

Absorptive patterns of the colon in normal  
and swine dysentery affected pigs

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1981

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A Thesis Submitted to the  
Graduate Faculty in Partial Fulfillment of the  
Requirements for the Degree of  
MASTER OF SCIENCE

Department: Veterinary Microbiology  
and Preventive Medicine  
Major: Veterinary Microbiology

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Iowa State University  
Ames, Iowa  
1981

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## TABLE OF CONTENTS

	Page
LITERATURE REVIEW	1
General	1
History and etiology	1
Clinical signs	7
Pathology	9
Clinical pathology	9
Gross pathology	10
Microscopic pathology	11
Diagnosis	12
Treatment and control	13
Immunity	15
Pathogenesis	16
Approach	16
Pathogenesis of Salmonellosis	17
Pathogenesis of Shigellosis	21
Pathogenesis of <u>Escherichia coli</u> and <u>Vibrio cholerae</u> diarrhea	24
Normal mammalian colonic physiology	28
Pathogenesis of swine dysentery	34
SECTION I. EXAMINATION OF CYCLIC NUCLEOTIDE INVOLVEMENT IN SWINE DYSENTERY	38

	Page
INTRODUCTION	39
MATERIALS AND METHODS	41
<u>In vivo</u> and <u>in vitro</u> Procedures	
Animals	41
Surgical preparation	41
Intragastric inoculation procedure and production of dysentery	42
Experimental solutions	42
Experimental design	44
Experimental procedures	45
Ligated colonic loops	45
Cyclic nucleotide assay	46
Chemical analyses	49
Calculations	50
Statistical analysis	52
Cultural techniques	52
Selective isolation	52
Viable count determinations	52
Histopathology	52
RESULTS	53
<u>In vivo</u> Experiments	53
Type I studies	53
Theophylline dose response, net effects of solutions 1-5 in ligated colonic loops of normal pigs	53

	Page
Type II studies	58
Effects of theophylline in sodium free solutions (1, 5, 6, and 7) on net transport in ligated colonic loops of normal pigs	58
Type III studies	61
Effects of theophylline on colonic transport in ligated colonic loops of dysentery affected pigs (Solutions 1, 3, and 5)	61
<u>In vitro</u> Experiments	65
Type IV studies	65
Measurements of cyclic AMP and cyclic GMP concentrations in normal and dysenteric pig colonic mucosa exposed <u>in vitro</u> to theophylline	65
DISCUSSION	74
<u>In vivo</u> and <u>in vitro</u> Experiments	74
The effects of theophylline on colonic transport in ligated colonic loops of normal pigs	74
The effects of theophylline on colonic transport in ligated colonic loops of dysentery affected pigs	79
SECTION II. EXAMINATIONS FOR ENTEROTOXIC, PERMEABILITY FACTOR, AND ENDOTOXIC ACTIVITY IN SWINE DYSENTERY	86
INTRODUCTION	87
MATERIALS AND METHODS	89
<u>In vivo</u> Experiments	89
Animals	89

	Page
Surgical preparation	89
Bacteria	90
Cultural techniques	90
Inocula	90
Viable count determinations	91
Selective isolation	91
Intragastric inoculation procedure	92
Histopathology	92
Assay of hemolytic activity	92
Chemical analyses	93
Calculations and statistical analysis	93
Preparation of test materials	94
Experimental procedures and design	98
Experiment I   Effects of <u>T. hyodysenteriae</u> alone and in combination with <u>B. vulgatus</u> in ligated colonic and ligated jejunal loops of normal pigs	98
Experiment II   Effects of crude colonic contents and sterile filtrates of crude colonic contents from dysentery affected pigs in ligated colonic loops of normal pigs	100
Experiment III   Comparison of the effects of hemolysins of <u>T. hyodysenteriae</u> and <u>T. innocens</u> to the effects of <u>T. hyo-</u> <u>dysenteriae</u> and <u>T. innocens</u> in ligated colonic loops of normal pigs	100

	Page
Experiment IV Effects of extracts of colonic mucosa and colonic contents of normal and dysentery affected pigs on normal colonic transport	102
Experiment V Effects of test materials in vascular permeability factor assay in rabbits	103
Experiment VI Endotoxic effects of test materials in embryonating hens' eggs	104
RESULTS	105
<u>In vivo</u> Experiments	
Experiment I	
Effects of <u>T. hyodysenteriae</u> alone and in combination with <u>B. vulgatus</u> in ligated colonic and ligated jejunal loops of normal pigs	105
Experiment II	109
Effects of crude colonic contents and sterile filtrates of crude colonic contents from dysentery affected pigs in ligated colonic loops of normal pigs	109
Experiment III	111
Comparison of the effects of hemolysins of <u>T. hyodysenteriae</u> and <u>T. innocens</u> to the effects of viable <u>T. hyodysenteriae</u> and <u>T. innocens</u> in ligated colonic loops of normal pigs	111
Experiment IV	113
Effects of extracts of colonic mucosa and colonic contents from normal and dysentery affected pigs on normal colonic transport	113

	Page
Experiment V	113
Effects of test materials in vascular permeability factor assay in rabbits	113
Experiment VI	113
Endotoxic effects of test materials in embryonating hens' eggs	113
DISCUSSION	119
LITERATURE CITED	124
ACKNOWLEDGMENTS	144
APPENDIX A. CALCULATIONS FOR PEG, $\text{HCO}_3^-$ , CYCLIC AMP AND CYCLIC GMP DETERMINATIONS	145
APPENDIX B. RAW AND PROCESSED NUMERICAL DATA	148

## LIST OF FIGURES

Figure 1.	Summary of proposed mammalian colonic epithelial transport mechanisms	32
Figure 2.	Effect of increasing doses of theophylline, solutions 1-5, on net water and solute transport and luminal pCO <sub>2</sub> in ligated colonic loops of normal pigs	55
Figure 3.	Absorption pattern of Na <sup>+</sup> . Sodium (mEq) remaining in ligated colonic loops over a 2 hour incubation period	57
Figure 4.	Effects of theophylline in sodium free solutions (1, 5, 6, and 7) on net water and solute transport and luminal pCO <sub>2</sub> in ligated colonic loops of normal pigs	60
Figure 5.	Effects of theophylline, solutions 1, 3, and 5, on net water and solute transport and luminal pCO <sub>2</sub> in ligated colonic loops of dysentery affected pigs	64
Figure 6.	Effect of theophylline on unidirectional Na <sup>+</sup> fluxes in ligated colonic loops of normal and swine dysentery affected pigs	68
Figure 7.	Cyclic AMP concentrations in colonic mucosa of normal and dysentery affected pigs exposed to Ringers solution + mannitol (20 mM) or Ringers solution + theophylline (20 mM)	70
Figure 8.	Cyclic GMP concentrations in colonic mucosa of normal and dysentery affected pigs exposed to a Ringers solution + mannitol (20 mM) or Ringers solution + theophylline (20 mM)	72
Figure 9.	Normal transport mechanisms of the pig colonic mucosal epithelium	76
Figure 10.	Relationship of net water and total solute transport in ligated colonic loops of normal pigs (N) exposed to solutions 1-5 and dysentery affected pigs (SD) exposed to solutions 1, 3, and 5	81
Figure 11.	Effect of extracts of colonic mucosa and colonic contents of normal and dysentery affected pigs on net water and solute transport in ligated colonic loops of normal pigs	115



## LIST OF TABLES

Table 1.	Composition of experimental solutions (mM or mosmoles)	43
Table 2.	Microscopic lesions observed in colonic sections from dysentery affected pigs	66
Table 3.	Composition of experimental solutions (mM or mosmoles)	99
Table 4.	Effects of <u>T. hyodysenteriae</u> alone and in combination with <u>B. vulgatus</u> in ligated colonic loops of normal pigs	106
Table 5.	Effects of <u>T. hyodysenteriae</u> alone and in combination with <u>B. vulgatus</u> in ligated jejunal loops of normal pigs	107
Table 6.	Effects of crude colonic contents and sterile filtrates of crude colonic contents from dysentery affected pigs in ligated colonic loops of normal pigs	110
Table 7.	Comparison of the effects of hemolysins of <u>T. hyodysenteriae</u> and <u>T. innocens</u> to the effects of viable <u>T. hyodysenteriae</u> and <u>T. innocens</u> in ligated colonic loops of normal pigs	112
Table 8.	Effects of test materials on vascular permeability as assayed by intradermal injection of rabbits	116
Table 9.	Endotoxic effects of test materials as assayed by intravenous injection of embryonating hens' eggs	117
Table B1.	Type I studies. Theophylline dose response, net effects of solutions 1-5	149
Table B2.	Sodium (mEq) remaining in ligated colonic loops (2 hr incubation)	150
Table B3.	Type II studies. Effects of theophylline in sodium free solutions (1, 5, 6, and 7) on colonic transport	151
Table B4.	Type III studies. Effects of theophylline on colonic transport in dysentery affected pigs (solutions 1, 3, and 5)	153

	Page
Table B5. Cyclic AMP concentrations (pmoles/mg mucosa) in normal and infected colonic mucosa exposed to mannitol (20 mM) or theophylline (20 mM)	155
Table B6. Cyclic GMP concentrations (fmoles/mg mucosa) in normal and infected colonic mucosa exposed to mannitol (20 mM) or theophylline (20 mM)	155
Table B7. Experiment VI. Effects of extracts of colonic mucosa and colonic contents of normal and dysentery affected pigs on normal colonic transport	156

## LITERATURE REVIEW

## General

History and Etiology

Swine dysentery was first described in 1921 (Whiting, et al.) although the disease was recognized as early as 1917 in Indiana (Whiting, et al., 1921) and 1918 in Iowa (Hofferd, 1936). It was reported to be an infectious disease, most commonly seen in weanling age swine, characterized by a mucohemorrhagic diarrhea (Whiting, et al., 1921; Whiting, 1924). Since the initial description, swine dysentery has been diagnosed in nearly all swine-producing areas of the United States (Warner, 1965; Sorenson, 1970) and in many foreign countries, including Canada (Lussier, 1962), Australia (Roberts, 1956a), and Netherlands (Terpstra, et al., 1968). Roncalli and Leaning (1976) have listed over forty countries where swine dysentery has been reported along with the dates of these reports. Although they questioned some of the diagnostic procedures, they concluded that the disease was found world-wide.

Over the years a great deal of confusion surrounded the etiology of dysentery. In early experiments hog cholera virus, Bacillus suipestifer (Salmonella), Bacillus coli (Escherichia coli), Bacillus necrophorum (Fusobacterium necrophorum), and an amebic organism were found not to be of etiologic significance (Whiting, et al., 1921; Whiting, 1924). This did not preclude the possibility of their

involvement as secondary agents once the disease was established. Balantidium coli and a spirochete were also not thought to be of importance even though both organisms were observed in high numbers in the colons of affected pigs. Unsuccessful attempts to reproduce the disease with a spirochete were made by Whiting, et al. (1921). The only reliable method of reproducing the disease was by inoculation of susceptible pigs with colonic contents and minced colon from infected swine (Whiting, et al., 1921; Whiting, 1928). In 1944, Doyle reported the isolation and propagation of an organism, which he named Vibrio coli (Campylobacter sp.), from the colonic mucosa of a naturally occurring case of swine dysentery. Susceptible pigs developed symptoms characteristic of swine dysentery when fed pure cultures of the organism. However, the clinical signs were not as severe as those seen in naturally occurring cases (Doyle, 1944). These results were later confirmed in a more extensive study (James and Doyle, 1947). Few other investigators were able to reproduce dysentery using Doyle's methods. Roberts (1956a) and Warner (1965) were not consistently able to produce the syndrome. Warner attributed his failures to either a loss of virulence during in vitro passage or, the use of avirulent strains. Most researchers using Vibrio coli were unable to reproduce the disease in conventional pigs (Deas, 1960; Davis, 1961; Terpstra, et al., 1968; Andress and Barnum, 1968; Harris, et al., 1972a; Sofrenovic, et al., 1973a; Hamdy and Glenn, 1974), or in gnotobiotic pigs (Andress, et al., 1968; Kashiwazaki, et al., 1971; Meyer,

et al., 1974a; Brandenburg, et al., 1977). The studies performed by Andress and Barnum utilized a number of different strains of Vibrio coli, with few in vitro passages, to avoid the problems suggested by Warner. Hughes, et al. (1972) using an isolated colonic loop, were also unable to produce lesions of swine dysentery with cultures of Vibrio coli. The conclusion of all these studies has been that Vibrio coli was not the primary etiologic agent of swine dysentery.

As mentioned, spirochetes had been observed associated with the lesions of dysentery and had even been tried unsuccessfully in experimental attempts to reproduce the disease (Whiting, et al., 1921). Little further attention was given the spirochete until Terpstra, et al. (1968) were able to stain a spirochete in fecal material and colonic mucosa from clinically affected animals using a fluorescent antibody technique with sera from swine dysentery recovered pigs. Soon after, additional reports of a spirochete observed in association with the lesions of dysentery began to appear. Vallejo (1969), saw large numbers of spirochetes by both light and electron microscopy in the lesions of swine dysentery, but was not sure of their importance. Others (Taylor, 1979; Blakemore and Taylor, 1979; Todd, et al., 1970; Roberts and Simmons, 1970) also observed spirochetes in association with the lesions of swine dysentery. Glock (1971) clearly demonstrated the presence of spirochetes both within and between the colonic epithelial cells of dysentery affected swine. A study by Taylor and Blakemore (1971), presented similar

results. This spirochete, which was at first only grown in mixed culture (Taylor, 1970; Todd, et al., 1970; Harris, et al., 1972c), was finally isolated and grown in pure culture by Taylor and Alexander in 1971. Using these Type 1 spirochete cultures, they were successful in reproducing dysentery in susceptible pigs. At approximately the same time, Harris, et al. (1972a) succeeded in isolating a similar organism, which they named Treponema hyodysenteriae, that was also capable of producing swine dysentery. This organism has been shown to be similar to the Type 1 spirochete isolated by Taylor and Alexander (Kinyon, 1974). Since these initial reports, isolation of a spirochete and reproduction of swine dysentery with the spirochete has been documented in many countries: Canada (Saheb and Richer-Massicotte, 1972), Netherlands (Akkermans and Pomper, 1973), Norway (Liven and Saxegaard, 1975), England (Hunter and Ross, 1972); and Yugoslavia (Sofrenovic, et al., 1973b; Oljuic, et al., 1973) to name a few. Although pure cultures of the spirochete did not produce disease in 100% of the cases, it was concluded to be the primary etiologic agent.

Some doubt concerning the role of T. hyodysenteriae in the disease process was raised when researchers were unable to produce clinical signs or lesions of dysentery with pure cultures of the organism in gnotobiotic pigs (Meyer, et al., 1974a; Brandenburg, et al., 1977; Harris, et al., 1978). It was suggested that Vibrio coli may participate in the pathogenesis or increase the severity of the disease (Hamdy and Glenn, 1974; Fernie, et al., 1975). The possibility of involvement

of other organisms was demonstrated when dysentery was produced in gnotobiotic pigs using colonic contents from dysentery affected pigs (Meyer, et al., 1974b). Further evidence of a synergism was revealed when Meyer, et al. (1975) produced the disease in gnotobiotic pigs using T. hyodysenteriae in combination with four other gram negative, non-spore forming anaerobes. Numerous organisms have been tried in combination with T. hyodysenteriae in studies attempting to clarify the nature of this synergism. Organisms which have been tried unsuccessfully include: Clostridium sp. (Meyer, et al., 1974b, Alexander, et al., 1976); Fusobacterium sp., Corynebacteria sp., Spirillum sp., Staphylococcus sp. (Alexander, et al., 1976), Lactobacillus sp., Escherichia coli (Meyer, et al., 1974b); Vibrio coli. (Meyer, et al., 1974b, Harris, et al., 1978); Peptostreptococcus sp. (Brandenburg, et al., 1977). Recent studies by Whipp, et al. (1979) and Harris, et al. (1978), have shown that several organisms are capable of producing lesions of swine dysentery when given orally in combination with T. hyodysenteriae to gnotobiotic pigs. Fusobacterium necrophorum, Bacteroides vulgatus (3 strains), Listeria denitrificans, and a Clostridia sp. were among the organisms used. The diversity of this group underscores the nonspecificity of the synergism between T. hyodysenteriae and the components of the normal colonic flora. The exact nature of this synergism remains to be fully determined.

The biochemical and physical characteristics of T. hyodysenteriae have also been studied. The organism is an S-shaped,

oxygen-tolerant, anaerobic spirochete. Although originally placed in the genus Treponema, recent DNA homology studies with other treponemes suggest this may not be entirely correct (Miao, et al., 1978).

Physically the organism is slender with 2 to 3 turns per cell, a length of 6-8.5 microns and a diameter of .32 to .38 microns. A thin envelope surrounds the protoplasmic cylinder. Eight to nine overlapping axial fibrils, are also present. These fibrils originate from implantation discs on each end of the organism (Glock and Harris, 1972). Darkfield and phase microscopy have been used for direct observation of the organism. Since the organism stains weakly gram negative, carbol fuchsin, Giemsa and Victoria blue 4-R (Olson, 1978) are stains more commonly used.

Initial in vitro characterization of T. hydysenteriae was performed using blood agar plates and blood agar with broth overlays under deoxygenated CO<sub>2</sub> (Harris, et al., 1972c). It was later grown as pure broth culture in trypticase soy broth without dextrose supplemented with 10% fetal calf serum under an atmosphere of deoxygenated H<sub>2</sub>:CO<sub>2</sub> (50:50) (Kinyon and Harris, 1974). Biochemically, the organism has been shown to be catalase and cytochrome oxidase negative, and capable of utilizing small amounts of oxygen (Harris, et al., 1976). It formed acetic and butyric acids from glucose fermentation, hydrolyzed esculin, and was stimulated by hydrogen gas (Kinyon and Harris, 1979).



Growth in agar medium was found to be optimal at 42°C under an 80:20 atmosphere of H<sub>2</sub>:CO<sub>2</sub> while broth growth was optimal at 37°C under a 50:50 mixture of H<sub>2</sub>:CO<sub>2</sub> (Kinyon, 1974).

Organisms which were morphologically identical to T. hydysenteriae were recognized in normal swine by Taylor and Alexander (1971). These organisms were not pathogenic for pigs (Kinyon, et al., 1977). Attempts have been made to separate these organisms from pathogenic types by biochemical (Hunter and Wood, 1979), serologic (Baum and Joens, 1979), and physical characteristics (Taylor and Alexander, 1971). All of these methods have had limited success. The most reliable method of separation used has been that based on hemolytic patterns on blood agar. Pathogenic strains cause beta hemolysis while nonpathogenic strains cause a weak beta hemolysis (Kinyon, et al., 1977). Recent work by Miao, et al. (1978) on DNA homology, and biochemical studies by Kinyon and Harris (1979), have identified the nonpathogen as a new species; Treponema innocens.

### Clinical signs

Several different terms have been applied to swine dysentery. Vibrionic dysentery, bloody scours, black scours, bloody diarrhea, and mucohemorrhagic disease are a few. The clinical signs associated with the disease, by whatever name, are essentially the same and are those first described by Whiting, et al. (1921). The classic signs are a bloody diarrhea with mucous and fibrin in the feces. The

animals become listless, anorectic, and emaciated. The animal may become prostrate and comatose prior to death. A slight febrile response may also be present but this finding is inconsistent. Numerous reports by a number of other researchers document the same syndrome with some minor variations (Hofferd, 1936; Whiting, 1928; Doyle, 1944; Doyle, 1945; Deas, 1960; Lussier, 1962; Glock, 1971; Terpstra, et al., 1968). Some of these reports give slight variations in the incubation time, morbidity, mortality, and overall severity of the disease. These differences will be discussed later.

Lussier (1962) characterized the disease process as four separate syndromes; peracute, acute, subacute, and chronic. Peracute cases are those where the animals are found dead with no prior clinical signs of illness. A similar situation was reported by Whiting, et al., (1921) but is not commonly observed. Acute cases are those animals exhibiting the classical signs of the disease, that is, diarrhea with blood and mucous in the feces. Subacute cases are characterized by continued weight loss or reduction of gain, associated with mild or intermittent diarrhea. Animals with chronic infection develop a watery diarrhea and undergo progressive emaciation showing no signs of recovery and eventually die. It may be that in these chronic cases secondary invaders assume an important role.

As mentioned, a variable period of incubation has been reported. Stress has been proposed as a contributing factor in this situation (Glock, 1971). The effect of diet on the susceptibility of pigs

has also received some attention (Saxegaard and Tiede, 1977; Tiede, et al., 1977; Tiede, et al., 1978). Treatment of animals with antibiotics can suppress the onset of the disease but may increase the severity once the disease begins (Olson and Rodabaugh, 1978). An endemic form of the disease resulting from continued medication has also been reported (Alexander and Taylor, 1969). All of these above mentioned factors lead to marked variations in the incubation period. The most common period given for incubation is from 10 to 14 days post exposure (Whiting, et al., 1921; Harris and Glock, 1975). Experimentally, the disease has been produced in as little as 48 hours after inoculation of starved pigs with crude colonic contents from dysentery affected swine (Glock, 1971). The morbidity and mortality associated with dysentery will vary with the age of the animal, the environmental conditions, and the speed of diagnosis and treatment. Breeding stock and suckling pigs, which do not commonly contract dysentery, exhibit a low morbidity and mortality (Deas, 1960; Doyle, 1955). Weanling age pigs and fattening hogs are those most severely affected by the disease. In these animals, the morbidity rates may be as high as 90% (Harris and Glock, 1972). The mortality rates will range from 40-60% in weanling animals, to 10-20% in older stock, with the average being around 25% (Whiting, et al., 1921; Doyle, 1955).

### Pathology

Clinical pathology      Death in swine dysentery has been associated with the loss of electrolytes and fluid (Ruth, 1967; Glock, 1971).

Decreased serum  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{HCO}_3^-$  levels have all been demonstrated. The loss of extracellular  $\text{Na}^+$  and fluid appear to be the critical factors. The loss of  $\text{HCO}_3^-$  may lead to a metabolic acidosis but this may be partially compensated by development of a respiratory alkalosis. The loss of  $\text{Na}^+$  causes a shift of  $\text{K}^+$  into the extracellular fluid. This increase in serum  $\text{K}^+$  may produce cardiotoxic effects in the terminal stages. The loss of fluid from the extracellular compartment will result in hypovolemic shock and subsequent death. Hematologic changes, such as an increased hematocrit, increased white blood cell count, and increased fibrinogen levels could all be observed associated with inflammatory disease processes and were not specific for dysentery (Glock, 1971).

Gross pathology      The lesions of swine dysentery are limited primarily to the cecum and colon. Lesions have been seen occasionally in the rectum and stomach, but these are not considered typical (Whiting, et al., 1921; Roberts, 1956b; Glock, 1971; Harris and Glock, 1972; Olson, 1974). Grossly, the serosa of the colon may appear edematous and hyperemic. Distended submucosal glands may be visible through the serosa (Glock, 1971; Glock and Harris, 1972). Mucosal lesions may range from catarrhal, to hemorrhagic, mucohemorrhagic, or fibrinonecrotic enteritis. In advanced cases, the fibrinonecrotic exudate often forms diphtheritic pseudomembranes. Areas of mucosal ulceration, erosion, and edema of the colonic wall are common in acute

cases of dysentery (Taylor and Alexander, 1971). Deas (1960) reports that in the chronic cases the entire colonic wall may become thickened. A description of the lesions associated with dysentery has been made by a number of investigators (Whiting, et al., 1921; Lussier, 1962; Roberts, 1956b).

