosa: A morphological study. Gut 11:217-222.

Powell, D. W. 1981. Muscle or mucosa: The site of action of anti-diarrheal opiates. Gastroenterology 50:406-407.

Powell, D. W., and S. J. Malawer. 1968. Relationship between water and solute transport from isosmotic solutions by rat intestine in vivo. Am. J. Physiol. 215:49-55.

Powell, D. W., and E. J. Tapper. 1979. Intestinal ion transport: Cholinergic-adrenergic interactions. Pages 175-192 in H. J. Binder, ed. Mechanisms of intestinal secretion. Kroc Foundation Series, Vol. 12. Alan R. Liss, Inc., New York.

Prince, W. T., and M. J. Berridge. 1973. The role of calcium in the action of 5-hydroxytryptamine and cyclic AMP on salivary glands. J. Exp. Biol. 58:367-384.

Prince, W. T., M. J. Berridge, and H. Rasmussen. 1972. Role of calcium and adenosine 3',5'-cyclic monophosphate in controlling fly salivary gland secretion. Proc. Nat. Acad. Sci. USA 69:533-534.

Prince, W. T., H. Rasmussen, and M. J. Berridge. 1973. The role of calcium in fly salivary gland secretion analyzed with the ionophore A-23187. Biochim. Biophys. Acta 329:98-107.

Quastler, H., and F. G. Sherman. 1959. Cell population kinetics in the intestinal epithelium of the mouse. Exp. Cell Res. 17:420-438.

Quill, J., and M. M. Weiser. 1975. Adenylate and guanylate cyclase activities and cellular differentiation in the small intestine. Gastroenterology 69:470-478.

Racusen, L. C., and H. J. Binder. 1979. Adrenergic interaction with ion transport across colonic mucosa: Role of both alpha and beta adrenergic agonists. Pages 201-215 in H. J. Binder, ed. Mechanisms of intestinal secretion. Kroc Foundation Series, Vol. 12. Alan R. Liss, Inc., New York.

Racusen, L. C., H. J. Binder, and J. W. Dobbins. 1978. Effect of exogenous and endogenous opiate compounds on ion transport in rabbit ileum in vitro. Gastroenterology 74:1081. (Abstr.)

Randles, J., and G. A. Kimmich. 1978. Effects of phloretin and theophylline on 3-O-methylglucose transport by intestinal epithelial cells. Am. J. Physiol. 234:C64-C72.

Rao, M. C., S. Guandalini, P. L. Smith, and M. Field. 1979. Heat-stable Escherichia coli enterotoxin effects on guanylate cyclase. Tissue and subcellular specificity and relation to ion transport. Clin. Res. 27:456A.

Rašková, H., and K. Raška. 1980. Enterotoxins from gram-negative bacteria relevant for veterinary medicine. Vet. Res. Comm. 4:195-224.

Reiser, S., and P. A. Christensen. 1971. The properties of the preferential uptake of L-leucine by isolated intestinal epithelial cells. Biochim. Biophys. Acta 225:123-139.

Richards, K. L., and S. D. Douglas. 1978. Pathophysiological effects of Vibrio cholera and enterotoxigenic Escherichia coli and their exotoxins on eucaryotic cells. Microbiol. Rev. 42:592-613.

Rimele, T. J., M. S. O'Dorisio, and T. S. Gaginella. 1981. Evidence for muscarinic receptors on rat colonic epithelial cells: Binding of [^{3}H]quinuclidinyl benzilate. J. Pharmacol. Exp. Ther. 218:426-434.

Robinson, J. W. L., and J. P. Felber. 1966. The effect of tissue aging on intestinal uptake of amino-acids in vitro. Gastroenterologia 105: 17-26.

Roggin, G. M., J. G. Banwell, J. H. Yardley, and T. H. Hendrix. 1972. Unimpaired response of rabbit jejunum to cholera toxin after selective damage to villus epithelium. Gastroenterology 63:981-989.