Microscopic pathology Microscopically, the lesions are those that would be suggested from observation of the gross lesions with the upper one-third of the mucosa usually being the area primarily involved (Glock, 1971). In early stages, the goblet cells undergo hyperplasia and large amounts of mucous are secreted, while later, these cells may become exhausted and collapse. Congestion of the vessels of the lamina propria and the submucosa, and edema of the lamina propria were also seen. (Whiting, et al., 1921; Glock, 1971). The epithelial surface of the mucosa suffers the most damage where cells lose their attachment to the underlying tissues and eventually are sloughed into the lumen of the colon. These cells can combine with fibrin, leukocytes, bacteria, cellular debris, and a few erythrocytes to form pseudomembranes. Focal areas of necrosis and erosion into the deeper layers may be found in the more severe cases (Taylor and Blakemore, 1971; Glock and Harris, 1972).

Electron microscope studies have shown cellular lesions in association with the penetration of the spirochete either into, or between the epithelial cells. Swollen and degenerating microvilli and swollen mitochondria and endoplasmic reticulum have been observed

(Glock, 1971). Large numbers of spirochetes have been observed in close association with the lesions of dysentery (Glock, et al., 1974; Kennedy and Strafuss, 1977; Hughes, et al., 1976; Hughes, et al., 1977), and in the colonic crypts of infected animals (Glock and Harris, 1972).

### Diagnosis

A definitive diagnosis of swine dysentery is based on clinical signs, history, isolation of the organism, and histopathology. Observation of the spirochetes by phase microscopy or immunofluorescence from fecal samples is helpful, but not positive confirmation. Stains of fecal samples or colonic mucosa from affected animals may also be of some help, but again are not completely reliable because of nonpathogens which are morphologically identical to T. hyo-dysenteriae (Hudson, et al., 1976a). The history, clinical signs, and histopathology of dysentery have already been considered. The isolation of the bacterium was greatly facilitated by the introduction of a selective medium (Songer, et al., 1976). A trypticase soy agar base supplemented with 5% citrated bovine blood and 400 mcg/ml of spectinomycin has been shown to be an effective medium for the suppression of over 99% of the normal fecal bacteria. A fluorescent antibody test has also been used (Terpstra, et al., 1968), but false positive reactions with Treponema innocens occur (Hudson,

et al., 1976a) A microagglutination test (Joens, et al., 1978a) which may be of use in identifying infected herds but not individual animals (Joens, et al., 1979), and a serum agglutination test (Hunter and Saunders, 1973). have also been developed.

#### Treatment and control

Early dietary treatments for dysentery, such as soaked oats (Hofferd, 1936), were replaced by arsenicals in the 1950s (Carpenter and Larson, 1952). Arsenicals in turn have been replaced in recent years by a number of antibiotics. Several antibiotics which were effective when first used are now of little benefit. Kunesh (1969) describes a number of these; nitrofurazone, penicillin, tetracyclines, neomycin, bacitracin, sulfonamides, and tylosin. Those antibiotics which have been shown efficacious in recent years are: gentamicin (Harris, et al., 1972b), virginiamycin (Brandenburg and Wilson, 1974), lincomycin (DeGeeter, et al., 1976), lincomycin-spectinomycin combination (Hamdy, 1974; DeGeeter and Harris, 1975), carbadox (Pearce and Smith, 1975; Wilson and Roe, 1971), and a group of antibiotics known as the nitroimidazoles. This group is comprised of dimetridazole, ronidazole, and ipronidazole. The efficacy of dimetridazole (Bech and Hyldgard-Jensen, 1972; Griffin, 1972; Miller and Fox, 1973; Davis, et al., 1973; Wellington and Aucock, 1977; Griffin, 1979), ronidazole (Taylor, 1974; Taylor, 1976; Olson and Rodabaugh, 1976b; Moeller and Pulliam, 1977), and ipronidazole (Stadler, 1976; Messersmith, et al.,

1973; Messersmith, et al., 1976) has been well-established. A comparison of the in vivo effectiveness of the nitroimidazoles and a number of other antibiotics has been compiled by Olson and Rodabaugh (1976a). A study of the in vitro activity of 39 different antimicrobials against T. hyodysenteriae has also been performed (Kitai, et al., 1979). The efficacy of these compounds in field cases will depend on the interval between onset of the disease, and diagnosis and initiation of treatment.

Control of swine dysentery has centered mainly on the treatment of affected animals. Studies on the transmission of the disease, and the survival of the organism under a variety of conditions, have recently been made. Glock, et al. (1975) found that dysentery could be transmitted by lagoon water from a farm with affected animals. Songer, et al. (1978) reported isolation of the organism from the holding pit beneath the slotted floor of a confinement unit. Both of these experiments indicated the possibility of spread via the waste disposal systems, especially on those farms where waste water was used as flush water in the confinement units. Dysentery has also been shown to be spread by contact via caretakers (Terpstra, et al., 1968), and also probably by vehicles (Harris and Glock, 1975). Animals other than swine have been studied for their possible role in transmission of the organism. Dogs have been shown to harbor the organism for up to 2 weeks and starlings for 8 hours (Glock, et al., 1978a). Mice



have also been implicated as possible vectors and have been shown to shed the organism in the feces for prolonged periods of time (Joens and Glock, 1979).

Survival of the organism outside the host has been investigated under a variety of conditions. It can survive up to 48 days at 0-10°C in pig feces (Chia and Taylor, 1978). Since fecal shedding of the organism is the only known route for transmission, this becomes quite significant, especially in the northern climates. When the fecal material was diluted with tap water the survival time was further enhanced. The organism survived for shorter periods of time at higher temperatures (Chia and Taylor, 1978).

One of the more important means of spreading the disease is probably via inapparent carrier animals. The presence of these animals has been suspected for many years (Hofferd, 1936). Spread from these animals was first experimentally documented when Songer was able to transmit the disease from recovered pigs to sentinel pigs in 28 days (Songer and Harris, 1978). The recovered animals had shown no clinical signs of dysentery for 70-90 days. The organism has also been isolated directly from recovered pigs 62 days after cessation of clinical signs (Chia, 1977). Detection of these carriers would seem to be of primary importance if the disease is to be controlled.

### Immunity

Immunity to dysentery has also been studied extensively in recent years in efforts to produce an effective vaccine. Originally,

it was believed that there was no immunity to dysentery since outbreaks of the disease reoccur in the same herd (Hofferd, 1936). Recovered swine, have now been shown to be resistant to reinfection (Joens, et al., 1979), although the exact nature of this resistance has not been determined. Circulating antibody has been detected (Terpstra, et al., 1968), but its role is not understood.

Attempts have been made at immunizing pigs with a variety of agents. Antiserum, while not providing protection, did delay the onset of the disease (Schwartz and Glock, 1976). Attenuated strains, administered orally, have not been successful in inducing immunity (Hudson, et al., 1974; Jenkins, et al., 1979). Intravenous vaccination (Glock, et al., 1978b) or a combination of oral and parenteral administration (Hudson, et al., 1976b) with attenuated T. hyo-dysenteriae induced a degree of immunity. While not completely preventing dysentery, it delayed the onset and the disease was less severe in vaccinated animals. There still remains a great deal of work to be done in both the development of an efficacious vaccine and in the characterization of the immunity.

## Pathogenesis

### Approach

The discussion of the pathogenesis of swine dysentery has been delayed to this point to allow for separate consideration. This

section will deal not only with swine dysentery, but will also consider other intestinal diarrheal disorders to obtain an understanding of mechanisms that may be involved in swine dysentery. This discussion will include normal mammalian colonic physiology and the work on pathogenesis of swine dysentery to date. Those selected diarrheal diseases and their pathogenic mechanisms which will be considered for purposes of comparison are:

- 1) Salmonellosis
- 2) Shigellosis
- 3) Escherichia coli diarrhea
- 4) Vibrio cholerae diarrhea

#### Pathogenesis of Salmonellosis

Salmonellosis is a diarrheal disease characterized by invasion of the ileal and colonic mucosa by the organism (Angrist and Moller, 1948; Takeuchi, 1967; Takeuchi and Sprinz, 1967), and production of diarrhea with net secretion of fluid and electrolytes (Powell, et al., 1971). Net chloride secretion and decreased sodium absorption have been documented in in vitro studies of Salmonella infected rabbit ileum (Fromm, et al., 1974). An additional characteristic, the ability to spread via the reticuloendothelial system has been described (Giannella, et al., 1973), but will not be considered. Unless noted otherwise, all studies cited were performed using strains of Salmonella typhimurium.

Although not the only factor, a definite correlation between the ability to invade the mucosa and the production of diarrhea has been established (Giannella, et al., 1973). There are, however, strains of Salmonella capable of invading the mucosa that do not produce diarrhea. It has been postulated that quantitative differences in the inflammatory response of the mucosa may be the reason for this difference (Rout, et al., 1974).

Clinical signs observed in experimentally infected monkeys reflected the transport abnormalities, morphologic damage, and concentrations of the organism present in different sections of the intestinal tract (Rout, et al., 1974). Animals with mild diarrhea had severely impaired colonic and jejunal transport and mildly impaired ileal transport. Severe diarrhea was the result of secretion by the jejunum, ileum, and colon. Transport dysfunctions correlated with morphologic damage and bacterial invasion of the mucosa in the colon and ileum but not the jejunum. In the jejunum, marked secretion occurred with minimal morphologic damage and little bacterial invasion (Rout, et al., 1974). Either an enterotoxin produced by the organism, or a "colonic factor," released from the inflamed colon, was postulated as the mediator of jejunal secretion.

Because of the histologic appearance of the lesions, increased mucosal permeability was also considered as a possible mechanism of fluid loss. Two separate studies both concluded that an increase in mucosal permeability was not associated with salmonellosis (Kinsey,

et al., 1976b; Giannella, et al., 1976).

The majority of the evidence was in favor of the "colonic factor," in this case prostaglandins. Prostaglandins, which are released from inflamed tissues, have been shown to induce jejunal secretion in dogs and humans (Pierce, et al., 1971; Matuchansky and Bernier, 1973). They were thought to mediate their action through the adenylyl cyclase-cyclic adenosine monophosphate (cyclic AMP) system (Kimberg, et al., 1971; Kimberg, et al., 1974), which had been demonstrated to induce ileal secretion in rabbit ileum in vitro (Field, 1971a). If prostaglandins were involved in the pathogenesis, an elevation in concentrations of adenylyl cyclase-cyclic AMP should accompany Salmonella induced secretion. Inhibitors of prostaglandin action should block any increase in adenylyl cyclase-cyclic AMP and consequently net secretion. Participation of prostaglandins could possibly explain the lack of secretion observed with invasive strains that do not produce inflammation.

Direct measurements in ligated ileal loops of rabbits infected with a Salmonella strain producing severe inflammation and secretion showed elevations of adenylyl cyclase-cyclic AMP concentrations. A strain of Salmonella causing a less intense inflammatory reaction and no secretion did not affect adenylyl cyclase-cyclic AMP concentrations (Giannella, et al., 1975). Indomethacin, a potent prostaglandin inhibitor, was used to abolish net secretion and elevations of adenylyl

cyclase activity in infected rabbit intestinal mucosa (Gots, et al., 1974), and as a pretreatment to inhibit secretion and elevations in adenylyl cyclase in experimentally infected animals (Giannella, et al., 1977). It did not affect the invasive capabilities of the organism or its dissemination to other organs (Gots, et al., 1974). A recent study by Giannella (1979) in ligated ileal loops of Salmonella infected rabbits has further implicated inflammation as being of primary importance. When the inflammatory response was blocked by intravenous administration of nitrogen mustard, no net secretion occurred in Salmonella infected loops. Nitrogen mustard inhibited neither the epithelial penetration by the Salmonella organisms, nor the secretory effects induced by cholera toxin.

The possibility of an enterotoxin mediated pathogenesis has received some attention and the isolation of an enterotoxin from Salmonella cultures has been reported (Koupal and Diebel, 1975). This substance, which was capable of inducing secretion in ligated rabbit ileal loops has been partially characterized (Sedlock, et al., 1978). Factors capable of causing increased vascular permeability in rabbit skin have also been reported (Sandefur and Peterson, 1976). The role of these factors in the process of fluid secretion or in eliciting inflammation is not understood.

The most probable hypothesis of Salmonella induced secretion is inflammation, causing release of prostaglandins, which induce elevations

in adenylyl cyclase and cyclic AMP concentrations causing net secretion of fluid and electrolytes.

### Pathogenesis of Shigellosis

Shigellosis is an infectious disease characterized by watery diarrhea, dysentery, or both. Three species of the organism: Shigella dysenteriae Type 1, Shigella flexneri 2a and Shigella sonnei are those most commonly associated with disease.

In early studies, researchers were able to isolate a neurotoxin from sterile, autolyzed Shigella dysenteriae (Flexner and Sweet, 1906). Characterization of the toxin proved it to be an entity separate from endotoxin (Olitsky and Kligler, 1920). The damage produced by this toxin was most evident in rabbits where the lesions were seen in the central nervous system. These lesions were not primary, but were due to vascular damage caused by the toxin (Bridgewater, et al., 1955). A complete review of the research on the neurotoxin has been provided by W. E. van Heyningen (1972).

Although isolated, the neurotoxin had been tested for biological activity in the intestine only once (Branham, et al., 1953). The invasion of the intestinal mucosa was established as an essential step, and probably the primary factor, in determining pathogenicity (LaBrec, et al., 1964; Formal, et al., 1965). It was not until 1972 that Keusch, et al. succeeded in demonstrating and isolating an enterotoxin from Shigella dysenteriae. It was later shown that the neurotoxin and

enterotoxin were closely related, if not identical (Keusch and Jacewicz, 1975). However, the role of the enterotoxin was questioned since Shigella flexneri 2a produced the same disease syndrome but did not elaborate a detectable enterotoxin (Rout, et al., 1975).

Additional studies with noninvasive toxigenic, and invasive non-toxigenic strains of Shigella dysenteriae reinforced the concept that epithelial cell penetration was of primary importance (Gemski, et al., 1972; Levine, et al., 1973).

Two other studies indicated that an enterotoxin may have a role in the pathogenesis of Shigellosis. In Shigella flexneri 2a, experimentally infected monkeys, Rout, et al. (1975) found that the disease could be divided into two syndromes. Animals were classified as having dysentery alone or dysentery and diarrhea. Those with dysentery alone had inhibition of colonic absorption or colonic secretion and mucosal invasion by the organism. Cases with dysentery and diarrhea had the same colonic lesions and transport abnormalities and in addition net secretion by the jejunum. In contrast to the colon, no epithelial invasion of the jejunal mucosa was observed, despite high concentrations of the organism in the lumen. The ileum of affected animals had neither transport abnormalities nor morphologic alterations of the mucosa (Rout, et al., 1975). An enterotoxin, or a factor released from the damaged colonic mucosa was postulated as the mediator of the jejunal secretion. Further support for an enterotoxin or factor capable of inducing jejunal secretion was provided by the finding



that intracecal inoculation of monkeys with Shigella flexneri 2a produced only dysentery while oral inoculation produced dysentery and diarrhea (Kinsey, et al., 1976a).

It was only recently that an enterotoxin, biologically and immunologically similar to that of Shigella dysenteriae, was isolated from Shigella flexneri 2a (O'Brien, et al., 1977). A major difference was that Shigella flexneri 2a produced the toxin in a 1000 fold less quantity.

Studies have been conducted with the enterotoxin to determine its mechanism of action. In in vivo rabbit ileum, the toxin induced secretion of fluid and electrolytes while in vitro net sodium secretion was elicited (Donowitz, et al., 1975). Sugar and amino acid transport were also inhibited in in vivo rabbit ileum (Binder and Whiting, 1977). However, studies with the enterotoxin in the rat colon revealed no activity (Donowitz and Binder, 1976). It was first believed that the adenylyl cyclase-cyclic AMP system did not participate in the action of the enterotoxin (Flores, et al., 1974). However, more recent evidence indicates that this system may be involved (Charney, et al., 1976), but since secretion occurs well before detectable elevations in cyclic AMP concentrations, correlation between secretion and cyclic AMP increases has not been possible (Paulk, et al., 1977). Cytotoxic activity has also been associated with the enterotoxin (Keusch, et al., 1972).

Although an enterotoxin has been isolated and partially

characterized, its role in the overall disease process has not been clearly demonstrated. The ability of the organism to invade the mucosal epithelium must still be regarded as the primary pathogenic determinant.

#### Pathogenesis of *Escherichia coli* and *Vibrio cholerae* diarrhea

Because of similarities in mechanisms of action and other characteristics, the enterotoxins of *Vibrio cholerae* and *Escherichia coli* will be discussed simultaneously. Since there is more than one syndrome associated with *E. coli* infection (Moon, 1974), it should be stated that only the enterotoxigenic form will be considered.

The capability of pure cultures of *E. coli* and *V. cholerae* to cause dilatation when inoculated into ligated rabbit ileal loops has been known for some time (De and Chatterje, 1953; De, et al., 1956). The same effect had been noted in ligated swine intestinal loops with cultures of *E. coli* pathogenic for swine (Moon, et al., 1966). Cell free supernatants of both of these organisms have also been shown to cause fluid accumulation in ligated intestinal loops (De, et al., 1960; Smith and Halls, 1967). It is known that *E. coli* produces two enterotoxins; one heat labile (LT), the other heat stable (ST) (Smith and Halls, 1967; Gyles and Barnum, 1969). *Escherichia coli* LT is quite similar to the enterotoxin of *V. cholerae* in its activity and possibly in chemical nature (Finkelstein, et al., 1976), while ST differs from cholera toxin in a number of ways.

Cholera toxin was isolated, purified, and partially characterized in 1972 (LoSpalluto and Finkelstein). The toxin was found to be heat

labile, acid and pronase sensitive, trypsin resistant, and highly antigenic. The heat labile toxin of E. coli is similar to cholera toxin in that it too was heat sensitive and highly antigenic, while the ST was not antigenic (Smith and Gyles, 1970a). In vivo, the heat labile enterotoxin was neutralized by antisera to cholera enterotoxin, but cholera enterotoxin was not neutralized by antisera to LT. Neither antisera had any effect on ST activity (Smith and Gyles, 1970a; Gyles, 1974).

The in vivo activity of these 3 enterotoxins was to produce an accumulation of water and electrolytes in the intestinal tract of various animals. By comparison of the fluid, it appeared that the toxins exerted a similar effect (Moon, et al., 1971). This was true for both the animal and human isolates of E. coli (Moon, et al., 1970; Smith and Gyles, 1970b). Fluid produced by the action of these toxins was the result of active secretion. Cholera toxin elicited active secretion of chloride and decreased sodium absorption (Field, et al., 1972). The two toxins of E. coli varied as to their effect on specific transport processes by species and the segment of intestine involved (Evans, et al., 1973a; Hamilton, et al., 1977) but appeared to activate similar secretory processes.

The toxins do share several common characteristics. None of the toxins has been observed to cause morphologic damage to the mucosa of the affected intestine (Elliot, et al., 1970; Moon, et al., 1971); Steinberg, et al., 1975). No permeability changes have been associated with cholera toxin (Scherer, et al., 1974) or the ST of E. coli (Presnell, et al., 1979). Both cholera toxin and LT appear to mediate

their actions through the adenylyl cyclase-cyclic AMP system (Field, 1971b; Sharp and Hynie, 1971; Evans, et al., 1972). These two toxins also have similar effects on cell cultures (Donta, et al., 1973; Donta, et al., 1974) and in vascular permeability assays (Craig, 1965; Evans, et al., 1973c). Direct measurements of cyclic AMP in tissue exposed to cholera toxin and LT have demonstrated marked increases in cyclic AMP concentrations (Kimberg, et al., 1971; Sharp and Hynie, 1971; Evans, et al., 1972; Kantor, et al., 1974; Kimberg, et al., 1974). These experiments have shown that inflammatory agents, such as prostaglandins, did not have a role in the elevations of cyclic AMP induced by cholera toxin (Kimberg, et al., 1974). The actual role of the adenylyl cyclase-cyclic AMP system in the secretory processes activated by cholera toxin and LT has been questioned. Secretion by in vivo pig jejunum exposed to cholera toxin did not consistently correlate with mucosal cyclic AMP or cyclic guanosine monophosphate (cyclic GMP) levels (Forsyth, et al., 1978b). An inhibitor of adenylyl cyclase activity had no effect on cholera induced secretions in in vivo rabbit jejunum (Taub, et al., 1977). Similar results were found when adenylyl cyclase activity was inhibited in in vivo pig jejunum exposed to cholera toxin (Forsyth, et al., 1978a). Indomethacin, a prostaglandin inhibitor, decreased secretion in in vivo rabbit jejunum exposed to cholera toxin, but did not inhibit the elevation of cyclic AMP concentrations (Wald, et al., 1977). Theophylline, a phosphodiesterase inhibitor, which should have potentiated the

secretory effects when used in combination with LT or cholera toxin, did not do so (Hamilton, et al., 1978a). These results suggest a mechanism other than, or in addition to, elevations of cyclic AMP and cyclic GMP concentrations may be involved in the active secretory processes.

As mentioned, E. coli ST differs from cholera toxin and LT, not only antigenically, but also in mechanism of action. While ST does cause secretion in ligated jejunal loops of the pig, it does not produce elevations of cyclic AMP. ST did not induce steroidogenesis in Y-1 adrenal cell cultures, an activity caused by increased cyclic AMP concentrations, as did both cholera toxin and LT (Donta, et al., 1974). Recent studies have shown that ST elevated cyclic GMP in both in vivo (Hughes, et al., 1978; Giannella and Drake, 1979) and in vitro (Field, et al., 1978) experiments.

Although the enterotoxins mediate their effects through two separate systems, they produce similar effects. Newsome, et al., (1978) have proposed that the overall control of the secretory processes may reside, not in the absolute concentrations, but the relative ratio of the concentrations of cyclic AMP to cyclic GMP.

Although the exact mechanisms for each of these toxins is not fully understood, their role in the production of diarrhea has been firmly established. The mechanisms of cholera and E. coli diarrhea are different from those of Salmonella and Shigella. No invasion of the epithelium with either E. coli or cholera enterotoxin induced

diarrhea was observed. Any transport abnormalities were due to the actions of the enterotoxins.

The pathogenic mechanisms of salmonellosis, shigellosis, and cholera and E. coli involve different mechanisms. Salmonellosis involves prostaglanin activation of cyclic AMP, shigellosis epithelial cell penetration and possible enterotoxin activity, and cholera and E. coli diarrhea activation of the cyclic nucleotide system by enterotoxins. These three different mechanisms, along with other possible mechanisms, must be considered when studying the pathogenesis of swine dysentery.

#### Normal mammalian colonic physiology

Only recently have colonic epithelial transport processes received study. The direct involvement of the colon in salmonellosis and shigellosis, and the possibility of a role in cholera and E. coli secretory diarrheas, have led to increased research. Both quantitative and qualitative aspects of colonic transport have been investigated by in vivo and in vitro techniques in a number of species.

Based on in vivo studies in the pig, the colon functions in the net absorption of sodium (Partridge, 1978) and water (Hamilton and Roe, 1977). Absorption of chloride and secretion of potassium have also been observed (Hamilton and Roe, 1977), with different diets producing variations in the absorption pattern (Partridge, 1978). Net absorption of water and sodium was also observed in the human colon

(Phillips, 1969; Billich and Levitan, 1969). Using perfusion techniques, it has been estimated that the human colon is capable of transporting 5.7 L of water, and 0.8 moles of  $\text{Na}^+$  per day (De Bongnie and Phillips, 1978) and the pig colon capable of absorbing 8.6 L water and 1.9 moles of  $\text{Na}^+$  each day (Argenzio and Whipp, 1979).

Acetate, which is absorbed by the pig colon, affects the absorption of  $\text{Na}^+$  by a mechanism which will be discussed later (Argenzio and Whipp, 1979). The transport capabilities of the colon are clearly allied with the anatomical properties of the mucosal epithelium. The intercellular junctions near the apical membrane are relatively "tight." As a result, the colon has low osmotic permeability (Billich and Levitan, 1969) and is capable of transporting sodium against an electrochemical gradient (Frizzell, et al., 1976). The tight junctions also give rise to other electrical and physical properties which will not be considered. Powell (1979) provides a more complete review of these properties.