Rubin, C. E. 1966. Electron microscopic studies of triglyceride absorption in man. Gastroenterology 50:65-77.

Rubin, W., A. S. Fauci, M. H. Sleisenger, and G. H. Jeffries. 1965. Immunofluorescent studies in adult celiae disease. J. Clin. Invest. 44:475-485.

Sabesin, S. M. 1980. Structure of the gastrointestinal tract as it relates to nutrient movement. Pages 3-27 in M. Wimick, ed. Nutrition and gastroenterology. Wiley-Interscience Publication, New York.

Sack, R. B. 1980. Enterotoxigenic Escherichia coli: Identification and characterization. J. Infect. Dis. 142:279-286.

Sack, R. B., I. L. Gorbach, J. B. Banwell, B. Jacobs, B. D. Chatterjee, and R. C. Mitra. 1971. Enterotoxigenic Escherichia coli isolated from patients with severe cholera-like disease. J. Infect. Dis. 123: 378-385.

Said, S. I., and V. Mutt. 1972. Isolation from porcine-intestinal wall of a vasoactive octacosapeptide related to secretion and to glucagon. European J. Biochem. 28:199-204.

Sato, K. 1977. The physiology, pharmacology, and biochemistry of the eccrine sweat gland. Rev. Physiol. Biochem. Pharmacol. 79:51-131.

Schrank, G. D., C. E. Stager, and W. F. Verway. 1973. Differential medium for Vibrio cholerae. Infect. Immun. 7:827-829.

Schultz, S. G. 1977. Sodium-coupled solute transport by small intestine: A status report. Am. J. Physiol. 233:E249-E254.

Schultz, S. G. 1978. Is a coupled Na-K exchange pump involved in active transepithelial Na transport? A status report. Pages 213-227 in J. F. Hoffman, ed. Membrane transport processes. Raven, New York.

Schultz, S. G. 1980. Cellular models of sodium and chloride absorption from mammalian small and large intestine. Pages 1-9 in M. Field, J. S. Fordtran, and S. G. Schultz, eds. Secretory diarrhea. American Physiological Society, Bethesda, MD.

Schultz, S. G., and P. F. Curran. 1970. Coupled transport of sodium and organic solutes. Physiol. Rev. 50:637-718.

Schultz, S. G., R. E. Fuisz, and P. F. Curran. 1966. Amino acid and sugar transport in rabbit ileum. J. Gen. Physiol. 49:849-866.

Schwartz, C. J., D. V. Kimberg, H. E. Sheerin, M. Field, and S. I. Said. 1974. Vasoactive intestinal peptide stimulation of adenylate cyclase and active electrolyte secretion in intestinal mucosa. J. Clin. Invest. 54:536-544.

Scoot, A., G. W. Forsyth, R. A. Kapitany, W. E. Roe, and D. L. Hamilton. 1980. Effects of isolated heat-stable enterotoxin produced by Escherichia coli on fluid secretion and cyclic nucleotide levels in the jejunum of the weanling pig. Can. J. Physiol. Pharmacol. 58: 772-777.

Sharp, G. W. G. 1973. Action of cholera toxin on fluid and electrolyte movement in the small intestine. Ann. Rev. Med. 24:19-28.

Shorter, R. G., C. G. Moertal, J. L. Titus, and R. J. Reitemeier. 1964. Cell kinetics in the jejunum and rectum of man. Am. J. Dig. Dis. 9:760-768.

Sjostrand, F. S. 1968. A simple and rapid method to prepare dispersions of columnar epithelial cells from the rat intestine. J. Ultrastruct. Res. 22:424-442.

Smith, H. W., and S. Halls, 1967a. Observations by the ligated segment and oral inoculation methods on Escherichia coli infections in pigs, calves, lambs and rabbits. J. Pathol. Bacteriol. 93:499-531.

Smith, H. W., and S. Halls. 1967b. Studies on Escherichia coli enterotoxin. J. Pathol. Bacteriol. 93:531-543.

Smith, H. W., and S. Halls. 1968a. The production of edema disease and diarrhea in weaned pigs by the oral administration of Escherichia coli: Factors that influence the course of the experimental disease. J. Med. Microbiol. 1:45-59.

Smith, H. W., and S. Halls. 1968b. The transmissible nature of the genetic factor in Escherichia coli that controls enterotoxin production. J. Gen. Microbiol. 52:319-334.

Smith, H. W., and M. A. Linggood. 1971. Observations on the pathogenic properties of the K88, Hly and Ent plasmids of Escherichia coli with particular reference to porcine diarrhea. J. Med. Microbiol. 4:467-485.

Smith, H. W., and M. A. Linggood. 1972. Further observations on Escherichia coli enterotoxins with particular regard to those produced by atypical piglet strains and by calf and lamb strains: The transmissible nature of these enterotoxins and of a K antigen possessed by calf and lamb strains. J. Med. Microbiol. 5:243-250.

Smith, N. W., and R. B. Sack. 1973. Immunologic cross-reactions of enterotoxins from Escherichia coli and Vibrio cholerae. J. Infect. Dis. 127:164-170.

Smith, R. J., and L. J. Ignarro. 1975. Bioregulation of lysomal enzyme secretion from human neutrophils: Role of guanosine 3':5'-cyclic monophosphate and calcium in stimulus-secretion coupling. Proc. Nat. Acad. Sci. USA 72:108-112.

Staley, M., and Trier, J. S. 1965. Morphologic heterogeneity of mouse Paneth cell granules before and after secretory stimulation. Am. J. Anat. 117:365-384.

Staley, T. E., E. Wynn Jones, and L. D. Corley. 1969. Attachment and penetration of Escherichia coli into intestinal epithelium of the ileum in newborn pigs. Am. J. Pathol. 56:371-392.

Stern, B. K. 1966. Some biochemical properties of suspensions of intestinal epithelial cells. Gastroenterology 51:855-867.

Stern, B. K., and R. W. Reilly. 1965. Some characteristics of the respiratory metabolism of suspension of rat intestinal epithelial cells. Nature 205:563-565.

Takeda, Y., T. Takeda, T. Yano, K. Yamamoto, and T. Miwatani. 1979. Purification and partial characterization of heat-stable enterotoxin of enterotoxigenic Escherichia coli. Infect. Immun. 25:978-985.

Tapper, E. J., D. W. Powell, and S. M. Morris. 1976. Effect of high dose carbachol on intestinal electrolyte transport. Gastroenterology 70: 942. (Abstr.)

Tapper, E. J., D. W. Powell, and S. M. Morris. 1978. Cholinergic-adrenergic interactions on intestinal ion transport. Am. J. Physiol. 235:E402-E409.

Tapper, E. J., A. S. Bloom, and D. L. Lewand. 1981. Endogenous nor-epinephrine release induced by tyramine modulated intestinal ion transport. Am. J. Physiol. 241:G264-G269.

Taraszka, M. J. 1971. Transfer of clindamycin and 1'-demethyl-4'-dephosphyl-4'-pentylclindamycin by the cannulated-everted rat gut. J. Pharm. Sci. 60:946-948.

Tidball, C. S. 1961. Active chloride transport during intestinal secretion. Am. J. Physiol. 200:309-312.

Tomasi, T. B., and J. Bienenstock. 1968. Secretory immunoglobulins. Pages 1-96 in F. J. Dixon, Jr. and H. G. Kunkel, eds. Advances in immunology, Vol. 9. Academic Press, New York.

Tomasini, J. T., and W. O. Dobbins. 1970. Intestinal mucosal morphology during water and electrolyte absorption: A light and electron microscopic study. Am. J. Dig. Dis. 15:226-238.