Transport mechanisms have been studied in in vitro human, rat, and rabbit colonic mucosa, and by in vivo perfusion of the pig colon.

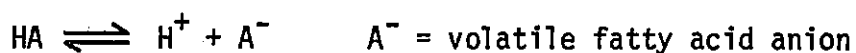
Two processes, electrogenic  $\text{Na}^+$  absorption, possibly in exchange for  $\text{K}^+$ , and a neutral  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange have been proposed as the mechanisms for absorption of  $\text{Na}^+$  and  $\text{Cl}^-$  in the human colon (Hawker, et al., 1978).

In the rat colon,  $\text{Na}^+$  absorption is thought to occur via two separate processes. The first is  $\text{Na}^+$ - $\text{Cl}^-$  coupled transport, and the

second electrogenic  $\text{Na}^+$  transport. The coupled process may represent the net effects of a  $\text{Na}^+$ - $\text{H}^+$  exchange and  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange (Binder and Rawlins, 1973a).

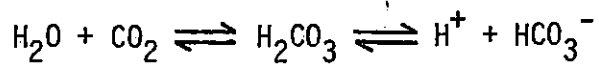
In vitro studies of rabbit colon indicate separate transport processes for  $\text{Na}^+$  and  $\text{Cl}^-$ . Electrogenic  $\text{Na}^+$  absorption and neutral  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange processes are proposed as the major mechanisms (Frizzell, et al., 1976; Frizzell and Turnheim, 1978). These studies also indicated  $\text{K}^+$  movement across the epithelium was passive and not linked to  $\text{Na}^+$  absorption. In vivo net secretion of  $\text{K}^+$ , observed in the human colon, was probably driven by the electrochemical gradient (+20 mv) created by  $\text{Na}^+$  absorption.

Net absorption of  $\text{Na}^+$  and  $\text{Cl}^-$  in the pig colon are thought to occur by two separate mechanisms. In vivo perfusion studies using ion replacement solutions have demonstrated  $\text{Na}^+$ - $\text{H}^+$  and  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange processes (Argenzio and Whipp, 1979). An observation made in the pig, and also in the horse (Argenzio, et al., 1977) and goat (Argenzio, et al., 1975), was that acetate, as well as other volatile fatty acids, facilitated the absorption of  $\text{Na}^+$ . This effect is thought to be caused by an increase in intracellular  $\text{H}^+$  available to exchange for  $\text{Na}^+$ . The volatile fatty acids, which are absorbed in the unionized form, dissociate once inside the cell, making the  $\text{H}^+$  available for the exchange process.





In the luminal solution,  $H^+$  is made available for transport of VFA's by hydration of  $CO_2$



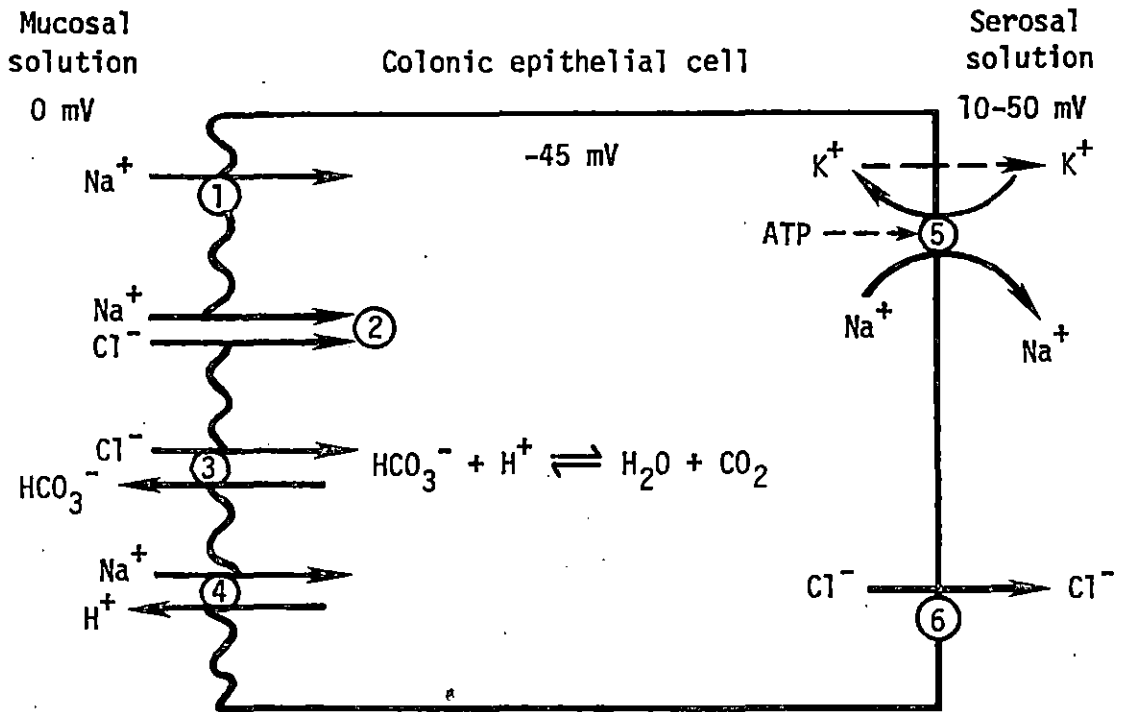
The pH of the luminal and cellular compartments regulate transport in accordance with the Henderson-Hasselbach equation.

In all species studied, colonic water absorption has been a passive process following an osmotic gradient.

A summary of the various transport processes believed to exist is presented in Figure 1. Although the absorption occurs against the electrical gradient (+10-50 mv), it is possible because of the active  $Na^+$  pump mechanism and relatively tight junctional complexes of the colonic mucosa (Powell, 1979).

Control of colonic transport is not well-understood. Cyclic nucleotides have been directly linked to secretory processes in the small intestine (Field, 1971a; Hughes, et al., 1978). Frizzell, et al., (1976) induced active  $Cl^-$  secretion, without affecting  $Na^+$  absorption, by exposing in vitro rabbit colonic mucosa to cyclic AMP. Experiments with ionophore A 23187, which mimics the effect of cyclic AMP through mobilization of intracellular calcium, produced similar results (Frizzell, 1977).

In the rat colon, where  $Na^+$  and  $Cl^-$  absorption are coupled, (Binder and Rawlins, 1973a) increased cyclic AMP concentrations,



1. Electrogenic or carrier mediated Na<sup>+</sup> absorption.
2. Coupled Na<sup>+</sup>-Cl<sup>-</sup> absorption; could be net result of Na<sup>+</sup>-H<sup>+</sup>, and Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchange processes.
3. Cl<sup>-</sup>-HCO<sub>3</sub><sup>-</sup> exchange process; could be Cl<sup>-</sup>-OH<sup>-</sup> exchange or coupled Cl<sup>-</sup>-H<sup>+</sup> absorption.
4. Na<sup>+</sup>-H<sup>+</sup> exchange process.
5. Na<sup>+</sup>-K<sup>+</sup> ATPase pump.
6. Passive Cl<sup>-</sup> movement across serosal border.

Figure 1. Summary of proposed mammalian colonic epithelial transport mechanisms (Powell 1979, p. 786).

abolished net  $\text{Na}^+$  absorption and elicited  $\text{Cl}^-$  secretion (Binder and Rawlins, 1973b). In the rat colon, cholera toxin, which increased cyclic AMP concentrations, produced secretion of  $\text{Na}^+$  and water and decreased  $\text{Cl}^-$  absorption, while Shigella dysenteriae enterotoxin and E. coli LT, both of which elevate cyclic AMP levels in other systems, had no effect.

No studies have been performed in the pig colon to determine if cyclic nucleotides exert any control over transport processes. Hamilton, et al. (1978b) were not able to demonstrate an increase in cyclic AMP concentrations in pig jejunum exposed to the LT or ST of E. coli or cholera toxin, even though net secretion occurred. They did demonstrate increased cAMP levels in rabbit jejunum exposed to the same toxins. The role of cyclic nucleotides, if any, in controlling transport processes of the pig colon awaits further study.

The importance of the absorptive capabilities becomes apparent when the amounts of fluid, in the form of ingested water or endogenous secretions that are presented to the colon, are considered. It has been calculated that in the pony, the entire extracellular fluid volume (Argenzio, et al., 1974) and in the pig at least 50% of the extracellular fluid volume are presented to the colon for reabsorption each day (Low, et al., 1978). Drastic shifts of intracellular fluid and electrolytes to the extracellular compartment would accompany the loss of this fluid due to colonic malfunction. Since swine dysentery,

histologically, involves almost exclusively the colon of the pig, an understanding of the normal transport processes and the control mechanism must be considered when studying pathogenesis of this disease.

#### Pathogenesis of swine dysentery

The pathogenesis of swine dysentery, especially from the aspect of physiopathology, has received little attention until recently. The etiologic studies discussed earlier have definitely established the synergistic relationship between T. hyodysenteriae and other components of the normal colonic flora in disease production. Several animal models, the guinea pig (Joens, et al., 1978b), conventional swine, mice (Joens and Glock, 1979), and isolated (Hughes, et al., 1972) and ligated colonic loops (Whipp, et al., 1978) of swine have been used.

The fluid and electrolyte loss of dysentery could be explained by at least four possible mechanisms:

- 1) an increase in colonic mucosal permeability
- 2) an enterotoxin eliciting colonic secretion, possibly through activation of cyclic nucleotides
- 3) prostaglandin release, which may increase small intestinal secretion and overwhelm the colonic absorptive capacity, or act locally and induce colonic secretion, or
- 4) abolition of normal colonic absorptive processes and loss of fluid usually reabsorbed by the colon.

Cytotoxin or other inflammatory agents, could also possibly be involved in the pathogenesis.

Using ligated colonic loops, in experimentally infected pigs, Argenzio, et al. (1980) were not able to demonstrate an increase in mucosal permeability. This was in contrast to what was expected based on the histologic appearance of infected colonic mucosa.

No enterotoxin activity has been detected in a number of experiments. In ligated colonic loops, neither inactivated whole cells, sterile filtrates of broth cultures, or sonicates of *T. hyodysenteriae* caused fluid accumulation or mucosal injury (Whipp, et al., 1978). Wilcock and Olander (1979b) obtained similar results in ligated colonic loops using sterile filtrates of both broth cultures and colonic contents from dysentery affected pigs. Other methods of testing for enterotoxins, the Y-1 adrenal cell assay and the suckling mouse model (Whipp, et al., 1978), were negative using sterile filtrates of broth cultures. Only with pure cultures of *T. hyodysenteriae* or crude colonic material from infected pigs, and only after 46-72 hours of incubation, was it possible to produce fluid accumulations and lesions resembling dysentery in ligated colonic loops (Whipp, et al., 1978; Wilcock and Olander, 1979a). The same findings have been reported by Hughes, et al. (1972) using an isolated colonic loop procedure. Similar results have been reported for rabbit ileal loops inoculated with pure cultures of *T. hyodysenteriae* (Knopp, 1979). An enterotoxin may be involved in the

mediation of the fluid secretion in loops inoculated with *T. hyodysenteriae*, but the negative results reported in a number of systems routinely used to assay for enterotoxins make this possibility unlikely. The long incubation periods (48-72 hrs.) required to produce fluid accumulation in ligated colonic loops would also appear to argue against enterotoxin mediated activity. Other microbial and physiologic factors may exert an influence. In addition, examination of net and unidirectional ion fluxes in ligated colonic loops of dysentery affected pigs have shown no increased secretion by the colonic mucosa. No increases in the blood to lumen fluxes of either  $\text{Na}^+$  or  $\text{Cl}^-$  were observed (Argenzio, et al., 1980).

Prostaglandins, which have not been studied for their effects on the normal swine intestinal tract, may participate in dysentery. If so, such a role would be different from that of salmonellosis where prostaglandins, possibly released from the inflamed colonic mucosa, induce secretion by the small intestine. Argenzio (1980) has established that the small intestine functions normally in dysentery affected pigs. The possibility still remains that prostaglandins are involved in a locally mediated response involving the colonic mucosa. Although net secretion does not occur, prostaglandins could function in reducing net transport.

The last possibility, that of decreased colonic absorption and loss of fluid, is the most likely. No net secretion, only a decrease in net absorption, has been observed in ligated colonic loops of

infected pigs. Prostaglandins, decreased mucosal blood flow, cytotoxins, inflammatory agents, or a combination of these factors may produce a decrease in net colonic transport. No cytotoxic activity has been reported for broth cultures of T. hyodysenteriae, culture filtrates of these broth cultures, or the filtrates of colonic contents of infected pigs (Wilcock and Olander, 1979b). The hemolysins of T. hyodysenteriae and T. innocens, which might be expected to contain cytotoxic agents, have received preliminary characterization (J. M. Kinyon, Veterinary Medical Research Institute, Iowa State University, Ames, Iowa, personal communication), but have not been examined in animal models. Little work has been done searching for possible inflammatory agents.

It should also be noted, that in contrast to salmonellosis and shigellosis, epithelial cell penetration does not appear to be of primary importance. However, attachment by the organism in vitro to mammalian epithelial cells has been demonstrated (Knopp, et al., 1979; Wilcock and Olander, 1979b).

From the studies performed, loss of normal colonic absorption appears to be the physiopathological dysfunction of swine dysentery. The pathogenic mechanism which is responsible for these changes, whether mediated by prostaglandins, cyclic nucleotides, cytotoxins, or other inflammatory agents, remains unclear.

SECTION I. EXAMINATION OF CYCLIC NUCLEOTIDE INVOLVEMENT  
IN SWINE DYSENTERY



## INTRODUCTION

Previous studies have indicated no passive or active colonic secretory components associated with the fluid loss of swine dysentery (Argenzio, et al., 1980). These same studies also showed increased colonic permeability not to be involved. The only finding was an abolition of the normal colonic absorptive processes.

This decrease in net transport could be linked to increases in cyclic nucleotide concentrations. It may be that a submaximally stimulated cyclic nucleotide activated secretory process may be involved. Cyclic nucleotides may also participate in the regulation of the transport processes and act to inhibit net absorption. The increases in cyclic nucleotides could be mediated by bacterial enterotoxin or possibly through the local release and activity of prostaglandins.

To investigate these possibilities, it was decided to attempt to potentiate any cyclic nucleotide activity already present in dysentery affected pigs. If cyclic nucleotides were participating in the disease process, such potentiation should result in further changes in the transport processes. Theophylline was chosen as the agent to increase concentrations of cyclic nucleotides. Theophylline functions by inhibiting the phosphodiesterases which break down cyclic nucleotides. It has been used to mimic the effects of cyclic AMP in in vitro rabbit ileum (Field, 1971a), to induce net secretion in the

rabbit ileum (Field, 1971a), to induce net secretion in the small intestine (Pierce, et al., 1971), and in in vivo attempts to potentiate the effects of cholera toxin and the LT and ST enterotoxins of E. coli in pig jejunal loops (Hamilton, et al., 1978a).

The effects of theophylline on transport will be compared in the ligated colonic loops of normal and dysentery affected pigs. Such treatment should expose any underlying cyclic nucleotide linked secretory component.

Concentrations of cyclic nucleotides in colonic mucosa of normal and dysentery affected pigs will be measured after exposure in vitro to either a control solution or a solution containing theophylline. Comparison of the basal levels and the response of infected and normal mucosa when stimulated by theophylline should provide evidence for or against participation of cyclic nucleotides in the pathophysiology of swine dysentery.

## MATERIALS AND METHODS

In vivo and in vitro ProceduresAnimals

Experimental animals were naturally farrowed pigs, 8-14 weeks of age, obtained from the National Animal Disease Center source herd. Each animal was fed a drug free, high protein ration once daily with water ad libitum. Pigs were housed together until after surgical preparation when they were individually penned.

Surgical preparation

Each pig used in loop studies was surgically prepared by implantation of a silastic cecal cannula. Anesthesia was induced and maintained by inhalation of halothane (Fluothane), Ayerst Lab., Inc., N.Y., NY., and the left paralumbar fossa clipped and prepared for surgery. The cecum was exteriorized through a 10-15 cm incision in the left flank region. Two purse string sutures of 00 Mersilene (Ethicon Inc., Somerville, NJ.) were placed in the muscle layers of the cecum. The cecum was then incised, the cannula inserted, and the purse string sutures tied. The cannula was then brought out through a surgical opening in the body wall. The incision was closed with 00 catgut in the peritoneum and muscle and Vetafil in the skin. Each pig received 300 mg Clindamycin (Cleocin, Upjohn, Kalamazoo, MI.) intraperitoneally before closure. No other antibiotics were administered. The cannula was kept plugged and clamped at all times. A post-operative

recovery period of 7-10 days was allowed before experimental use.

#### Intragastric inoculation procedure and production of dysentery

Pigs to be infected were starved for 48 hours prior to intragastric inoculation, via stomach tube, with 100 ml of pure culture of I. hyodysenteriae isolate B204. The inoculation was repeated on the following day. Cultures were grown in trypticase soy broth without dextrose (BBL Inc., Cockeysville, MD.) supplemented with 10% fetal calf serum (Gibco, Grand Island, NY.) under a 50:50 atmosphere of deoxygenated H<sub>2</sub>:CO<sub>2</sub> according to the method of Kinyon and Harris (1974). Inoculum in all cases was less than 15th in vitro passage of the organism and viable counts ranged from  $5 \times 10^7$  colony forming units (cfu)/ml to  $1 \times 10^8$  cfu/ml. Pigs were used for experiment within 36 hours of exhibiting clinical signs of dysentery.

#### Experimental solutions

Composition of the solutions is given in Table 1. All were prepared daily from concentrated stock solutions and were gassed for 15 minutes at 37°C with 95% O<sub>2</sub>, 5% CO<sub>2</sub>. Osmolality and pH of each solution was monitored. Polyethylene glycol 4000 (PEG 4000) was used as a nonabsorbable marker to measure net water movement. <sup>14</sup>C PEG at 10 μ Ci/L was used in solutions not containing <sup>22</sup>Na. Mannitol was used to bring solutions to isotonicity. <sup>22</sup>Na was added to solutions 1 and 5 in Type II experiments and solutions 1, 3, and 5 in Type III

Table 1. Composition of experimental solutions (mM or mosmoles)

	1	2	3	4	5	6	7
Na <sup>+</sup>	125	125	125	125	125		
Cl <sup>-</sup>	100	100	100	100	100	100	100
HCO <sub>3</sub> <sup>-</sup>	25	25	25	25	25	25	25
Choline	-	-	-	-	-	100	100
Mannitol	50	49	40	30	10	50	10
Theophylline	-	1	10	20	40	-	40
PEG 4000	1g/L	1g/L	1g/L	1g/L	1g/L	1g/L	1g/L
Osmolality	298 ± 2.2	299 ± 2.3	273 ± 1.7	292 ± 2.0	283 ± 3.4	303 ± 2.6	289 ± 2.7
pH	7.55 ± .03	7.52 ± .01	7.51 ± .02	7.48 ± .02	7.50 ± .02	7.59 ± .03	7.56 ± .04

experiments. Theophylline (1, 3 dimethylxanthine) was purchased from Sigma Chemical Co. (St. Louis, MO.).  $^{22}\text{Na}$  and  $^{14}\text{C}$  PEG were obtained from New England Nuclear (Boston, MA.). All other chemicals were purchased from Fisher Scientific Co. (Pittsburgh, PA.), and were of reagent grade.

### Experimental design

Three types of experiments were performed in ligated colonic loops with the solutions in Table 1.

In Type I experiments, solutions 1, 2, 3, 4, and 5 were injected into ligated colonic loops of normal pigs to determine the dose response of the colon to theophylline.

In Type II experiments, solutions 1 and 5, with  $10\ \mu\text{Ci}\ ^{22}\text{Na}/\text{L}$ , and solutions 6 and 7 in which choline was substituted for  $\text{Na}^+$ , were incubated in ligated colonic loops of normal pigs. Solutions 6 and 7 were used to determine the effects on net transport of theophylline in  $\text{Na}^+$  free solutions. However, significant  $\text{Na}^+$  absorption, from  $\text{NaHCO}_3$  in the solutions and residual  $\text{NaCl}$  used to flush the colon, caused complications in interpreting the results. Low volumes (ca. 25 ml) of solutions 1 and 5 were also placed in loops to determine if any hydrostatic driving forces were affecting absorption.

Ligated colonic loops in infected pigs were used in Type III studies, and solutions 1, 3, and 5, again with  $10\ \mu\text{Ci}\ ^{22}\text{Na}/\text{L}$ , were incubated in the loops. Sections of colonic mucosa were taken for

selective isolation of T. hyodysenteriae and for histopathologic examination.

In all three types of experiments, net movements of  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ , total solute, and water and pH and  $\text{pCO}_2$  changes were measured over a one hour period.

In vitro measurements of cyclic AMP and cyclic GMP were made in Type IV experiments on normal and infected colonic mucosa exposed to a Ringers solution containing 20 mM mannitol or Ringers solution containing 20 mM theophylline.

An additional experiment with three pigs, where samples were taken every twenty minutes from cannulas placed in colonic loops, was used to determine the absorption pattern of  $\text{Na}^+$ .

### Experimental procedures

Ligated colonic loops After a 24 hour fast, surgically prepared animals were placed in a metabolic cage and the colon of each pig flushed via the cecal cannula with warmed isotonic saline at a rate of 40-80 ml/min until the anal effluent ran clear. Anesthesia was induced and maintained by inhalation of halothane. A midline laparotomy was performed and the colon exteriorized. Ligated colonic loops were placed in the colon by the method of Whipp, et al., (1978). Residual saline was manually expressed from each loop before ligatures were tied and care was taken not to compromise the blood supply. Each 10-15 cm loop was separated from adjacent loops by a 2.5 cm

interloop. Weighed test solutions, ca. 50 ml, were injected into the loops, the solutions mixed and a time zero sample, ca. 10 ml, withdrawn. The colon was then replaced in the abdominal cavity, the incision closed, and the animal allowed to recover from anesthesia. At the end of one hour, the animals were euthanized by intramuscular injection of succinylcholine chloride followed by intracardiac injection of sodium pentobarbital. The loops were exposed and samples (10 ml) again taken from each loop. The loops were then opened, the mucosa stripped from the muscle layers, dried at 100°C for 24 to 36 hours, and weighed. All net movements of electrolytes and fluid were then calculated and expressed as amount per gram of dry mucosa per hour. The same procedure was followed for both normal and infected tissues.

In three pigs, two 20 cm loops were made. Each loop had a cannula that passed through the body wall and was filled with either solution 1 or 5 to 5 cm water pressure. Samples were removed from the cannulas every 20 minutes over a 2 hour period. The results were used to determine the  $\text{Na}^+$  absorption pattern and time course of theophylline response.

Cyclic nucleotide assay Cyclic nucleotide concentrations were measured in colonic mucosa, from normal and dysentery affected pigs, exposed in vitro to a Ringers solution containing either 20 mM mannitol or 20 mM theophylline. Composition of the Ringers solution in mMoles was  $\text{Na}^+$  140.0,  $\text{Cl}^-$  144.8,  $\text{Ca}^{++}$  1.2,  $\text{Mg}^{++}$  1.2,  $\text{K}^+$  12.4,



$\text{H}_2\text{PO}_4$  0.2,  $\text{HPO}_4^-$  1.2,  $\text{HCO}_3^-$  10.0, and glucose 10.0.

A section of colon from normal and dysenteric pigs was harvested, opened, and the muscle layers stripped from the mucosa while the tissue was kept in warmed (39°C), gassed (95%  $\text{O}_2$ , 5%  $\text{CO}_2$ ), Ringers solution. After removal of the muscle, the mucosa was allowed to equilibrate for 15 minutes in Ringers solution before being placed in the test solutions. The time interval from removal of the colonic section to placement of the mucosa in the Ringers did not exceed 10 minutes.

After equilibration, a portion of the mucosa from each pig was placed in the test solutions. Samples were taken after 0, 15 and 30 minutes of incubation in the solution with mannitol and 0, 2, 5, 10 and 30 minutes of incubation in theophylline solution. All samples were clamp frozen between aluminum blocks, which had been kept in liquid nitrogen, and the samples stored in liquid nitrogen until assayed for cyclic nucleotides. Extraction of cyclic nucleotides for assay was performed after homogenization of the mucosa. To obtain the homogenate, the tissue, while being kept under liquid nitrogen, was ground with mortar and pestle (Hess and Brand, 1974). A portion of the homogenized mucosa was transferred immediately to a tared tube, weighed, and 1 ml of cold 6% trichloroacetic acid added to precipitate cellular proteins and inhibit phosphodiesterase activity. The material, along with 5 nCi/ml of  $^3\text{H}$ -cyclic AMP or  $^3\text{H}$ -cyclic GMP added as a recovery marker, was mixed and allowed to stand for 15-30 minutes in a 4°C ice bath. The tubes were then centrifuged at 3500 x g for 15 minutes at 4°C, and the

supernatant removed. The supernatant was then extracted four times with 5 ml washes of water saturated ether (Fisher Chemical Co., Pittsburgh, PA.). The excess ether was driven off by heating in 70°C water bath, and the samples shell frozen in dry ice and 95% ethanol and lyophilized. The lyophilized samples were redissolved in 1 ml of the sodium acetate buffer supplied in the radioimmunoassay kit (New England Nuclear, Boston, MA.). The radioimmunoassay, for both cyclic AMP and cyclic GMP was a competitive binding reaction utilizing  $^{125}\text{I}$  as the marker. Each sample was extracted and measured according to the procedure described in the kit. Duplicate samples of each tissue were assayed for cyclic AMP and triplicate samples of each tissue assayed for cyclic GMP.

Direct counts of  $^3\text{H}$ -cyclic AMP or  $^3\text{H}$ -cyclic GMP, in 100  $\mu\text{l}$  aliquots removed before and after extraction, were used to measure recovery rates. These samples were diluted in 15 ml Biofluor scintillation cocktail and counted to less than 5% relative standard deviation in a Beckman CS 8000 LCS (Beckman Inst. Inc., Irvine, CA.).

Counts of  $^{125}\text{I}$ , used to calculate cyclic nucleotide concentrations, were determined to 2% relative standard deviation by solid scintillation spectrometry on a Searle Automated Gamma Counter with a NaI(TL) crystal (G. D. Searle Co., Des Plaines, IL.). Equations used for calculation of cyclic nucleotides are given in Appendix A.

### Chemical analyses

Concentrations of  $\text{Na}^+$  were determined by flame photometry (Beckman Model 105 Flame Photometry, Beckman Inst. Inc., Irvine, CA.) and  $\text{Cl}^-$  by automated colorimetry (Technicon Auto Analyzer, Technicon Inst. Corp., New York, NY.). PEG 4000 concentrations were measured by the method of Hyden (1956) (See Appendix A) or by direct counts of  $^{14}\text{C}$  labeled PEG. Triplicate samples were diluted in 15 ml Brays or Bioflour scintillation cocktail (New England Nuclear, Boston, MA.) and counted to less than 2% relative standard deviation in a Packard Tri-Carb #3380 LCS (Packard Inst. Co., Downers Grove, IL.) or a Beckman LS 8000 LCS. Osmolality was obtained from freezing point depression (Fiske Osmometer, Fiske Associates, Uxbridge, MA.). Final pH and  $\text{PCO}_2$  were measured prior to exposure to air on a Corning Model 165 blood gas analyzer (Corning Glass Works, Medfield, MA.). Acidification and back titration to a pH 7.0 end point was used to determine  $\text{HCO}_3^-$  concentration (Dobson, 1959) (See Appendix A).  $^{22}\text{Na}$  counts were measured on triplicate samples to less than 2% relative standard deviation by solid scintillation spectrometry on a Searle #1185 Auto Gamma Counter with a  $\text{NaI(Tl)}$  crystal.

Calculations

Net water movement was calculated from the following equation:

$$J_v = \left[ \frac{[\text{PEG}_i] (V_i)}{[\text{PEG}_o]} - V_o \right] \times \left[ \frac{[\text{PEG}_o]}{[\text{PEG}_f]} - 1 \right]$$

$J_v$  = net movement of water (ml)

$\text{PEG}_i$  = concentration of PEG 4000 or counts per minute (cpm)/ml  $^{14}\text{C}$  PEG in the initial solution

$\text{PEG}_o$  = concentration of PEG 4000 or cpm/ml of  $^{14}\text{C}$  PEG in time zero sample

$\text{PEG}_f$  = concentration of PEG 4000 or cpm/ml of  $^{14}\text{C}$  PEG in final sample (1 hour)

$V_i$  = volume injected into the loop

$V_o$  = volume removed as time zero sample

Net ion fluxes were determined by:

$$\text{Net ion} = [V_f (\text{ion}_f)] - [V_o (\text{ion}_o)]$$

where  $V_f$  = final volume of the loop (ml)

$\text{ion}_f$  = final ion concentration (mEq/L)

$V_o$  = initial volume at the loop (ml)

$\text{ion}_o$  = initial ion concentration (mEq/L)

Net solute movement was measured as:

$$\text{Net solute} = [(\text{osmolality}_f) (V_f)] - [(\text{osmolality}_o) (V_o)]$$

where osmolality<sub>f</sub> = final osmolality (mosm)

V<sub>f</sub> = final volume of loop (ml)

osmolality<sub>o</sub> = initial osmolality (mosm)

V<sub>o</sub> = initial volume of the loop (ml)

Unidirectional fluxes were determined according to the methods of Berger and Steele (1958) using the following equations and calculations.

$$J_{L \rightarrow B}^{Na^+} = \left[ \left( \frac{[\ln (\phi A_f / \phi A_o)]}{[-\ln (A_t / A_o)]} \right) - 1 \right] (A_f - A_o)$$

$J_{L \rightarrow B}^{Na^+}$  = lumen to blood flux of Na<sup>+</sup>

$\phi A_f$  = specific activity of <sup>22</sup>Na<sup>+</sup> at final time  
(cpm <sup>22</sup>Na<sup>+</sup>/mEq Na<sup>+</sup>)

$\phi A_o$  = specific activity of <sup>22</sup>Na<sup>+</sup> at time zero  
(cpm <sup>22</sup>Na<sup>+</sup>/mEq Na<sup>+</sup>)

A<sub>t</sub> = A<sub>f</sub> = Na<sup>+</sup> (mEq) at final time (one hour)

A<sub>o</sub> = Na<sup>+</sup> (mEq) at time zero

$$J_{B \rightarrow L}^{Na^+} = \text{blood to lumen flux of Na}^+ = J_{Net}^{Na^+} - J_{L \rightarrow B}^{Na^+}$$

Net movements are expressed on the basis of ml/g mucosa/hr for water, mEq/g mucosa/hr for ions, and mosm/g mucosa/hr for total solute.

### Statistical analysis

Computer assisted one way analysis of variance (Statistical Analysis System, Cary, NC.), and Student's t tests (Snedecor and Cochran, 1967) were used to determine significance between groups.

### Cultural techniques

Selective isolation T. hyodysenteriae was selectively isolated by the method of Songer, et al., (1976) on trypticase soy agar (BBL Inc., Cockeysville, MD.) supplemented with 5% citrated bovine blood, and 400 mcg/ml spectinomycin (Upjohn, Kalamazoo, MI.). Plates were incubated at 42°C under an atmosphere of 80:20, H<sub>2</sub>:CO<sub>2</sub> in an anaerobic jar containing palladium catalysts. Plates were examined every 48 hrs. over a 6 day period for growth of T. hyodysenteriae.

Viable count determinations All viable counts of T. hyodysenteriae were performed in duplicate on trypticase soy agar plates supplemented with 5% citrated bovine blood. Plates were incubated as for selective isolation and observed for growth after 48 and 72 hrs.

### Histopathology

All sections taken for histopathology were formalin fixed, paraffin embedded, sectioned, and stained with hematoxylin and eosin, and Warthin-Starry stains.

## RESULTS

In vivo ExperimentsType I studies

Theophylline dose response, net effects of solutions 1-5 in ligated colonic loops of normal pigs The net fluxes of water, solute,  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{HCO}_3^-$ , and the final  $\text{pCO}_2$  for colonic loops exposed to increasing concentrations of theophylline are presented in Figure 2 (Appendix, Table B1). At low doses, net absorption of solute, water and  $\text{Na}^+$  decrease while at high doses, net secretion occurs. Net absorption of  $\text{Cl}^-$  decreases, as theophylline concentration increases but no secretion was observed. With increasing doses, net secretion of  $\text{HCO}_3^-$  increases while the  $\text{pCO}_2$  of the loop decreases. Although plasma  $\text{pCO}_2$  was not measured, the  $\text{pCO}_2$  values at the higher concentrations are near expected plasma values.

All changes in net water and net ion movements with the exception of  $\text{HCO}_2^-$  were significant at the 10 mM or greater dose of theophylline. Changes in  $\text{HCO}_3^-$  and  $\text{pCO}_2$  were significant at a dose of 20 mM theophylline.

The absorption pattern of  $\text{Na}^+$  was established in the acute colonic loops incubated two hours. The results are presented in Figure 3 (Appendix Table B2). Net absorption from control solution followed a linear pattern becoming significant after 60 minutes incubation. This pattern of  $\text{Na}^+$  absorption in the control loop was confirmed by linear regression analysis ( $r = .85$ ).

Figure 2. Effect of increasing doses of theophylline, solutions 1-5, on net water and solute transport and luminal pCO<sub>2</sub> in ligated colonic loops of normal pigs

Positive values indicate net absorption, negative values net secretion ( $\pm$  s.e.).

\* p < .05 Significant differences from control solution (#1) with no theophylline.  
\*\* p < .01



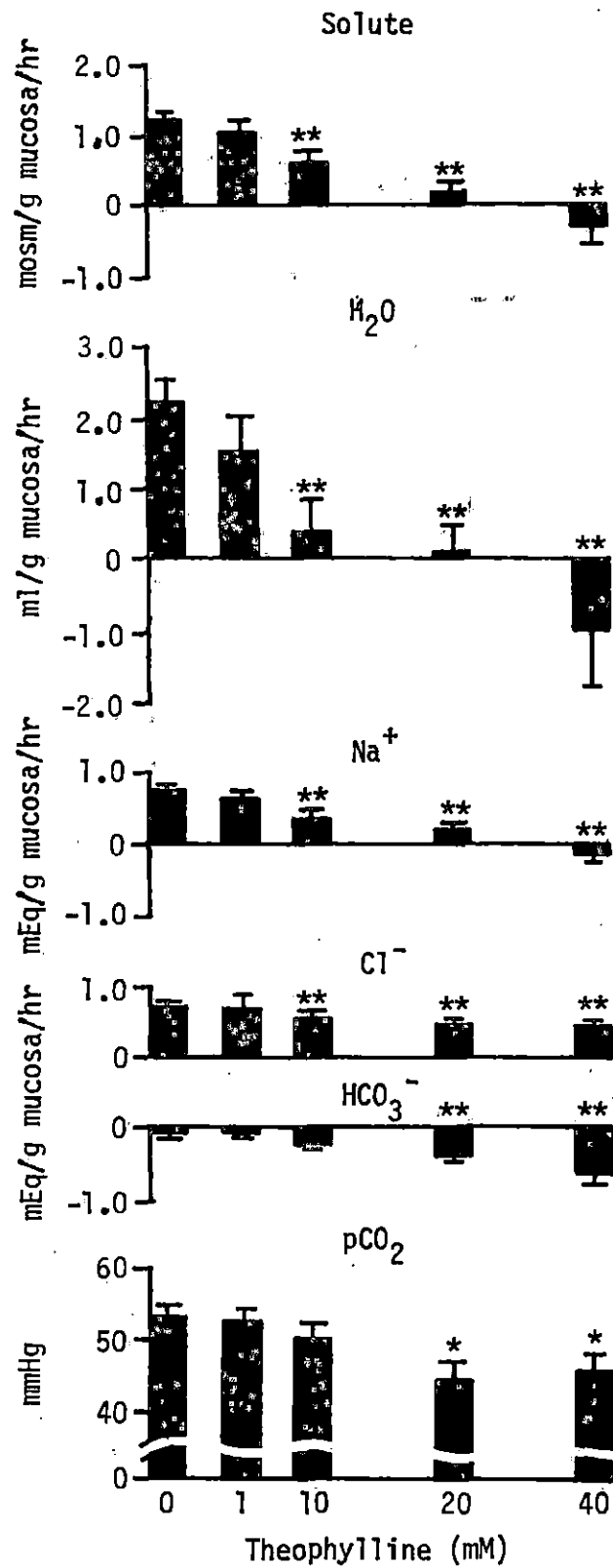
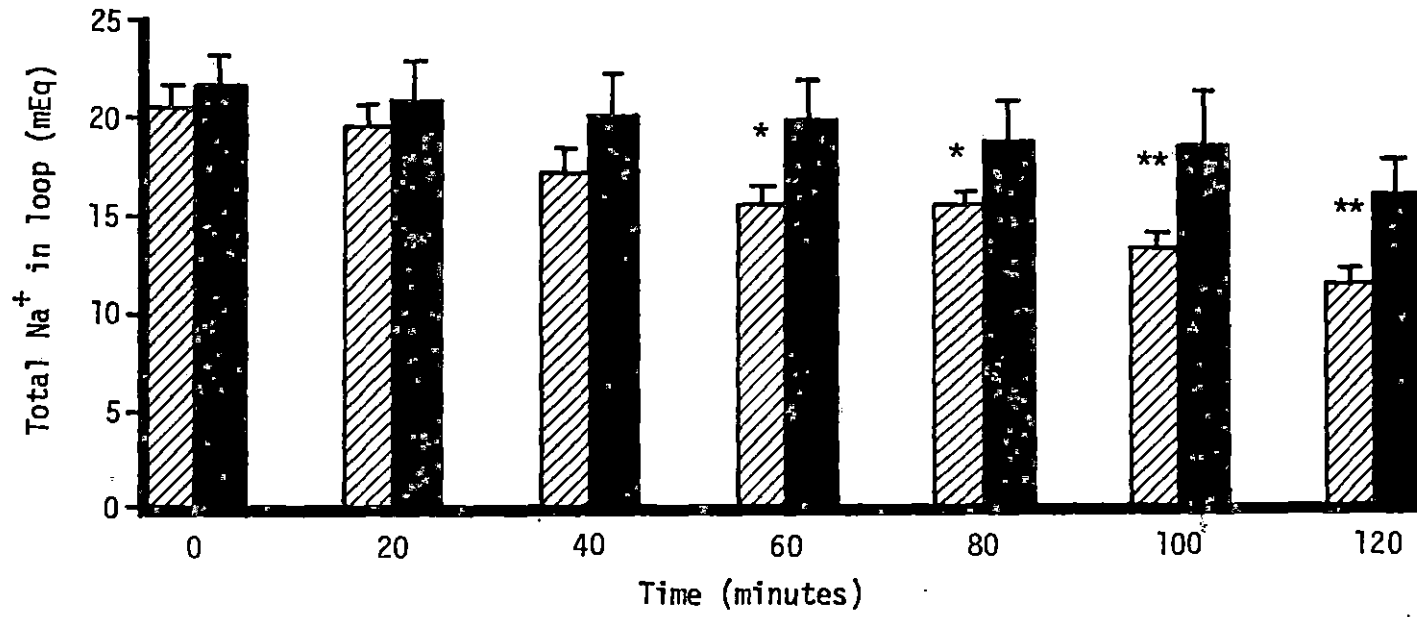



Figure 3. Absorption pattern of Na<sup>+</sup>. Sodium (mEq) remaining in ligated colonic loops over a 2 hour incubation period (± s.e.)

\* p < .05 Significant differences from time zero.

\*\* p < .01



 Control

 Theophylline (40 mM)

Absorption of  $\text{Na}^+$  from the loop exposed to 40 mM theophylline was not significant over the two hour time period. These results are important for two reasons. The first is that the pattern of  $\text{Na}^+$  absorption validates the use of the unidirectional flux equations. The other is that the effects of theophylline were present during the incubation period used in these loop studies.

### Type II studies

Effects of theophylline in sodium free solutions (1, 5, 6; and 7) on net transport in ligated colonic loops of normal pigs The results of the Type II studies are shown in Figures 4 and 6 (Appendix Table B3). It should be noted, that with the exception of net  $\text{Cl}^-$  movement, all net ion and water movements were significant in tissues exposed to 40 mM theophylline (solution #5) when compared to control solution (#1). These findings are identical to those of Type I studies.

The studies with choline replacement solutions present confusing results. Since some  $\text{Na}^+$  absorption occurred from the  $\text{NaHCO}_3$  and residual  $\text{NaCl}$  flush solution, the solutions were not entirely  $\text{Na}^+$  free. Comparison of loops incubated with choline to those with control solution showed some differences. Net solute and  $\text{Na}^+$  absorption were significantly reduced. Net water and  $\text{Cl}^-$  movements were relatively unaffected.

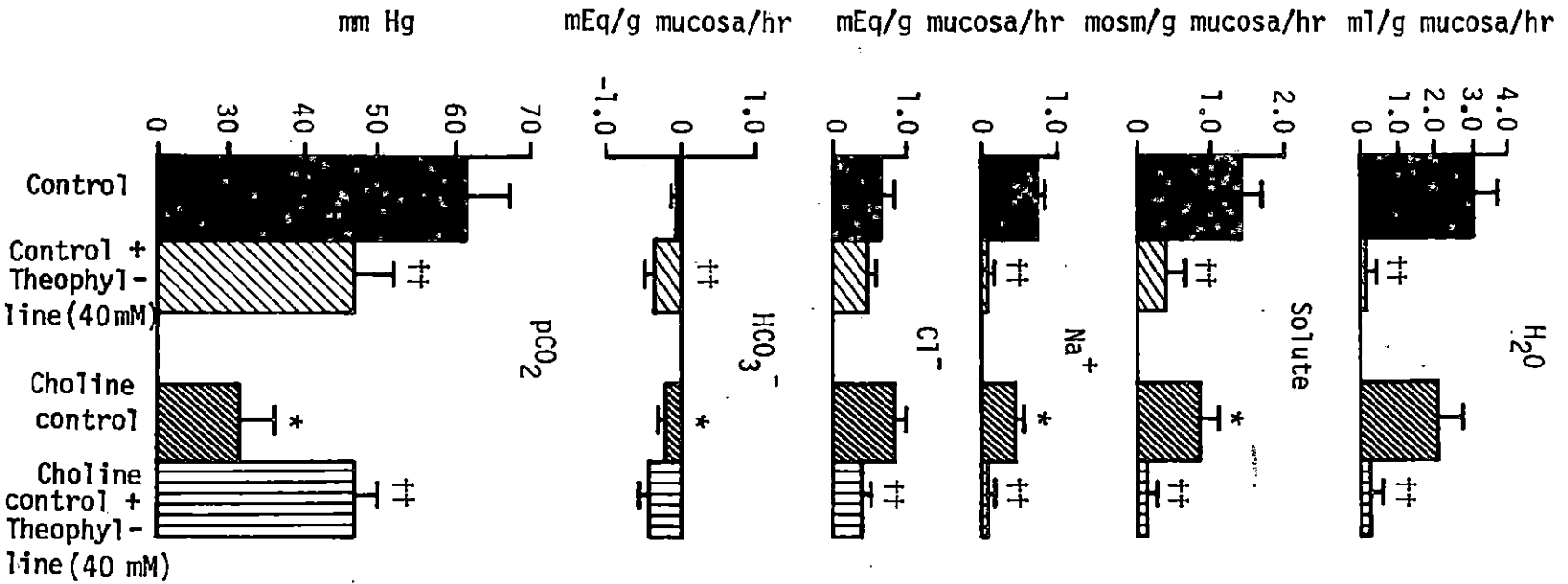
Addition of 40 mM theophylline to the choline solution produced significant additional effects on all parameters with the exception of

Figure 4. Effects of theophylline in sodium free solutions (1, 5, 6, and 7) on net water and solute transport and luminal  $pCO_2$  in ligated colonic loops of normal pigs

Positive values indicate net absorption,  
negative values net secretion ( $\pm$  s.e.).

\*  $p < .05$  Significant differences between corresponding solutions: control (#6), control + theophylline (#5) vs choline control + theophylline (#7).

†  $p < .05$  Significant differences between control (#1) vs control + theophylline (#5) or choline control (#6) vs choline control + theophylline (#7).



$\text{HCO}_3^-$  secretion. Net absorption of water, solute,  $\text{Na}^+$  and  $\text{Cl}^-$  were all significantly decreased in loops with choline plus theophylline when compared to loops with choline alone. Final  $\text{pCO}_2$  levels increased unexpectedly in loops with choline plus theophylline.

No significant changes were found between the control solution with 40 mM theophylline (solution 5) and the choline solution with 40 mM theophylline.

Unidirectional movements of  $\text{Na}^+$  were determined by adding  $10\mu\text{Ci/L}^{22}\text{Na}$  to solutions 1 and 5. Figure 6 shows that addition of 40 mM theophylline decreases the lumen to blood movement of  $\text{Na}^+$  without affecting the blood to lumen flux. The effect of theophylline on  $\text{Na}^+$  transport was to abolish net  $\text{Na}^+$  absorption by decreasing lumen to blood movement, rather than increasing blood to lumen movement.

The low volume loops (ca. 25 ml) used in this study with solutions 1 and 5 produced similar results as the larger volumes routinely employed. Luminal hydrostatic driving forces did not appear to alter net colonic transport.

### Type III studies

Effects of theophylline on colonic transport in ligated colonic loops of dysentery affected pigs (Solutions 1, 3 and 5) Results of net transport from solutions 1, 3 and 5 in ligated colonic loops of

infected pigs compared to values obtained from normal pigs are presented in Figure 5 (Appendix Table B4).

Comparison of control solutions (#1) in the two groups of ligated loops shows significant net secretion of water and solute in infected loops. Net absorption of  $\text{Na}^+$  was decreased by almost 80%, net movement of  $\text{Cl}^-$  was abolished, and  $\text{HCO}_3^-$  was absorbed.  $\text{pCO}_2$  levels were greatly elevated in infected loops. Unidirectional  $\text{Na}^+$  fluxes (Figure 6) indicate a significant decrease in lumen to blood movement but no increased blood to lumen movement. These results are slightly different from those of Argenzio, et al. (1980), since net  $\text{Na}^+$  absorption, although at a much reduced rate, still occurred in the ligated colonic loops of the infected pigs.