Trier, J. S. 1964. Studies on small intestinal crypt epithelium. II. Evidence for and mechanisms of secretory activity by undifferentiated crypt cells of the human small intestine. Gastroenterology 47:480-495.

Trier, J. S. 1968. Morphology of the epithelium of the small intestine. Pages 1125-1176 in C. F. Charles, ed. Handbook of physiology, Vol. III. American Physiological Society, Washington, DC.

Troughton, W. D., and J. S. Trier. 1969. Paneth and goblet cell renewal in mouse duodenal crypts. J. Cell Biol. 41:251-268.

Turnberg, L. A., F. A. Bieberdorf, S. G. Morawski, and J. S. Fordtran. 1970. Interrelationships of chloride, bicarbonate, sodium and hydrogen transport in the human ileum. J. Clin. Invest. 49:557-567.

Valiulis, E., and J. F. Long. 1973. Effects of drugs on intestinal water secretion following cholera toxin in guinea pigs and rabbits. Physiologist 16:475. (Abstr.)

Vaughan, M., N. F. Pierce, and W. B. Greenough, III. 1970. Stimulation of glycerol production in fat cells by cholera toxin. Nature (London) (London) 226:658-659.

Walling, M. W., A. K. Mircheff, C. H. Vanos, and E. M. Wright. 1978. Subcellular distribution of nucleotide cyclases in rat intestinal epithelium. Am. J. Physiol. 235:E539-E545.

Webster, H. L., and D. D. Harrison. 1969. Enzymic activities during the transformation of crypt to columnar intestinal cells. Exp. Cell. Res. 56:245-253.

Weiser, M. M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. I. An indicator of cellular differentiation. J. Biol. Chem. 248:2536-2544.

Weiss, S. D., P. D. McNamara, L. M. Pepe, and S. Segal. 1978. Glutamine and glutamic acid uptake by rat renal brush border membrane vesicles. J. Memb. Biol. 43:91-105.

Wheeler, H. O. 1963. Transport of electrolytes and water across wall of rabbit gallbladder. Am. J. Physiol. 205:427-438.

Williams, J. A., and M. Lee. 1974. Pancreatic acinar cells: Use of a Ca^{++} ionophore to separate enzyme release from the earlier steps in stimulus-secretion coupling. Biochem. Biophys. Res. Commun. 60:542-548.

Wilson, T. H., and G. Wiseman. 1954. The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. J. Physiol. 123:116-125.

Wohlgemuth, K. 1977. Diarrhea in calves. Diagnosis and incidence in the North Central States. Pages 131-140 in Proceedings of 81st Annual Meeting, U.S. Animal Health Association.

Wolfe, D. L., S. C. Forland, and L. Z. Benet. 1973. Drug transfer across intact-rat intestinal mucosa following surgical removal of serosa and muscularis externa. J. Pharm. Sci. 62:200-205.

Worthington, B. B., and D. O. Graney. 1973a. Uptake of adenovirus by intestinal absorptive cells of the suckling rat. I. The neonatal ileum. Anat. Rec. 175:37-62.

Worthington, B. B., and D. O. Graney. 1973b. Uptake of adenovirus by intestinal absorptive cells of the suckling rat. II. The neonatal jejunum. Anat. Rec. 175:63-76.

Wright, E. M., C. H. van Os, and A. K. Mircheff. 1980. Sugar uptake by intestinal basolateral membrane vesicles. Biochim. Biophys. Acta 597:112-124.

Wright, R. D., M. A. Jennings, H. W. Florey, and R. Lium. 1940. The influence of nerves and drugs on secretion by the small intestine and an investigation of the enzymes in intestinal juice. Q. J. Exp. Physiol. 30:73-120.

Yousef, I. M., and A. Kuksis. 1972. Release of chylomicrons by isolated cells of rat intestinal mucosa. Lipids 7:380-386.

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