Comparison of corresponding solutions containing either 10 mM or 40 mM theophylline in ligated colonic loops of normal and infected pigs also shows significant differences. All net movement, with the exception of net  $\text{HCO}_3^-$  flux, are significantly less in loops of infected animals than those of normal animals when exposed to 10 mM theophylline. At 40 mM theophylline, only net transport of  $\text{Cl}^-$  and  $\text{HCO}_3^-$  were significantly different in ligated loops of infected pigs. Significant changes in ligated loops of normal pigs exposed to theophylline are those observed in Type I experiments. When compared to the control solution, addition of theophylline produced few changes in net transport in ligated loops of infected pigs.  $\text{Na}^+$  transport was decreased with 10 mM but not 40 mM level of theophylline. Net  $\text{HCO}_3^-$



Figure 5. Effects of theophylline, solutions 1, 3, and 5, on net water and solute transport and luminal pCO<sub>2</sub> in ligated colonic loops of dysentery affected pigs

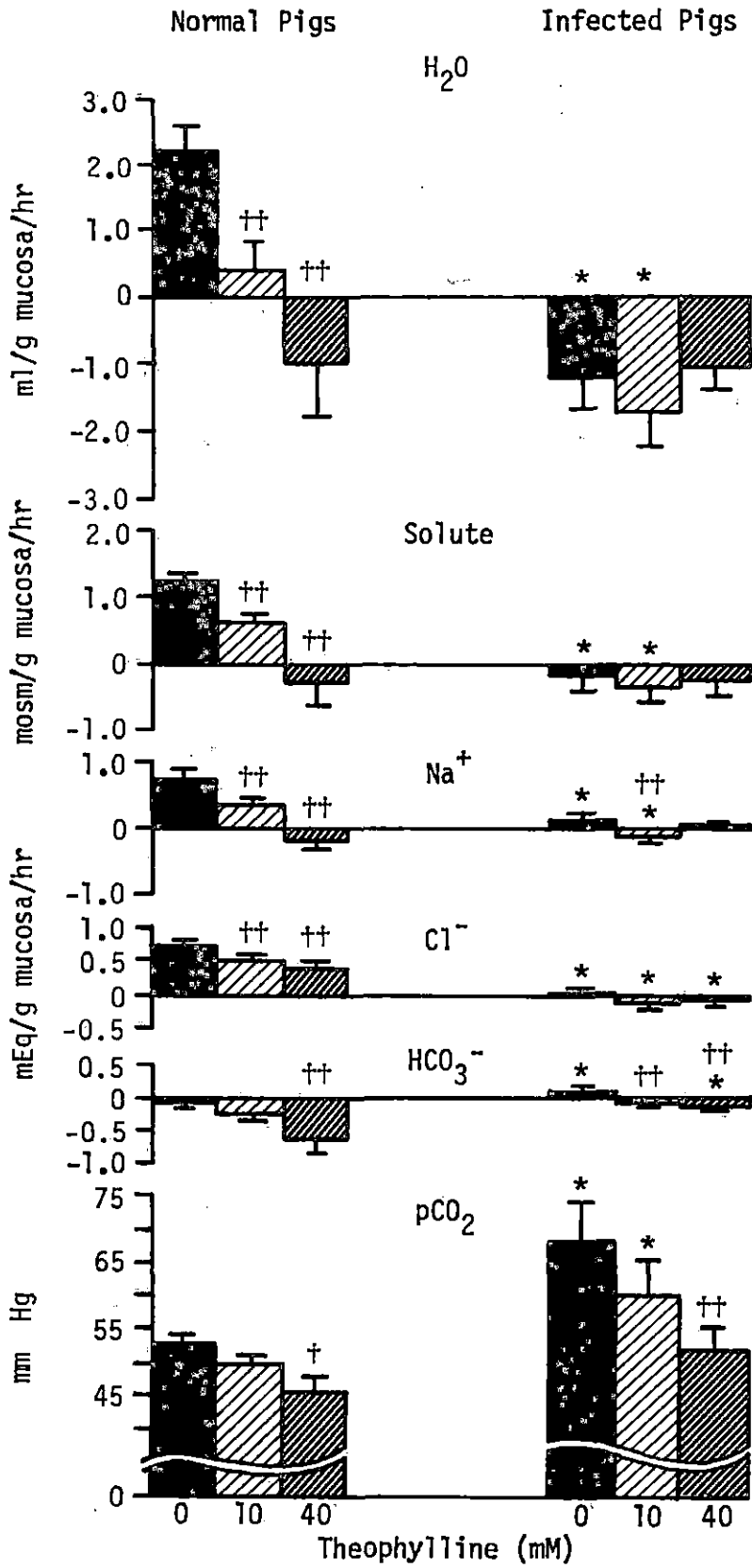
Positive values indicate net absorption, negative values net secretion ( $\pm$  s.e.).

\*  
p < .05 Significant differences between corresponding solutions in normal and infected pigs.

\*\*  
p < .01

†  
p < .05 Significant differences from control solution (#1) in normal and infected pigs.

††  
p < .01



secretion was increased with both levels. Final  $pCO_2$  decreased significantly only with 40 mM theophylline as did the lumen to blood flux of  $Na^+$ .

Dysentery was confirmed in each of the seven pigs used in this study by observation of gross lesions, selective isolation of *I. hyodysenteriae*, and histopathologic examination of a section of colon from each animal. Gross lesions ranged from a mucohemorrhagic to fibrinonecrotic colitis with diphtheritic pseudomembrane formation. Lesions tended to be diffuse rather than focal but small areas of normal appearing colonic mucosa could be found in some animals. An evaluation of the microscopic lesions observed in the colonic sections of each of the animals is presented in Table 2. The most common lesions were mucosal hyperemia and edema, crypt dilation, and mucosal erosion. Where mucosal erosion had occurred, varying amounts of necrotic debris were present in the lumen of the colon.

### In vitro Experiments

#### Type IV studies

Measurements of cyclic AMP and cyclic GMP concentrations in normal and dysenteric pig colonic mucosa exposed *in vitro* to theophylline  
Concentrations of cyclic AMP and cyclic GMP in normal and infected colonic mucosa are presented in Figures 7 and 8 (Appendix Tables B5 and B6).

In normal mucosa, both cyclic AMP and cyclic GMP increased significantly within two minutes of exposure to theophylline, with

Table 2. Microscopic lesions observed in colonic sections from  
dysentery affected pigs

Pig #	Mucosal Hyperemia	Mucosal Swelling	Crypt Dilatation	Mucosal Erosion
1	++ <sup>a</sup>	+++	++	++
2	+	++	+	+
3	+++	+++	+++	++
4	+++	+++	+++	+++
5	+	+	+	++
6	+	++	+	++
7	+++	++	++	++

<sup>a</sup>+ → +++ least severe → most severe.

Figure 6. Effect of theophylline on unidirectional  $\text{Na}^+$  fluxes in ligated colonic loops of normal and swine dysentery affected pigs ( $\pm$  s.e.)

$J_{LB}^{\text{Na}^+}$  Lumen to blood flux.

$J_{BL}^{\text{Na}^+}$  Blood to lumen flux.

\*  $p < .05$  Significant differences between corresponding solutions in normal and infected pigs.

†  $p < .05$  Significant differences from control solution (#1) in normal and infected pigs.

††  $p < .01$

Unidirectional  $\text{Na}^+$  flux

Normal Pigs

Infected Pigs

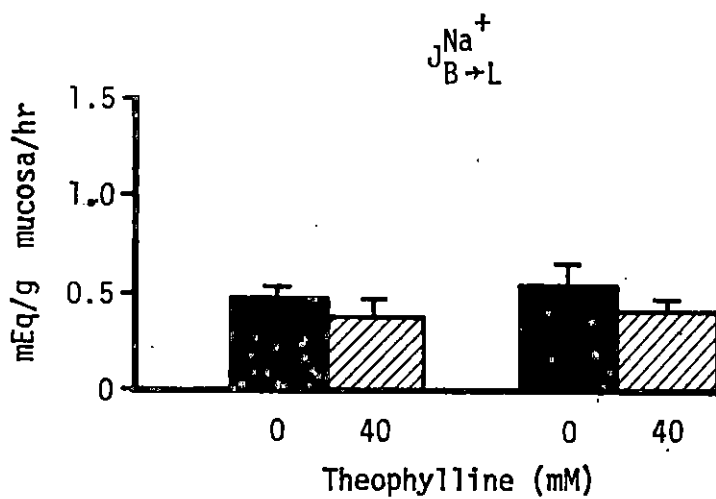
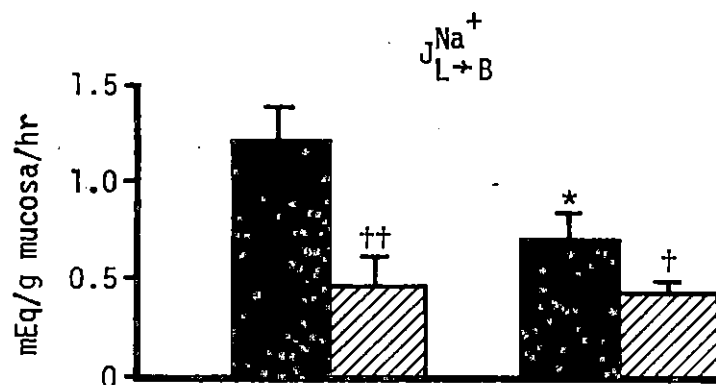


Figure 7. Cyclic AMP concentrations in colonic mucosa of normal and dysentery affected pigs exposed to Ringers solution + mannitol (20 mM) or Ringers solution + theophylline (20 mM) (± s.e.)

(pmoles = picomoles  $10^{-12}$  M).

\* p < .05 Significant differences from time 0 in either normal or infected mucosa.

\*\* p < .01

p < .05 Significant differences between corresponding solutions in normal

p < .01 and infected mucosa.

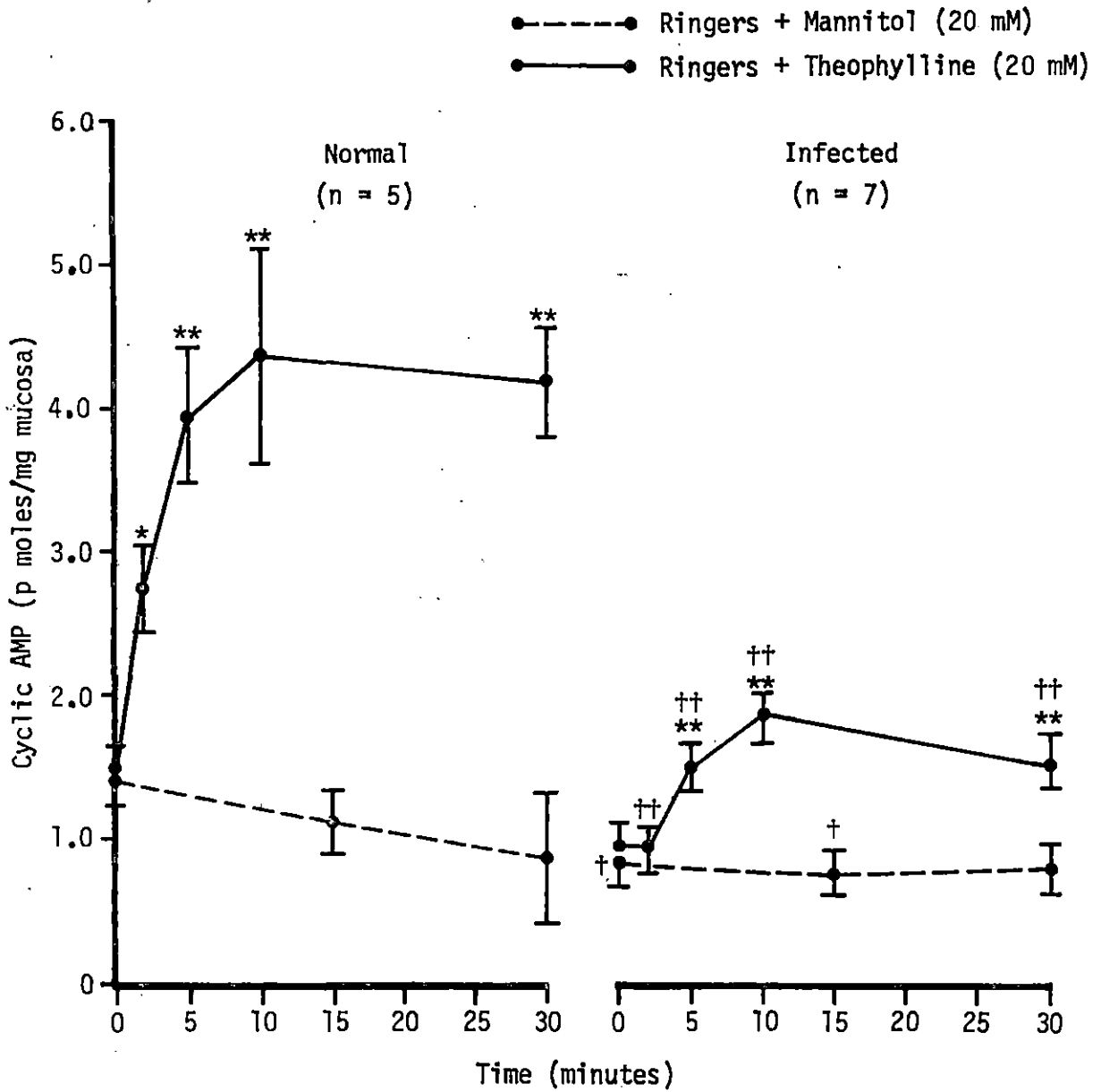




Figure 8. Cyclic GMP concentrations in colonic mucosa of normal and dysentery affected pigs exposed to a Ringers solution + mannitol (20 mM) or Ringers solution + theophylline (20 mM) (± s.e.)

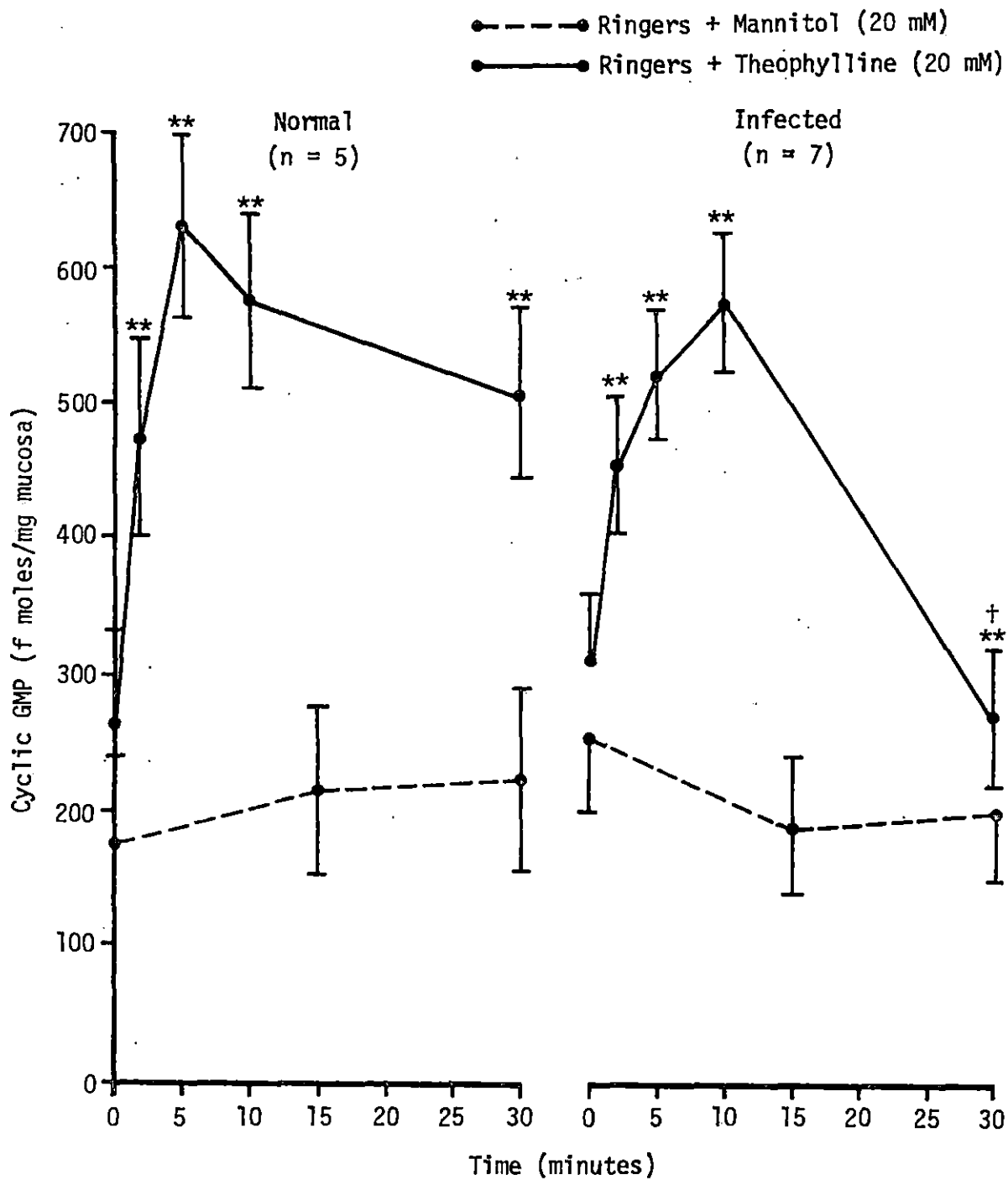
(f moles = femtomoles  $10^{-15}$  M).

\*  $p < .05$  Significant differences from time 0 in either normal or infected mucosa.

\*\*  $p < .01$

†  $p < .05$  Significant differences between corresponding solutions in normal and infected mucosa.

††  $p < .01$



cyclic AMP increasing 3.6 fold within 10 minutes and cyclic GMP 3.7 fold within 5 minutes. Significant changes were not present in the levels of either cyclic nucleotide exposed to the Ringers solution with 20 mM mannitol.

Incubation of infected colonic mucosa in Ringers plus 20 mM theophylline produced significant, though smaller, elevations of cyclic nucleotides. Increases in cyclic AMP levels were significantly less at all times in infected mucosa exposed to theophylline than in uninfected mucosa. The only significant difference in cyclic GMP levels was after 30 minutes of incubation. Quantitatively, cyclic AMP increased 2.1 fold and cyclic GMP 2.6 fold, both after 10 minutes incubation. No increased concentrations of either cyclic AMP or cyclic GMP were observed in infected mucosa incubated with mannitol. Concentrations of cyclic AMP were significantly less in infected control than uninfected control mucosa while no significant differences were observed in concentrations of cyclic GMP in the control tissues.

## DISCUSSION

In vivo and in vitro ExperimentsThe effects of theophylline on colonic transport in ligated colonic loops of normal pigs

Theophylline (1, 3 dimethylxanthine) has been demonstrated to elevate levels of cyclic nucleotides by inhibiting the activity of phosphodiesterase (Butcher and Sutherland, 1962). Therefore, the effects of theophylline mimic those of increased concentrations of cyclic AMP (Field, 1971a).

In ligated colonic loops of the pig, the net effects of theophylline were to abolish net absorption of  $\text{Na}^+$ , total solute, and water, decrease absorption of  $\text{Cl}^-$ , increase net  $\text{HCO}_3^-$  secretion, and decrease luminal  $\text{pCO}_2$  levels. These results are in contrast to those reported in other segments of the pig intestine and in intestinal tissues of other species.

Pig (Hamilton, et al., 1977) and dog (Pierce, et al., 1971) jejunum under in vivo conditions, and rabbit ileum (Field, 1971a) under in vitro conditions responded by secreting  $\text{Na}^+$ ,  $\text{Cl}^-$ , and water when exposed to theophylline. Increased concentrations of cyclic AMP abolish net  $\text{Na}^+$  absorption and elicit  $\text{Cl}^-$  secretion in in vitro studies of the rat colon (Binder and Rawlins, 1973b). In contrast, in vitro studies of rabbit colon exposed to increased cyclic AMP concentrations demonstrated active  $\text{Cl}^-$  secretion but no effect on  $\text{Na}^+$  absorption (Frizzell, et al., 1976). Under in vivo conditions, colonic mucosa of the rat exposed to cholera toxin secreted  $\text{Na}^+$ ,  $\text{Cl}^-$ , and water, while exposure to E. coli LT had no effect (Donowitz and Binder, 1976). This was

surprising since both enterotoxins were thought to mediate their effects through elevations of cyclic AMP. Thus the effect of elevated cyclic AMP concentrations on ion transport in these various species and tissues contrasts markedly from that presently observed.

Before discussing the effects of theophylline on the transport processes of the pig colon, it should be noted that values for net absorption of ions and water from the control solution, if extrapolated to a 2 hour incubation period, are similar to those reported previously for this model (Argenzio, et al., 1980). The two basic transport mechanisms postulated for the pig colon are a  $\text{Na}^+-\text{H}^+$  and a  $\text{Cl}^--\text{HCO}_3^-$  exchange process (Fig. 9). Water is thought to be absorbed passively as a result of decreased luminal osmolality owing to the titration of  $\text{H}^+$  and  $\text{HCO}_3^-$  to  $\text{H}_2\text{O}$  and  $\text{CO}_2$ . Potassium ( $\text{K}^+$ ) is distributed passively according to its electrochemical gradient across the epithelium. A blood side positive 20 mV potential difference is present across the epithelium. Based on the proposed transport mechanisms, the decrease in net  $\text{Na}^+$  absorption, increased  $\text{HCO}_3^-$  secretion, and reduced  $\text{pCO}_2$  produced by theophylline, could be explained simply by interference with the  $\text{Na}^+-\text{H}^+$  exchange mechanism. Unidirectional  $\text{Na}^+$  fluxes are consistent with this possibility. A marked decrease in lumen to blood with no increase in blood to lumen movement of  $\text{Na}^+$  was observed. In the absence of the  $\text{Na}^+-\text{H}^+$  exchange,  $\text{HCO}_3^-$  would accumulate and  $\text{pCO}_2$  decrease in the luminal solution (see Fig. 9). This model does not account for all the observed

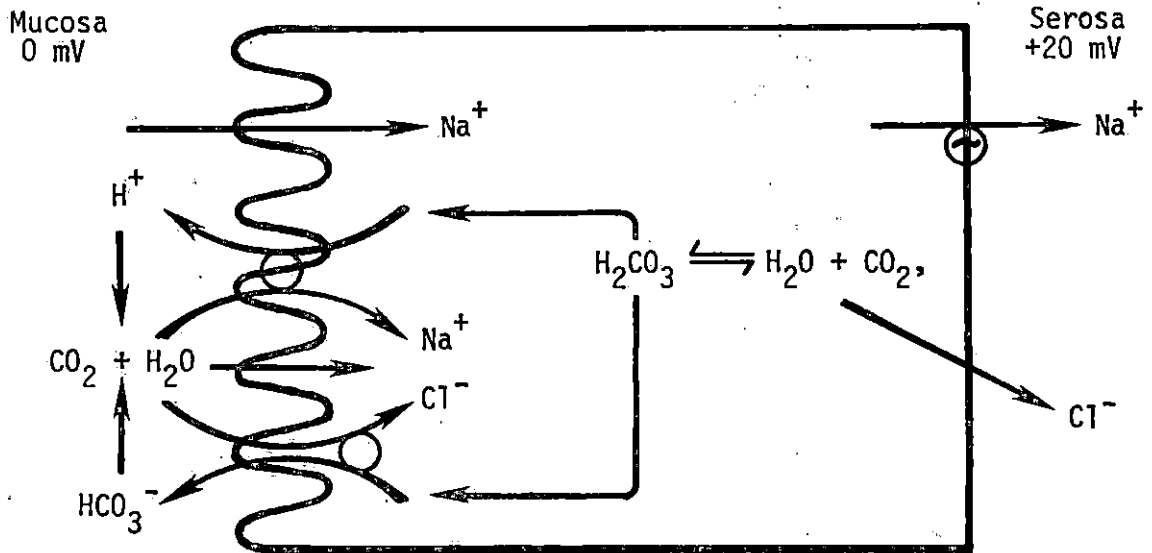


Figure 9. Normal transport mechanisms of the pig colonic mucosal epithelium. [One third of net  $\text{Na}^+$  absorption is by electrogenic pathway and two thirds by the  $\text{Na}^+-\text{H}^+$  exchange process. The  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange accounts for absorption of  $\text{Cl}^-$  which then diffuses across serosal border. The  $\text{HCO}_3^-$  is titrated by  $\text{H}^+$  to  $\text{H}_2\text{O}$  and  $\text{CO}_2$  which then diffuse back into the cell where the reverse reaction may occur]

(Argenzio, R. A., and Whipp, S. C., 1979).

effects since decreased net  $\text{Cl}^-$  absorption would not be expected in the presence of a normal  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange. It may be that theophylline has an effect on the  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange or affects secretion of either  $\text{Cl}^-$  or  $\text{HCO}_3^-$ .

The replacement studies were designed to eliminate net  $\text{Cl}^-$  absorption that may be electrically coupled to  $\text{Na}^+$  absorption. The action of theophylline on the net absorption of  $\text{Cl}^-$  alone could then be examined. However, significant  $\text{Na}^+$  absorption occurred from the solutions making interpretation of the results difficult if not impossible. Further problems with interpretation were encountered due to inexplicably low  $\text{pCO}_2$  values from loops exposed to a choline solution without theophylline. Although these studies cannot confirm the mechanisms involved in decreased net  $\text{Cl}^-$  absorption, several possibilities should be considered.

Either an active or passive blood to lumen movement of  $\text{Cl}^-$  accompanied by a decrease in the transepithelial potential difference, would be possible explanations. Such movement should be detectable using isotopically labelled  $\text{Cl}^-$  and by measuring the potential difference across the epithelium. This model would assume the normal function of the  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange to account for net  $\text{HCO}_3^-$  secretion. An alternative would be the inhibition of the  $\text{Cl}^-$ - $\text{HCO}_3^-$  exchange process and production of active  $\text{HCO}_3^-$  secretion. Electrogenic secretion of  $\text{HCO}_3^-$  would increase the transepithelial potential difference and create a driving force for passive  $\text{Cl}^-$  absorption.

Redistribution of a passively distributed ion, such as  $K^+$ , would be expected to accompany this change in potential difference.

Further in vivo studies with ion replacement solutions and measurement of the potential difference, and possibly in vitro studies, are necessary to assess the effects of theophylline on  $Cl^-$  absorption.

The results of the cyclic nucleotide assay definitely establish the ability of the pig colon to respond to theophylline with increases in both cyclic AMP and cyclic GMP. These results were obtained from in vitro studies, and were not directly correlated to transport changes observed in vivo. The rapid elevations and sustained levels over a 30 minute interval suggest that the effect on transport processes was equally as rapid and sustained for the majority of the one hour ion flux period. The absorption pattern of  $Na^+$  over a 2 hour period in loops exposed to theophylline also indicate the activity of theophylline to last for well over the one hour incubation period used in the ligated loop model. However, studies correlating the time course of the effects of theophylline on transport and cyclic nucleotide elevations would be of value.

The present results demonstrate that decreased net absorption of  $Na^+$  and fluid, and net secretion of  $HCO_3^-$  accompany exposure of the pig colonic mucosa to theophylline. Even at high doses, marked net fluid and electrolyte secretion, such as has been reported in the small intestine of the pig, does not occur in the pig colon. These



results indicate that either the pig colon is not capable of net secretion, as is the small intestine, or that cyclic AMP and cyclic GMP do not control the secretory processes that are present.

If cyclic AMP and cyclic GMP exert influences over the  $\text{Na}^+ - \text{H}^+$  exchange and  $\text{Cl}^- - \text{HCO}_3^-$  exchange processes, bacterial enterotoxins, that mediate the action through cyclic nucleotides, may have an effect on colonic transport. Prostaglandin induced elevations of cyclic nucleotides, as have been documented in the small intestines in cases of salmonellosis in other species may also function in inflammatory diseases of the pig colon. In either instance, the decrease in net electrolyte and fluid absorption by the colon would be deleterious to the pig.

#### The effects of theophylline on colonic transport in ligated colonic loops of dysentery affected pigs

Before examining the effects of theophylline, the net transport of fluid and electrolytes from a control solution in ligated colonic loops of infected pigs should be considered. The results obtained in this study are in close agreement with those of Argenzio, et al. (1980). In loops of infected pigs, net absorption of all electrolytes was abolished or severely impaired and small amounts of water and total solute secreted. Significant increases in luminal  $\text{pCO}_2$  were also observed when compared to control values. Net  $\text{Na}^+$  absorption was reduced by 80% due to a decrease in the lumen to blood movement. No

increase in the blood to lumen flux was observed. That net  $\text{Na}^+$  absorption did occur indicates some functional mucosa remains. A correlation may exist between severity of the lesions and the decrease in net  $\text{Na}^+$  absorption. However, such a correlation is not possible based on the results of this study, since only one section of colonic mucosa from each animal was examined for microscopic lesions.

The microscopic lesions and the net  $\text{Na}^+$  absorption in experimental colonic loops of a number of infected animals should be compared to provide statistical correlation.

The net secretion of water which was observed should not be interpreted as an active secretory process. Argenzio and Whipp (1979) have demonstrated that a fluid circuit, such as has been documented in the small and large intestine of the rat (Munck and Rasmussen, 1977; Wanitschke, et al., 1977) probably functions in the pig colon. Under this situation, secretion of water will take place when net solute transport is abolished. This was observed in normal pigs where theophylline abolished net solute transport (Fig. 10). In the infected colonic loops, where net solute absorption is zero, net water secretion would be expected. This was, in fact, exactly what was observed in the ligated colonic loops of the infected pigs (Fig. 10). It should also be noted that the slopes and intercepts of these two lines are not significantly different. The 95% confidence interval on the data from normal pigs includes that from the swine dysentery affected pigs. Wilcock and Olander (1979a) have reported that net fluid secretion occurs in ligated colonic loops of pigs inoculated with pure cultures

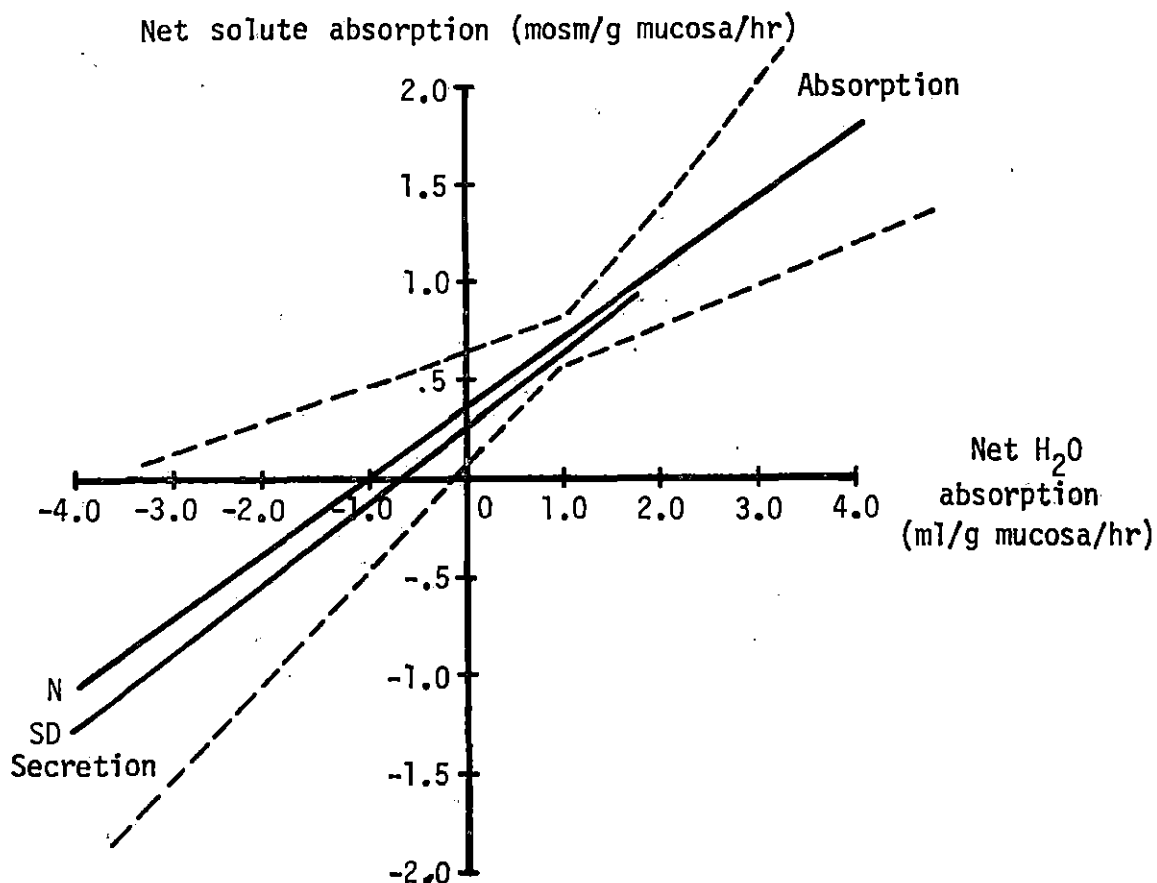


Figure 10. Relationship of net water and total solute transport in ligated colonic loops of normal pigs (N) exposed to solutions 1-5 and dysentery affected pigs (SD) exposed to solutions 1, 3, and 5

The entire range of values are shown. Positive values indicate net absorption, negative values net secretion. The 95% confidence limits are shown for line N.

$$[N = .36x + .35 (r = .91), \text{intercept} = .35 \pm .05$$

$$SD = .39x + .27 (r = .91), \text{intercept} = .27 \pm .06].$$

of *T. hyodysenteriae* or crude colonic material from infected pigs, and have suggested this may be due to an active secretory process. It is more probable that the net fluid accumulation which took 48-72 hours to occur, was a result of damage to the mucosal epithelium, which decreased net solute absorption and allowed fluid to enter the loop via the fluid circuit.

The significantly elevated  $pCO_2$  levels in the ligated colonic loops of infected pigs could not be interpreted since plasma  $pCO_2$  was not measured. Swine dysentery affected pigs have been shown to develop a metabolic acidosis and this may account for the increased  $pCO_2$ . The mucosal edema, present in dysentery, could also lead to increased  $pCO_2$  due to a decrease in mucosal perfusion. There was no evidence to suggest that the increase in  $pCO_2$  could be accounted for by the titration of  $HCO_3^-$  by  $H^+$  or other acid secreted by the epithelium. The  $Na^+-H^+$  and  $Cl^- - HCO_3^-$  exchanges are abolished in dysentery removing the possibility of titration by  $H^+$ .

In contrast to normal colon exposed to theophylline, in which decreased  $Cl^-$  absorption and  $HCO_3^-$  secretion was present, infected colon exposed to theophylline demonstrated complete abolition of all net trans-epithelial ion movements. These results suggest that simply an elevation of cyclic nucleotides cannot account for the functional changes observed in swine dysentery. However, it is clear that theophylline did not produce any significant additive effects on electrolyte transport in the infected colon. The only significant changes produced when theophylline was added to solutions in infected loops, was a decreased

$\text{Na}^+$  absorption at the 10 mM level, and a decrease in  $\text{pCO}_2$  and increased  $\text{HCO}_3^-$  secretion at the 40 mM level. These changes are difficult to interpret unless they can be attributed to variations in severity of the lesion in the ligated loops.

The measurement of cyclic nucleotides in infected tissues exposed to mannitol and theophylline is another indication of an altered colonic mucosal metabolism. Elevations of cyclic nucleotide levels in infected mucosa exposed to theophylline were not of the same magnitude as for normal mucosa. Increases in cyclic AMP were significantly less in the infected tissue. It should be noted that basal concentrations of either cyclic nucleotide were not elevated in the infected tissue, in fact, cyclic AMP concentrations were significantly lower in the infected control tissue. These results do, however, indicate that at least portions of the colonic mucosa in swine dysentery are capable of responding to a stimulus, theophylline, and that some metabolic activity may be occurring. The relatively unchanged basal levels and the fact that infected mucosa responds to theophylline with increases in both cyclic nucleotides are evidence that increased levels of cyclic nucleotides are not associated with swine dysentery.

Results of these studies indicate the pathophysiology of swine dysentery is related to abnormal mucosal metabolism and loss of normal transport processes. As in previous studies, (Argenzio, et al., 1980) no evidence for active or passive secretory components has been found in

association with swine dysentery. Exposure of infected colonic mucosa to theophylline, which should have augmented an underlying cyclic nucleotide mediated secretory process, produced no change in transport. The absence of increases in basal concentrations of cyclic AMP and cyclic GMP on infected colonic mucosa indicates no participation by an enterotoxin which mediates its effects via cyclic nucleotides in swine dysentery. Additional support for a lack of cyclic nucleotide mediated pathogenesis is provided by the fact that infected mucosa can respond to a stimulus, theophylline, with increased levels of both cyclic AMP and cyclic GMP. However, this response is not as marked or as prolonged as in uninfected mucosa, suggesting some inhibition of normal cellular metabolism. The pathogenic mechanism of swine dysentery does not appear to be related to those previously discussed for other diarrheal diseases. Active secretion via cyclic nucleotide activation, such as occurred with cholera toxin, the enterotoxins of E. coli or Shigella enterotoxin, did not take place in swine dysentery. Prominent mucosal invasion by the etiologic agent, and net colonic secretion, which has been observed in shigellosis, was not found. The active secretion due to salmonella infection was reduced by mucosal invasion and inflammation causing prostaglandin release and elevations of cyclic AMP. This does appear to occur in swine dysentery. This is not to say that prostaglandins do not have a role in the normal pig colon. The function of prostaglandins has not been investigated. The inflammatory lesions of dysentery may cause a local release of prostaglandins that mediate an action through mechanisms other than cyclic nucleotides. Salmonellosis and shigellosis, in addition to their direct actions on the colon,

elicit net secretion by the small intestine. Argenzio (1980) has demonstrated no such activity in the small intestine in swine dysentery. The invasive properties of both Salmonella sp. and Shigella sp. have been firmly associated with the production of disease. This same property has not been definitely established with T. hyodysenteriae, although the organism does attach to cell cultures (Knopp, et al., 1979; Wilcock and Olander, 1979b).

The pathophysiology of swine dysentery, including decreased net absorption of water and electrolytes, does not correlate well with any of the previously described pathogenic mechanisms. What produces the mucosal damage, transport alterations, and inflammation is unclear. Cytotoxins, inflammatory agents or other unknown mechanisms may be involved. What is clear, is that the clinical syndrome, diarrhea, dehydration, hypovolemic shock, and death, can be attributed to the loss of the normal colonic absorptive processes.

SECTION II. EXAMINATIONS FOR ENTEROTOXIC, PERMEABILITY FACTOR,  
AND ENDOTOXIC ACTIVITY IN SWINE DYSENTERY



## INTRODUCTION

As yet, no pathogenic mechanism has been firmly established for swine dysentery. Previous experiments searching for enterotoxic or cytotoxic activity in sterile filtrates of broth cultures of T. hyodysenteriae, or crude colonic contents from infected pigs have been unsuccessful (Whipp, et al., 1978; Wilcock and Olander, 1979a, 1979b). Studies of the physiopathology of dysentery indicate no active or passive secretory components, no increase in colonic permeability (Argenzio, et al., 1980), and no involvement of the small intestine (Argenzio, 1980). The experiments presented here were designed to investigate a number of possible mechanisms that may be involved in dysentery.

Work in gnotobiotic pigs has shown a definite synergism between T. hyodysenteriae and components of the normal colonic flora (Harris, et al., 1978; Whipp, et al., 1979). Bacteroides vulgatus, a gram negative, nonsporing, anaerobe, has been demonstrated to fulfill the synergistic requirement (Whipp, et al., 1979). In this study, the role of B. vulgatus, alone and in combination with T. hyodysenteriae, was investigated in ligated colonic, and ligated jejunal loops of normal pigs.

Hemolysins, which are produced by T. hyodysenteriae and T. innocens, and which produce characteristic patterns of hemolysis on blood agar, have been the primary means of separating the two

organisms. These hemolysins have not previously been tested for biological activity in vivo and were studied in these experiments.

All of the evidence from physiopathologic studies indicates decreased mucosal absorption as the primary transport abnormality associated with swine dysentery. Extracts from the colonic contents and colonic mucosa of infected pigs were tested for the ability to cause a decrease in absorption in ligated colonic loops.

Factors capable of increasing vascular permeability, when injected intradermally in rabbits, have been demonstrated in association with virulent strains of Vibrio cholera (Craig, 1965), E. coli (Moon and Whipp, 1971; Evans, et al., 1973c), and Salmonella typhimurium (Sandefur and Peterson, 1976). Extracts of T. hyodysenteriae and colonic material from infected pigs were examined for similar activity.

Endotoxin has been extracted from numerous bacterial organisms, E. coli and Salmonella sp. among them. Phenol extracts of T. hyodysenteriae and extracts of colonic contents and mucosa of infected pigs were assayed for endotoxic activity by intravenous injection of embryonating hens' eggs. This procedure has been used to demonstrate endotoxic activity from a variety of bacterial organisms (Milner and Finkelstein, 1966).

## MATERIALS AND METHODS

In vivo ExperimentsAnimals

Pigs were obtained from either the Indian Creek (Ames, IA.), or North Central (Clear Lake, IA.) pig farms both of which are free of swine dysentery. Animals were naturally farrowed and weighed from 12-16 kilograms when purchased and were used for each experiment within four weeks. Rectal swabs were taken from each animal upon arrival and cultured for T. hyodysenteriae, T. innocens, and Salmonella sp. Pigs in which ligated colonic loops were prepared were housed together until after surgical preparation when they were penned individually. Control pigs were housed by group. Individually housed animals were fed a drug-free, high protein ration once daily, while those in groups were self-fed. All had water ad libitum.

Adult male New Zealand white rabbits were used in the vascular permeability factor assay. They were individually caged and fed a commercial pelleted ration with water ad libitum.

Eleven day embryonating hens' eggs were obtained from the research flock of the Veterinary Medical Research Institute (Ames, IA.). Fertilized eggs were set and rotated once daily while held at 38°C in a humidified incubator.

Surgical preparation

All pigs used in ligated colonic loop studies were surgically prepared by implantation of a silastic cecal cannula by the procedure

previously described (See Section 1). Cleocin (Clindamycin, Upjohn Co. Inc., Kalamazoo, MI.) was given intraperitoneally (300 mg) at the time of surgery. A postoperative recovery period of 7-10 days was allowed before initiation of each experiment.

### Bacteria

Three bacterial organisms were used. Treponema hyodysenteriae, isolate B204, has been shown to be pathogenic (Kinyon, et al., 1977) and T. innocens, isolate B256, nonpathogenic (Kinyon and Harris, 1979) for swine. Isolate 32A of B. vulgatus was isolated from the colon of a pig with swine dysentery by Dr. D. L. Harris. All organisms were grown from stock freezer cultures and were passaged less than 15 times in vitro.

### Culture techniques

Inocula Treponema hyodysenteriae and T. innocens were grown in trypticase soy broth without dextrose (TSB) (BBL, Cockeysville, MD.) supplemented with 10% (v/v) fetal calf serum (Gibco, Grand Island, NY.) under a 50:50 atmosphere of deoxygenated H<sub>2</sub>:CO<sub>2</sub> by the method of Kinyon and Harris (1974). Large volumes were grown in flasks on a reciprocating incubator as described by Joens, et al., (1978a). Media for hemolysin production was further supplemented by addition of 1% sodium ribonucleate (Sigma Chemical Co., St. Louis, MO.). This

procedure has been demonstrated to enhance hemolysin production (Picard, et al., 1979). Bacteroides vulgatus was grown at 37°C in prereduced anaerobically sterilized peptone yeast glucose broth (PYGb) (Difco Lab., Detroit, MI.) under an atmosphere of deoxygenated CO<sub>2</sub>.

Viable count determinations      Viable counts of T. hyodysenteriae and T. innocens were made in duplicate on trypticase soy agar (BBL, Cockeysville, MD.) supplemented with 5% citrated bovine blood. Serial 1:10 dilutions were made in sterile phosphate buffered saline (PBS) and 0.01 ml of each dilution inoculated onto the agar medium. The plates were then incubated at 42°C under an atmosphere of H<sub>2</sub>:CO<sub>2</sub> (80:20) in an anaerobe jar with palladium catalysts. Plates were observed after 48 and 72 hours of incubation and the results recorded. Viable counts of T. hyodysenteriae, used as inoculum, for production of hemolysin, sonically disrupted cells, or for phenol extraction, ranged from 1 x 10<sup>7</sup> to 2 x 10<sup>8</sup> colony forming units per ml.

Selective isolation      Treponema hyodysenteriae and T. innocens were selectively isolated by the method of Songer, et al. (1976), on trypticase soy agar supplemented with 5% citrated bovine blood and 400 mcg/ml of spectinomycin (Upjohn, Kalamazoo, MI.). Plates were incubated at 42°C under an atmosphere of 80:20, H<sub>2</sub>:CO<sub>2</sub> in an anaerobic jar containing palladium catalyst. Plates were examined every 2 days over a six day incubation period for growth of the organisms.

Salmonella sp. were isolated on Tergitol 7 agar (Difco Labs.,

Detroit, MI.) and in Tetrathionate broth (Difco Labs., Detroit, MI.) incubated at 37°C. After 24 hours incubation, the Tergitol 7 was examined for suspect colonies and the Tetrathionate broth was inoculated on Brilliant Green agar (BBL, Cockeysville, MD.). The Brilliant Green agar was observed for suspect colonies after 24 hours incubation at 37°C. All Salmonella suspects were identified biochemically and were agglutinated by Polyvalent O antiserum. All Salmonella were serotyped to species by the National Animal Plant Health Inspection Service, Ames, IA.

#### Intragastric inoculation procedure

All pigs were not given feed for 48 hours prior to intragastric inoculation via stomach tube.

#### Histopathology

All tissues taken for histopathologic examination were formalin fixed, paraffin embedded, sectioned, and stained with hematoxylin and eosin. Colonic sections from infected pigs were also stained with Warthin-Starry stain to demonstrate spirochetes.

#### Assay of hemolytic activity

The hemolytic activity of sterile filtrates of I. *hyodysenteriae* and I. *innocens*, and fluid from ligated colonic loops in experiment III, was determined by the following procedure. Serial two fold dilutions

of the fluid to be assayed were made in saline and equal volumes of washed equine red blood cells (1% in saline) added. The mixture was incubated at 37°C for 30 minutes, centrifuged, and read to a 50% hemolysis endpoint. Water lysed equine red blood cells, at 100%, 50%, 25%, and 12% hemolysis, were used as standards. Hemolytic activity was expressed as a reciprocal of the dilution.

### Chemical analyses

Analyses of net water and electrolyte transport were made in experiment IV. Concentrations of  $\text{Na}^+$  were determined by flame photometry,  $\text{Cl}^-$  by automated colorimetry, and  $\text{HCO}_3^-$  by the method of Dobson (1959). The same procedures and instruments used previously (Section 1) were utilized. Net water movement was measured using polyethylene glycol 4000 (PEG) as a nonabsorbable marker. The method of Hyden (1956) was used to assay for PEG. Freezing point depression was used to determine osmolality (Osmette A, Automatic Osmometer, Precision Instruments Inc., Sudbury, MA.). Solution and sample pH were measured with a Beckman model 3360 digital pH meter (Beckman Instrument Inc., Irvine, CA.).

### Calculations and statistical analysis

All calculations of net electrolyte and water movement, and statistical analysis of data was performed as previously described (See Section 1).

### Preparation of test materials

Most test materials were tested for activity in ligated colonic loops, by intradermal injection in rabbits, and intravenous injection of embryonating hens eggs.

Crude colonic contents and sterile filtrates of the colonic contents were obtained from two pigs experimentally infected with I. hyodysenteriae. Both pigs showed clinical signs of dysentery by 6 days post inoculation. Treponema hyodysenteriae was isolated from the colon of both pigs at necropsy. Histopathologic examination revealed mucosal edema, hyperemia, and erosion, with fibrinonecrotic debris covering areas of the mucosal surface. The pigs were euthanized, the colons aseptically removed, the contents expressed, and the colon opened along its entire length. Using sterile glass slides, the mucosa was scraped from the underlying muscle and connective tissue layers. The mucosal scrapings and contents were then homogenized with mortar and pestle, added to 300 ml of sterile phosphate buffered saline (PBS), and further homogenized for one minute in a Waring blender. A portion of the homogenate was removed and refrigerated at 4°C. The remainder was centrifuged at 500 xg at 4°C for 15 minutes and the supernatant removed. The supernatant was then centrifuged at 10,000 xg at 4°C for 60 minutes. The supernatant was then serially passed through 8 µm, 3 µm, 1.2 µm, .8 µm, .45 µm, and .22 µm filters to obtain the sterile filtrate. This material was held at 4°C and used within 36 hours. Viable counts of I. hyodysenteriae in the



crude material were  $9 \times 10^7$  colony forming units/ml. No Salmonella sp. was cultured from the crude material or from the small intestine or mesenteric lymph nodes of the pigs.

Hemolysin, in the form of sterile filtrates was obtained by the passage of broth cultures of T. hyodysenteriae and T. innocens through a .22  $\mu$ m filter. Growth medium for the organisms was supplemented with 1% sodium ribonucleate to enhance hemolysin production. Hemolytic activity was assayed by the method described.

Extracts were prepared from the colonic mucosa and contents of both normal and dysentery affected pigs by the following method. The dysentery group consisted of 8 pigs which were intragastrically inoculated with 100 ml of pure broth culture T. hyodysenteriae on two successive days. Four pigs manifested clinical signs of dysentery within 7 days of inoculation and were euthanized. The colons were removed from the animals and the extraction performed at 4°C. The contents were expressed and mixed with cold sterile PBS in a 5:1 proportion. The colonic mucosa was stripped from the underlying layers and PBS added in the same proportions as with contents. The mucosa was then homogenized at 4°C in a Waring blender in three-ten second bursts separated by one minute intervals. Both the contents and the mucosa were then stirred for 3-4 hours at 4°C. Each portion was centrifuged at 500 xg for 30 minutes at 4°C and the supernatant removed. The supernatants were centrifuged at 2000 xg for 60 minutes at 4°C and the supernatant removed. These supernatants were then

centrifuged at 10,000 xg for 60 minutes at 4°C. The supernatant was drawn off and concentrated 10 fold by dialysis for 24-36 hours at 4°C against polyethylene glycol (PEG 6000, Sigma Chemical Co., St. Louis, MO.). A portion of each extract was then sterilized by serial filtration and by passage through a .22 µm filter. This material was stored at -78°C until used. The identical procedure was followed with the extraction of the colonic mucosa and contents of two normal pigs.

Concentrated lyophilized broth cultures of T. hyodysenteriae were used to prepare sonically disrupted cells and phenol extracts. One liter volumes of broth cultures were concentrated 10 fold by filtration with a Millipore Pellicon Cassette with a .22 µm filter, washed three times with sterile PBS, then shell frozen and lyophilized. Viable counts of T. hyodysenteriae were  $1.0 \times 10^8$  to  $1.2 \times 10^8$  colony forming units per ml prior to concentration. Ten milligrams of lyophilized cells were suspended in 20 ml of sterile PBS and the tube placed in an ice bath. The cells were sonicated for three minutes (Model W-225R Sonicator Cell Disruption, Heat Systems Ultrasonics, Inc., Plainview, L.I., New York, NY.). No whole cells were observed by phase microscopic examination of the suspension. The sonicate was sterilized by passage through a .22 µm filter and stored at 4°C.

The procedure of Westphal, et al. (1952) as modified by Baum and Joens (1979) was used for phenol extraction. Ten ml of distilled water (68°C) was added to 200 mg of lyophilized cells, mixed, and 10 ml of concentrated phenol (68°C) added. The mixture was

incubated at 68°C for 12-15 minutes, placed in an ice bath and brought to 10°C. The material was centrifuged at 2000 xg for 20 minutes and the water phase removed. Another 10 ml of water (68°C) was added and the incubation and centrifugation steps repeated. The second water phase was removed, combined with the first, and dialyzed for 24 hours at 4°C against 6 changes of distilled water. This material was concentrated to 20% of original volume by dialysis at 4°C against PEG 6000. Six volumes of 90% ethanol (-20°C) and a few sodium acetate crystals were added to each volume of concentrate and held at -20°C for 24 hours. The material was centrifuged at 16,000 xg at 4°C for 60 minutes and the supernatant discarded. The pellet was reconstituted in 2 ml of cold distilled water, dialyzed against distilled water at 4°C for 2-3 changes of water, filter sterilized, and stored at 4°C. The phenol phase and cellular material were collected and dialyzed for 48 hours at 4°C against 6 changes of distilled water. The dialyzed fluid and precipitate were homogenized by mortar and pestle and centrifuged at 16,000 xg at 4°C for 60 minutes. The supernatant was harvested, concentrated 10 fold by dialysis against PEG 6000, filter sterilized, and stored at 4°C.

Purified toxin from E. coli strain 263 was obtained from Dr. S. C. Whipp at the National Animal Disease Center (Ames, IA.). This toxin has previously been reported to increase vascular permeability when injected intradermally in rabbits (Moon and Whipp, 1971).

Control materials for experiments I, II, III, V, and VI consisted

of broth media, TSB and PYGb, the supplements, fetal calf serum (10% in PBS), and 1% sodium ribonucleate, and PBS.

The composition of solutions used in experiment IV are given in Table 3. These two solutions are of the same composition as solutions 1 and 5 used in earlier studies (See Section I). Solutions were freshly prepared from concentrated stock solutions, and gassed at 39°C for 15 minutes with 95% O<sub>2</sub>, 5% CO<sub>2</sub> prior to use. Theophylline was purchased from Sigma Chemical Co. (St. Louis, MO.). All other chemicals were obtained from Fisher Scientific Co., (Pittsburgh, PA.) and were of reagent grade.

#### Experimental procedures and design

Ligated colonic loops were used in experiments, I, II, III, and IV. In Experiment I, ligated jejunal loops were also used. Each pig was starved for 36-48 hours then placed in a metabolic cage on the day of experiment and the colon flushed via the cecal cannula with warmed isotonic saline at a rate of 40-80 ml/minute until the anal effluent ran clear. The animals were then anesthetized and ligated loops placed in the colon as previously described (See Section I). Ligated jejunal loops were made by a similar procedure.

Experiment I      Effects of *T. hyodysenteriae* alone and in combination with *B. vulgatus* in ligated colonic and ligated jejunal loops of normal pigs      Four ligated loops were placed in the colon

Table 3. Composition of experimental solutions (mM or mosmoles)

	1	2
Na <sup>+</sup>	125	125
Cl <sup>-</sup>	100	100
HCO <sub>3</sub> <sup>-</sup>	25	25
PEG 6000	1g/L	1g/L
Mannitol	50	10
Theophylline	-	40
pH	7.27 ± .02	7.23 ± .03
Osmolality	279 ± 1.0	262 ± 3.1

and jejunum of each of four pigs. Test materials consisted of:

- A) Viable T. hyodysenteriae + viable B. vulgatus (5 ml of each)
- B) Viable T. hyodysenteriae + PYGb (5 ml of each)
- C) Viable B. vulgatus + TSB (5 ml of each)
- D) TSB + PYGb (5 ml of each)

One pig was euthanized at 24, one at 48, and one at 72 hours post inoculation. Tissue samples were taken for selective isolation of T. hyodysenteriae, for culture of Salmonella sp., and for histopathology. Gross lesions and quantities of accumulated fluid were recorded. No analysis of the fluid was made.

Three groups of two control pigs were intragastrically inoculated on two consecutive days with the test materials to test for infectivity. Group 1 received viable T. hyodysenteriae and viable B. vulgatus (50 ml of each), Group 2, viable T. hyodysenteriae and PYGb (50 ml of each), and Group 3, TSB and PYGb (50 ml of each). All control pigs were observed daily for clinical signs of dysentery and cultured by rectal swab for T. hyodysenteriae every second day. When clinical signs of swine dysentery occurred, the animals were euthanized and necropsied. Tissue samples were taken for culture of T. hyodysenteriae, Salmonella sp., and for histopathologic examination.

Experiment II    Effects of crude colonic contents and sterile filtrates of crude colonic contents from dysentery affected pigs in ligated colonic loops of normal pigs    Four ligated colonic loops

of three pigs and the following amounts of materials tested for activity,

- E) Crude colonic contents from dysentery affected pigs (10 ml)
- F) Sterile filtrate of the crude colonic contents from dysentery affected pigs (10 ml)
- G) Viable I. hyodysenteriae (10 ml)
- H) TSB (10 ml)

One pig was euthanized at 24, one at 48, and one at 72 hours after inoculation of the loops. Samples were taken for culture and a section taken for histopathology from each loop. Fluid accumulations were measured and gross appearance of the mucosa recorded. Three groups, of two pigs each, were used as controls to check for infectivity of test materials. Each group was intragastrically inoculated with 50 ml of either E, G, or H on two successive days. Control pigs were observed daily for development of clinical signs of dysentery and euthanized and necropsied when signs appeared. Samples were taken from each animal for culture and histopathology.

Experiment III      Comparison of the effects of hemolysins of I. hyodysenteriae and I. innocens to the effects of viable I. hyodysenteriae and I. innocens in ligated colonic loops of normal pigs

Five pigs, each with five ligated colonic loops were used in this study. The test materials used were:

- I) Viable I. hyodysenteriae (10 ml)
- J) Viable I. innocens (10 ml)
- K) Hemolysin from I. hyodysenteriae (10 ml)

- L) Hemolysin from T. innocens (10 ml)
- M) TSB supplemented with 10% fetal calf serum and 1% sodium rubonucleate

Hemolytic activity was 1:128/ml and 1:32/ml for the hemolysins of T. hyodysenteriae and T. innocens respectively. The time intervals between inoculation and euthanasia were 2, 6, 18, 24, and 72 hours. Amounts and hemolytic activity of any accumulated fluid were measured. Samples were taken from each loop for culture and histopathologic examination.

Experiment IV      Effects of extracts of colonic mucosa and colonic contents from normal and dysentery affected pigs on normal colonic transport

The effects of the four extracts, from colonic contents and mucosa of both normal and dysentery affected pigs, were examined by combining 5 ml of each extract with 40 ml of Solution 1 and placing the combination in a ligated colonic loop. Loops containing 45 ml of Solution 1 and 45 ml of Solution 2 were also used in each test animal. Net absorption of electrolytes and water occurs from loops containing Solution 1. Solution 2, containing 40 mM theophylline has been shown to decrease net absorption of  $\text{Na}^+$ ,  $\text{Cl}^-$ , and water, and increase net secretion of  $\text{HCO}_3^-$  (See Section I). Any changes in net absorption produced by the extracts can be determined by comparison with the net absorption from loops containing Solutions 1 and 2. Six ligated loops were placed in the colons of six pigs and one of the following combinations injected into each loop.



- N) Solution 1
- O) Solution 2
- P) Solution 1 + 5 ml of extract of colonic contents of normal pigs
- Q) Solution 1 + 5 ml of extract of colonic mucosa of normal pigs
- R) Solution 1 + 5 ml of extract of colonic contents of dysentery affected pigs
- S) Solution 1 + 5 ml of extract of colonic mucosa of dysentery affected pigs.

After injection, solutions were mixed in the loops, and a time zero sample withdrawn. After a one hour incubation, samples were again taken, the loops opened, the mucosa stripped, dried, and weighed. Small sections were taken from each loop for histologic examination. Net absorption from each loop was determined and expressed on the basis of the amount per gram of mucosa per hour.

Experiment V      Effect of test materials in vascular permeability factor assay in rabbits      Two rabbits were prepared and injected intradermally by the following procedure to determine if any of the test materials produced an increase in vascular permeability.

The back of each rabbit was shaved and cleansed. The test materials (0.1 ml) were injected intradermally in duplicate on the back of the rabbits. At 18 and 24 hours post inoculation, Evans blue dye (40 mg/kg) was injected intravenously. Positive reactions were

determined by accumulation of the dye at the site of injection. The materials tested and the results of the tests are presented in Table 8.

Experiment VI      Endotoxic effects of test materials in  
embryonating hens' eggs      Eleven day embryonating hens' eggs were candled and the position of a large blood vessel marked on the shell. A window was made over the vessel without disturbing the chorio-allantoic membrane. The membrane was cleared by application of a small amount of mineral oil to which 1,000,000 units of Potassium Penicillin G/ml had been added. The test materials (0.1 ml) were then injected intravenously with a syringe and 30 gauge needle. The eggs were then placed back in the incubator and candled again at 4 and 24 hours after inoculation. Those dead at 4 hours were considered deaths due to manipulation. The number of deaths at 24 hours was recorded. The materials tested and the results are presented in Table 9.

## RESULTS

In vivo ExperimentsExperiment I

Effects of *T. hyodysenteriae* alone and in combination with *B. vulgatus* in ligated colonic and ligated jejunal loops of normal pigs

The results of this trial are presented in Tables 4 and 5. Neither ligated colonic loops incubated 24 hours, nor jejunal loops, regardless of incubation time, had fluid accumulations, or gross or microscopic lesions. It should be noted that the ligated jejunal loops were placed using anatomical references. Several of these loops appeared, histologically, to be from the ileum. By 48 hours, gross lesions of edema, mucosal hyperemia and excess mucus productions were present in colonic loops inoculated with either *T. hyodysenteriae* or *T. hyodysenteriae* in combination with *B. vulgatus*. Microscopically, mucosal erosion, swelling, and hyperemia, with leukocytic infiltration of the lamina propria were evident. At 72 hours, in addition to gross lesions, a large amount of viscous fluid had accumulated in those loops inoculated with *T. hyodysenteriae* alone or in combination with *B. vulgatus*. Edema, hyperemia, mucosal erosions, and in one instance a diphtheritic pseudomembrane were evident grossly. Areas of mucosal swelling, erosion, and hyperemia, and crypt dilatation were seen microscopically. Necrotic material composed of cellular debris, fibrin, and leukocytes was present across the mucosal surface. Selective isolation of *T. hyodysenteriae* was made from all affected loops

Table 4. Effects of T. hyodysenteriae alone and in combination with B. vulgatus in ligated colonic loops of normal pigs.

Pig #	Incubation Time	Loop #	Inoculum <sup>a</sup>	Gross Lesions	Microscopic Lesions	Phase Microscopic Observation (# Organisms/fld.)	Culture <u>T. hyodysenteriae</u>	<u>Salmonella</u> Sp. <sup>b</sup>
66	24 hours	1	C	-	-	ND <sup>c</sup>	+	-
		2	D	-	-	ND	+	-
		3	A	-	-	ND	+	-
		4	B	-	-	ND	+	-
65	48 hours	1	D	-	-	-	-	-
		2	C	-	-	-	-	-
		3	A	+	+	20	+	-
		4	B	+	+	10	+	-
67	72 hours	1	D	-	-	-	-	+ <sup>b</sup>
		2	C	-	-	-	-	-
		3	A	+(175 ml fluid)	+	10-15	+	+
		4	B	+(65 ml fluid)	+	10-15	+	+
68	72 hours	1	A	+(20 ml fluid)	+	20	+	-
		2	C	-	-	-	-	-
		3	D	-	-	-	-	-
		4	B	+(fluid lost)	+	5	+	-

<sup>a</sup>A) Viable T. hyodysenteriae + B. vulgatus.

B) Viable T. hyodysenteriae + PYGb.

C) Viable B. vulgatus + TSB.

D) TSB + PYGb.

<sup>b</sup>Salmonella derby.

<sup>c</sup>Not done.

Table 5. Effects of T. hyodysenteriae alone and in combination with B. vulgatus in ligated jejunal loops of normal pigs

Pig #	Incubation Time	Loop #	Inoculum <sup>a</sup>	Gross Lesions	Microscopic Lesions	Phase Microscopic Observations (# Organisms/fld.)	Culture <u>T. hyodysenteriae</u>	<u>Salmonella</u> <sup>b</sup> sp.
66	24 hours	1	D	-	-	ND <sup>c</sup>	-	-
		2	C	-	-	ND	-	-
		3	A	-	-	ND	+	-
		4	B	-	-	ND	+	-
65	48 hours	1	C	-	-	-	-	-
		2	D	-	-	-	-	-
		3	A	-	-	1	+	-
		4	B	-	-	1	+	-
67	72 hours	1	C	-	-	-	-	+
		2	A	-	-	1	+	+
		3	D	-	-	-	-	+
		4	B	-	-	2	+	+
68	72 hours	1	B	-	-	3	+	-
		2	D	-	-	-	-	-
		3	A	-	-	3	+	-
		4	C	-	-	-	-	-

<sup>a</sup>Inoculum A) Viable T. hyodysenteriae + B. vulgatus.  
 B) Viable T. hyodysenteriae + PYGb.  
 C) Viable B. vulgatus + TSB.  
 D) TSB + PYGb.

<sup>b</sup>Salmonella derby.

<sup>c</sup>Not done.

and also from two uninoculated loops. This unexpected isolation was probably due to contamination when one loop was accidentally opened during removal of the colon from the abdominal cavity. Salmonella derby was isolated from one animal, pig #67.

Of the six control pigs, the two inoculated with the combination of T. hyodysenteriae and B. vulgatus began showing clinical signs of dysentery at 11 and 23 days post inoculation. At necropsy, gross lesions of edema, mucofibrinous exudation, and focal necrosis and hemorrhage were present in the colon and in one instance, the cecum. Microscopically, mucosal erosion with leukocytic infiltration of the lamina propria was present. A fibrinonecrotic exudate covering portions of the mucosal surface was also observed. Treponema hyodysenteriae was isolated from the colons of both animals but no attempt was made to isolate B. vulgatus. No Salmonella were isolated from these pigs. The two pigs inoculated with T. hyodysenteriae alone did not develop clinical signs of dysentery and were necropsied 33 days post inoculation. The organism was isolated from rectal swabs taken from one of the pigs at 14 and 33 days post inoculation. No gross lesions were present in the colon of either animal and microscopic lesions consisted only of a mild leukocytic infiltration of the lamina propria. Treponema hyodysenteriae was isolated at necropsy from the colons of both animals. Salmonella agona was isolated from the intestinal tract of one pig. The two pigs inoculated with broth medium did not show clinical signs and were not euthanized or necropsied.

## Experiment II

### Effects of crude colonic contents and sterile filtrates of crude colonic contents from dysentery affected pigs in ligated colonic loops of normal pigs

The results from this experiment are presented in Table 6. In this experiment both the crude material from infected pigs and pure cultures of T. hyodysenteriae were capable of causing fluid accumulation and mucosal damage in the loops as early as 24 hours after inoculation. No mucosal damage was produced by either TSB or the sterile filtrate of the crude colonic material. Gross lesions ranged from edema and hyperemia in the early stages to a mucofibrinous enteritis with focal areas of hemorrhage and necrosis in the longer incubating loops. A viscous brownish-red to yellow fluid with shreds of fibrin was present. Microscopically, focal areas of mucosal erosion and leukocytic infiltration of the lamina propria were evident after 24 hours exposure to either the crude colonic contents of infected pigs or to the pure cultures of T. hyodysenteriae. By 48 hours incubation, marked epithelial detachment and erosion were occurring. Leukocytic infiltration of the lamina propria was also present. Some fibrinonecrotic debris was evident covering the mucosal surface. Lesions after 72 hours incubation were similar to those present after 48 hours. Isolation of T. hyodysenteriae was made only from those loops inoculated with the crude material or the pure culture of the organism. No Salmonella sp. were isolated from any of the three experimental animals.

Table 6. Effects of crude colonic contents and sterile filtrates of crude colonic contents from dysentery affected pigs in ligated colonic loops of normal pigs

Pig #	Incubation Time	Loop #	Inoculum <sup>a</sup>	Gross Lesions	Microscopic Lesions	Culture <u>T. hyo-dysenteriae</u>	<u>Salmonella sp.</u>
62	24 hours	1	E	+(with fluid)	+	+	-
		2	H	-	-	-	-
		3	G	+(with 50 ml fluid)	+	+	-
		4	F	-	+	-	-
61	48 hours	1	E	+(with 200 ml fluid)	+	+	-
		2	H	-	-	-	-
		3	G	+(with 75 ml fluid)	+	+	-
		4	F	-	-	-	-
63	72 hours	1	E	+(with fluid-lost)	+	+	-
		2	H	-	-	-	-
		3	G	+(with 230 ml fluid)	+	+	-
		4	F	-	-	-	-

- <sup>a</sup>Inoculum E) Crude colonic contents from dysentery affected pigs.  
 F) Sterile filtrate of crude colonic contents.  
 G) Whole cell T. hyodysenteriae.  
 H) TSB.



Each of the four control pigs inoculated with either the crude colonic contents from infected pigs, or the pure cultures of T. hyodysenteriae developed clinical signs of dysentery by 7 days post inoculation. At necropsy gross lesions of edema, hyperemia, mucofibrinous colitis, and formation of a diphtheritic pseudomembrane were present. Microscopic lesions observed were diffuse mucosal detachment and erosion, marked leukocytic infiltration of the lamina propria, focal areas of hemorrhage, and fibrinonecrotic debris along the mucosal surface. Treponema hyodysenteriae but no Salmonella sp. were isolated from these control animals.

### Experiment III

Comparison of the effects of hemolysins of T. hyodysenteriae and T. innocens to the effects of viable T. hyodysenteriae and T. innocens in ligated colonic loops of normal pigs The results of this study are given in Table 7. None of the test materials, with the exception of viable T. hyodysenteriae after 72 hours incubation, produced any damage in ligated colonic loops. In the loops inoculated with T. hyodysenteriae, approximately 10 ml of fluid, with a hemolytic activity of 1:256 had accumulated by 72 hours after inoculation. Grossly, slight hyperemia and a mild mucoid enteritis were seen in this loop. Focal areas of mucosal erosion and leukocytic infiltration of the lamina propria were observed microscopically. No other loops had microscopic lesions although small amounts of fluid with hemolytic activity were present in some loops after 2, 6, and 18 hours incubation. Isolation of T. hyodysenteriae

Table 7. Comparison of the effects of hemolysins of *T. hyodysenteriae* and *T. innocens* to the effects of viable *T. hyodysenteriae* and *T. innocens* in ligated colonic loops of normal pigs

Pig #	Incubation Time	Loop #	Inoculum <sup>a</sup>	Gross Lesions	Microscopic Lesions	Hemolytic Activity	Culture <i>T. hyodysenteriae</i>	Salmonella <sup>b</sup>
16	2 hrs.	1	I	-	-	1:128	+	+
		2	M	-	-	-	-	-
		3	L	-	-	-	-	-
		4	J	-	-	-	-	-
		5	K	-	-	1:32	-	-
15	6 hrs.	1	J	-	-	-	-	-
		2	K	-	-	-	-	-
		3	I	-	-	1:256	+	-
		4	M	-	-	-	-	-
		5	L	-	-	-	-	-
14	18 hrs.	1	K	-	-	-	-	+
		2	I	-	-	1:64	+	-
		3	M	-	-	-	-	-
		4	L	-	-	1:32	-	-
		5	J	-	-	1:32	-	-
13	24 hrs.	1	L	-	-	-	-	+
		2	J	-	-	-	-	-
		3	K	-	-	-	-	-
		4	I	-	-	-	+	-
		5	M	-	-	-	-	-
17	72 hrs.	1	M	-	-	-	-	+
		2	L	-	-	-	-	-
		3	J	-	-	-	-	-
		4	K	-	-	-	-	-
		5	I	+	+	1:256	+	-

<sup>a</sup>Inoculum I) Viable *T. hyodysenteriae*; J) Viable *T. innocens*; K) Hemolysin from *T. hyodysenteriae*; L) Hemolysin from *T. innocens*; M) TSB + 1% sodium ribonucleate.

<sup>b</sup>*Salmonella derby*.

was made only from those loops inoculated with the organism.

Salmonella derby was isolated from four of the experimental pigs.

#### Experiment IV

##### Effects of extracts of colonic mucosa and colonic contents from normal and dysentery affected pigs on normal colonic transport

Results of the studies with ligated colonic loops used to measure the effects of the extracts on colonic transport are presented in Figure 11 (Appendix Table B7). None of the extracts significantly decreased net absorption of electrolytes and water from the loops. Net values for absorption of water and electrolytes from the control solution are comparable to those previously reported (See Section 1). Theophylline decreased net absorption of water and electrolytes with the only significant change being the amount of  $\text{HCO}_3^-$  secreted.

#### Experiment V

Effects of test materials in vascular permeability factor assay in rabbits Test materials used in the assay and the results of the tests are given in Table 8. The only material significantly increasing vascular permeability, as evidenced by dye accumulation, was the toxin of E. coli strain 263.

#### Experiment VI

Endotoxic effects of test materials in embryonating hens' eggs Test materials and results of intravenous injection of these materials into embryonating hens' eggs are shown in Table 9. Sterile filtrates of TSB-fetal calf serum-1% sodium ribonucleate

Figure 11. Effect of extracts of colonic mucosa and colonic contents of normal and dysentery affected pigs on net water and solute transport in ligated colonic loops of normal pigs

Positive values indicate net absorption, negative values net secretion (± s.e.).

\*  $p < .05$  Significant differences from control solution.

N - Mannitol (Control) solution.

O - Theophylline.

P - Control + extract of colonic contents of normal pigs.

Q - Control + extract of colonic mucosa of normal pigs.

R - Control + extract of colonic contents of infected pigs.

S - Control + extract of colonic mucosa of infected pigs.

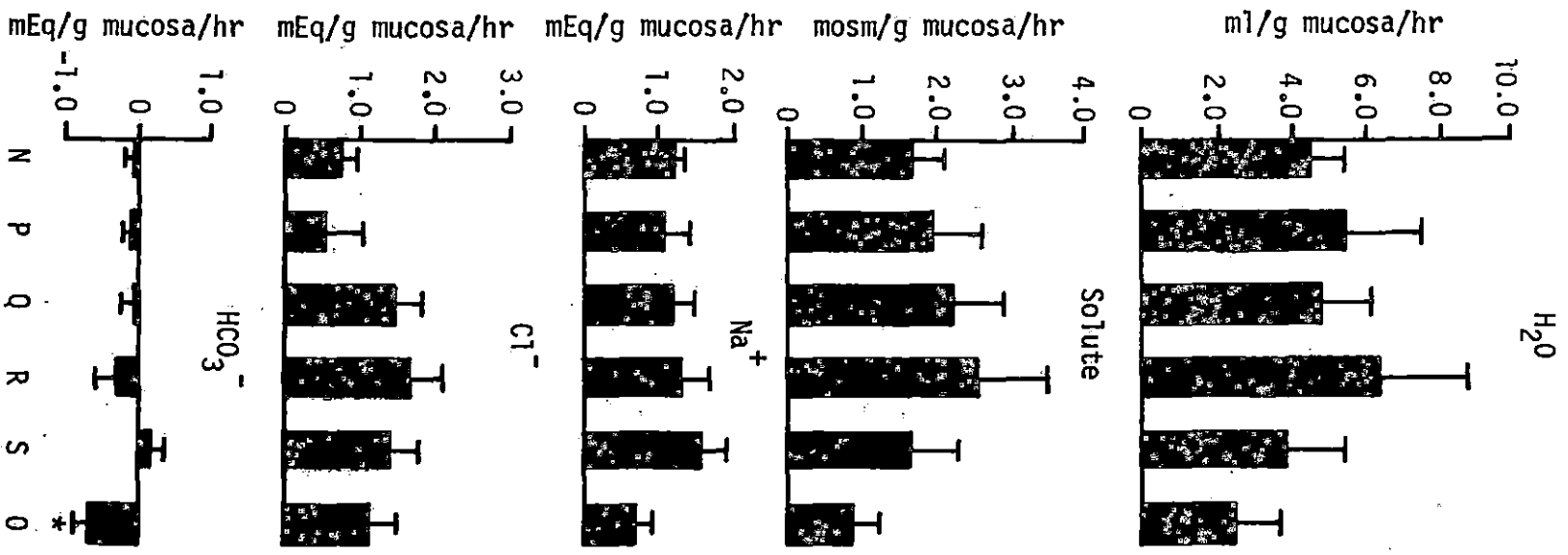


Table 8. Effects of test materials on vascular permeability as assayed by intradermal injection of rabbits

Test Materials	Positive Reactions <sup>a</sup>	
	18 hrs.	24 hrs.
Hemolysin from <u>T. hyodysenteriae</u>	0/4	0/4
Hemolysin from <u>T. innocens</u>	0/4	0/4
Sonicated <u>T. hyodysenteriae</u>	0/4	0/4
Phenol phase extract of <u>T. hyodysenteriae</u>	0/4	0/4
Water phase extract of <u>T. hyodysenteriae</u>	0/4	0/4
Extract of colonic contents of normal pigs	0/4	0/4
Extract of colonic contents of dysentery affected pigs	0/4	0/4
<u>E. coli</u> strain 263 toxin	3/4	3/4
PBS	0/4	0/4
TSB	0/4	0/4

<sup>a</sup>Positive reactions per number of injection sites as evidenced by accumulation of dye at the injection site.

Table 9. Endotoxic effects of test materials as assayed by intravenous injection of embryonating hens' eggs

Test Materials	Inoculated	Survived	% Survival
Hemolysin from <u>T. hyodysenteriae</u> <sup>a</sup>	3	0	0
Hemolysin from <u>T. innocens</u> <sup>a</sup>	3	1	33
Sonicated <u>T. hyodysenteriae</u>	21	17	86
Phenol phase extract of <u>T. hyodysenteriae</u>	4	4	100
Water phase extract of <u>T. hyodysenteriae</u>	4	3	75
Extract of colonic contents of normal pigs	4	0	0
Extract of colonic contents of dysentery affected pigs	20	0	0
TSB + fetal calf serum (10% v/v) + 1% sodium ribonucleate	3	0	0
TSB	3	3	100
Fetal calf serum	4	4	100
Sodium ribonucleate (1% in saline)	4	1	25
PBS	16	14	88
Uninoculated controls	9	8	89

<sup>a</sup>Sterile filtrates of the organisms grown in TSB + FCS (10% v/v) + 1% sodium ribonucleate.

medium in which T. hyodysenteriae and T. innocens had been grown and containing hemolysin were lethal to embryonating hens eggs. However, uninoculated media and 1% solutions of sodium ribonucleate were also lethal to embryonating eggs. Sonicated suspensions and phenol and hot water extracts of T. hyodysenteriae were not lethal for embryonating eggs.



## DISCUSSION

The results of ligated loop studies presented here are consistent with those previously reported. Only pure cultures of T. hyodysenteriae, or crude colonic contents from infected pigs, produced either fluid accumulations or lesions in ligated colonic loops of normal swine (Whipp, et al., 1978; Wilcock and Olander, 1979a). Combining B. vulgatus with T. hyodysenteriae produced no noticeable increase in the severity of the lesions, or decrease in the time required for lesion development. Sterile filtrates, both of broth cultures and colonic contents from infected pigs, hemolysins from T. hyodysenteriae and T. innocens, and extracts of colonic mucosa and contents from normal and dysentery affected pigs, were negative for production of lesions and fluid accumulation when assayed in ligated colonic loops. Extracts of colonic mucosa and contents from normal and dysentery affected pigs had no effect on net transport by the colonic mucosa. Lesions produced in ligated loops by T. hyodysenteriae or crude colonic material from infected pigs closely resembled those of naturally occurring dysentery.

None of the materials, with the exception of toxin from E. coli strain 263, produced increased vascular permeability. Since only two animals were used in this procedure, further testing may be warranted. The discovery of a vascular permeability factor with activity similar to the permeability factors of E. coli and V. cholerae in association with dysentery is not likely. The enterotoxigenic activity of both E. coli

and V. cholerae has been closely associated with vascular permeability factors (Evans, et al., 1973b; Mosley, et al., 1970; Craig, 1970).

It has been suggested that the enterotoxins and permeability factors may be the same entity.

Further effort should probably not be directed toward searching for an enterotoxin. No secretory component or activation of cyclic nucleotides, such as occurs with the enterotoxins of E. coli, V. cholerae, and Shigella sp., has been documented in dysentery. Sterile filtrates of broth cultures and of colonic contents from infected pigs have produced no changes in any of the systems used to assay for enterotoxic activity (Whipp, et al., 1978; Wilcock and Olander, 1979a, 1979b). The only materials capable of causing fluid accumulation in ligated loops are pure cultures of T. hyodysenteriae or crude material from the colons of infected pigs. This fluid accumulation should not be construed as a secretory process. Damage to the mucosal epithelium and decreased solute transport result in fluid accumulation in the loops by the mechanism discussed in Section I. An enterotoxin may be produced which is capable of causing a decrease in net absorption by the colon, but one was not detected in these studies. If further attempts are made, a model other than the ligated colonic loop of pigs should probably be employed. The long period of time required before mucosal changes are seen (48-72 hrs.), and the possible misinterpretation of fluid accumulations make this model unsuitable for enterotoxin assay in studies of dysentery. Other in vivo models, such as the ligated rabbit ileal loop used by Knopp (1979), or in vitro models

may be useful.

Results of the endotoxin assay indicate that an endotoxin-like activity may not occur in dysentery. Deaths produced by the hemolysins were probably due to the 1% sodium ribonucleate used to supplement the growth medium. No significance can be attached to the deaths produced by the extract from the colonic contents of infected pigs since the extracts from similar material in normal pigs also was lethal. No other materials produced lethal effects.

Future research on the pathogenesis of dysentery should consider cytotoxic activity, a possible inflammatory agent, and the physical relationship of the mucosal epithelium to T. hyodysenteriae.

Although no cytotoxin has been demonstrated (Wilcock and Olander, 1979b), it would seem likely that one does exist. A substance capable of producing changes in the metabolism of the mucosal epithelium leading to the death of the epithelial cell and erosion of the epithelial layer would be consistent with the physiopathology associated with dysentery. Reasons why this toxin may not have been found are that it is produced in close association with the epithelium, and as such was not extractable, or that the medium used for in vitro growth of the organism was lacking in an essential component. The time span from inoculation until clinical signs are observed may be an indication of a requirement that is fulfilled by the colonic environment. It may also be that this toxin attacks only younger maturing cells and it is necessary for these cells to reach the villous surface before disease

becomes evident. It must also be remembered that only one isolate, B204, was used in these studies. Four distinct serologic groups of I. hyodysenteriae have been identified (Baum and Joens, 1979) and since differences may exist in the ability of different serotypes to produce the active component in pathogenesis, other strains should be tested.

Any of the materials tested for cytotoxic activity should also be tested as possible inflammatory agents since the lesions of dysentery are primarily inflammation of the colon and cecum. The water phase, of the hot phenol and water extraction procedure, should be closely examined for each activity. The water phase material is also used for serotyping (Baum and Joens, 1979).

Before performing experiments searching for a cytotoxin or inflammatory agents, the role of inflammation in the disease process must be established. Does the inflammation precede mucosal edema, epithelial death, and erosion, or is it secondary to these processes? Is the inflammation produced directly by a cellular product or secondarily to exposure of the underlying layers to the normal colonic flora? An answer to these questions would provide presumptive evidence for or against a cytotoxin mediated pathogenesis for dysentery. One method would be to attempt to establish the disease in the absence of an inflammatory process. This has been performed in experiments studying the pathogenesis of salmonellosis (Giannella, et al., 1977; Giannella, 1979). Dysentery without inflammatory activity would indicate a cytotoxin may be involved, while the inability to produce

disease in the absence of the inflammatory process would indicate the necessity of inflammation in the pathogenesis.

A third possibility which must be considered is the effects produced simply by the association of T. hyodysenteriae with the colonic mucosa. Studies of mammalian epithelial cells in in vitro systems indicate penetration of the cells by the organisms may not be of primary importance (Knopp, et al., 1979). However, little is understood about the nature of the association of T. hyodysenteriae with the epithelial cells. A cytotoxin, inflammatory agent, or other factors, may be produced only when the organism is in close contact with the mucosal epithelium. Association may be necessary in allowing the organisms to grow to high enough numbers to exert pathogenicity. It is also possible that the association of the organism with the epithelial cell may alter cellular metabolism and transport sufficiently to lead to cellular death, and the lesions observed in dysentery.

The factors considered here, a cytotoxin, an inflammatory agent, and epithelial associations by the organism, may function independently or in combination in the pathogenesis of dysentery. Other host or bacterial related factors may also function in some capacity. Although the mechanisms of fluid loss during the acute stages of swine dysentery are now well-understood, further studies must discern the mechanisms by which T. hyodysenteriae contributes to the pathogenesis of the disease.

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## ACKNOWLEDGMENTS

I would like to acknowledge the many people who have assisted in the work described in this thesis.

Special thanks goes to Dr. D. L. Harris for his support throughout my graduate program. Drs. R. A. Argenzio and S. C. Whipp at the National Animal Disease Center were also generous in both time and effort supporting this research. The assistance of Mrs. J. M. Kinyon and Dr. R. D. Glock are also greatly appreciated.

The support of excellent technical help, both at the National Animal Disease Center and the Veterinary Medical Research Institute deserves mention. Bev Mullen, Deb Lebo, Robert Morgan, Iona Hegland, and Sean Phan are only a few of those who have assisted in this research.

Typist Marge Davis and illustrator Dean Beichler have provided excellent aid in preparing this manuscript.

Finally I wish to thank my wife Jill for her support.

APPENDIX A. CALCULATIONS FOR PEG,  $\text{HCO}_3^-$ , CYCLIC AMP AND CYCLIC  
GMP DETERMINATIONS

Calculations:Procedure for PEG analysis

Five standards- 0, .5, 1.0, 1.5, and 2.0 mg PEG/ml were used to obtain a standard curve

- 1) 1 ml samples or standard + 19 ml H<sub>2</sub>O + 1 ml 10% BaCl<sub>2</sub> + 2 ml filtered .3N Ba (OH)<sub>2</sub> + 2 ml .3N ZnSO<sub>4</sub>
- 2) Filter through #2 Whatman paper
- 3) Add 2 ml filtrate to 2 ml of filtered 5% BaCl<sub>2</sub> and 30% trichloroacetic acid
- 4) Mix
- 5) Read at 425 nm in exactly 5 minutes on spectrophotometer (Beckman Model DBG or Gilford Model 225 spectrophotometer)

PEG concentrations were determined from curve of five standards.

Procedure for HCO<sub>3</sub><sup>-</sup> determination

- 1) 2 ml sample + 1 ml .1 N HCl + 10 ml H<sub>2</sub>O + 1 drop phenol red indicator
- 2) Gas for 15 minutes at room temperature with N<sub>2</sub>
- 3) Titrate to endpoint (pH 7.0) with CO<sub>2</sub> free .05 N NaOH

HCO<sub>3</sub><sup>-</sup> concentrations were determined by the formula:

$$\text{HCO}_3^- = [(B_{\text{NaOH}} - S_{\text{NaOH}}) \times N] / S_v$$

where B<sub>NaOH</sub> = amount of NaOH required to titrate a blank of 10 ml water + 1 ml of .1 N HCL

$S_{\text{NaOH}}$  = amount of NaOH required to titrate the sample

$N$  = normality of the NaOH

$S_v$  = sample volume

### Cyclic AMP and cyclic GMP determination

Calculations for c-AMP were made as follows:

$$\frac{\text{p moles c-AMP (from assay)} \times \% \text{ recovery} - (\text{p moles C-AMP added as marker})}{\text{mg tissue}}$$

= p moles c-AMP/mg tissue

Calculations for c-GMP were made in a similar manner with results expressed as f moles/mg tissue. Results are given

plus or minus the standard error; p moles = picomoles =

$10^{-12}$ M, fmoles = femtomoles =  $10^{-15}$ M.

APPENDIX B. RAW AND PROCESSED NUMERICAL DATA

Table B1. Type I studies. Theophylline dose response, net effects of solutions 1-5

Solution	Na <sup>+</sup> <sup>a</sup>	Cl <sup>-</sup> <sup>a</sup>	HCO <sub>3</sub> <sup>-</sup> <sup>a</sup>	TS <sup>b</sup>	H <sub>2</sub> O <sup>c</sup>	pH	pCO <sub>2</sub> <sup>d</sup>
1 (n = 19) <sup>e</sup>	.75 ± .09	.74 ± .06	-.07 <sup>f</sup> ± .03	1.26 ± .10	2.23 ± .33	7.39 ± .02	51.3 ± 1.7
2 (n = 10)	.65 ± .09	.73 ± .14	-.06 ± .05	1.07 ± .17	1.57 ± .47	7.30 ± .02	51.7 ± 1.9
3 (n = 10)	.34 ± .07	.52 ± .06	-.24 ± .05	.62 ± .15	.40 ± .45	7.39 ± .02	49.9 ± 2.0
4 (n = 10)	.14 ± .09	.44 ± .05	-.42 ± .06	.18 ± .14	.06 ± .43	7.56 ± .04	44.2 ± 2.2
5 (n = 9)	-.15 ± .13	.44 ± .06	-.69 ± .13	-.29 ± .25	-1.02 ± .80	7.56 ± .05	45.8 ± 2.6

<sup>a</sup>Na<sup>+</sup>, Cl<sup>-</sup>, and HCO<sub>3</sub><sup>-</sup> are expressed as mEq/g mucosa/hr ± s.e.

<sup>b</sup>TS expressed as mosm/g mucosa/hr ± s.e.

<sup>c</sup>H<sub>2</sub>O expressed as ml/g mucosa/hr s.e.

<sup>d</sup>pCO<sub>2</sub> expressed in mm Hg ± s.e.

<sup>e</sup>n = number of loops.

<sup>f</sup>Negative values indicate net secretion, positive net absorption.

Table B2. Sodium (mEq) remaining in ligated colonic loops<sup>a</sup> (2 hr incubation)

Time (min)	<u>Solution</u>							
	<u>1</u>				<u>5</u>			
	<u>Pig #</u>							
	1	2	3	$\bar{X}$	1	2	3	$\bar{X}$
0	22.7	18.3	20.1	20.4	25.1	19.6	19.5	21.9
20	21.8	17.6	18.9	19.4	25.1	18.1	19.2	20.8
40	19.9	15.5	16.3	17.2	24.5	18.0	17.2	19.9
60	17.2	14.5	14.1	15.3	24.3	17.1	17.2	19.5
80	16.3	16.2	13.2	15.2	23.4	16.6	15.6	18.5
100	13.3	14.4	11.8	13.2	23.7	17.1	14.4	18.4
120	10.6	12.9	10.7	11.4	50.0	13.8	13.4	15.7

<sup>a</sup>No significant variation in dried weights of mucosa from loops exposed to each solution.



Table B3. Type II studies. Effects of theophylline in sodium free solutions (1, 5, 6, and 7) on colonic transport

	$\text{Na}^{\text{+a}}$	$\text{Cl}^{\text{-a}}$	$\text{HCO}_3^{\text{-a}}$	TS <sup>b</sup>
1 (n = 6) <sup>e</sup>	.73 ± .01	.68 ± .13	-.06 <sup>f</sup> ± .04	1.45 ± .24
1 (n = 6)	.63 ± .09	.45 ± .08	-.02 ± .03	1.13 ± .16
5 (n = 6)	.05 ± .07	.48 ± .11	-.39 ± .08	.41 ± .23
5 (n = 6)	.14 ± .16	.62 ± .21	-.43 ± .06	.39 ± .27
6 (n = 6)	.44 ± .06	.80 ± .11	-.26 ± .06	.85 ± .19
7 (n = 6)	.02 ± .10	.38 ± .12	-.48 ± .10	.12 ± .12

<sup>a</sup> $\text{Na}^{\text{+}}$ ,  $\text{Cl}^{\text{-}}$ ,  $\text{HCO}_3^{\text{-}}$   $J_{\text{LB}}^{\text{Na}^{\text{+}}}$   $J_{\text{BL}}^{\text{Na}^{\text{+}}}$  are expressed in mEq/g mucosa/hr ± s.e.

<sup>b</sup>TS is expressed in mosm/g mucosa/hr ± s.e.

<sup>c</sup> $\text{H}_2\text{O}$  is expressed in ml/g mucosa/hr ± s.e.

<sup>d</sup> $\text{pCO}_2$  is expressed in mm Hg ± s.e.

<sup>e</sup>n = number of loops.

<sup>f</sup>Negative values indicate net secretion, positive net absorption.

$H_2O^c$	$J_{LB}^{Na^+a}$	$J_{BL}^{Na^+a}$	pH	$pCO_2^d$
3.01 ± .71	1.22 ± .16	.49 ± .05	7.27 ± .04	60.8 ± 6.4
2.21 ± .42	1.06 ± .14	.43 ± .06	7.27 ± .03	63.0 ± 6.1
.07 ± .37	.44 ± .15	.39 ± .09	7.60 ± .05	46.9 ± 4.6
.81 ± .56	.48 ± .20	.34 ± .05	7.70 ± .04	51.5 ± 7.12
2.04 ± .71			7.73 ± .03	30.9 ± 5.0
.16 ± .32			7.69 ± .03	46.5 ± 3.5

Table B4. Type III studies. Effects of theophylline on colonic transport in dysentery affected pigs (solutions 1, 3, and 5).

Solution	$\text{Na}^{\text{a}}$	$\text{Cl}^{\text{a}}$	$\text{HCO}_3^{\text{a}}$	$\text{TS}^{\text{b}}$
1 (n = 10) <sup>e</sup>	.14 ± .10	.00 ± .08	.10 ± .04	-.16 ± .20
3 (n = 12)	-.02 <sup>f</sup> ± .16	-.08 ± .10	-.04 ± .03	-.34 ± .21
5 (n = 14)	.03 ± .07	-.03 ± .05	-.09 ± .02	-.22 ± .12

<sup>a</sup> $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$   $J_{\text{LB}}^{\text{Na}^+}$   $J_{\text{BL}}^{\text{Na}^+}$  are expressed in mEq/g mucosa/hr ± s.e.

<sup>b</sup> $\text{TS}$  is expressed in mosm/g mucosa/hr ± s.e.

<sup>c</sup> $\text{H}_2\text{O}$  is expressed in ml/g mucosa/hr ± s.e.

<sup>d</sup> $p\text{CO}_2$  is expressed as mmHg ± s.e.

<sup>e</sup>n = number of loops.

<sup>f</sup>Negative values indicate net secretion, positive net absorption.

$H_2O^c$	$J_{LB}^{Na^+a}$	$J_{BL}^{Na^+a}$	pH	$pCO_2^d$
$-1.22 \pm .43$	$.69 \pm .14$	$.55 \pm .10$	$7.08 \pm .06$	$68.9 \pm 6.3$
$-1.78 \pm .48$	$.48 \pm .23$	$.50 \pm .11$	$7.17 \pm .05$	$60.5 \pm 5.3$
$-1.05 \pm .34$	$.43 \pm .04$	$.40 \pm .06$	$7.31 \pm .04$	$52.8 \pm 3.4$

Table B5. Cyclic AMP concentrations (pmoles/mg mucosa)<sup>a</sup> in normal and infected colonic mucosa exposed to mannitol (20 mM) or theophylline (20 mM)

Time (minutes)	Mannitol (20 mM)		Time	Theophylline (20 mM)	
	Normal	Infected		Normal	Infected
0	1.49 ± .12	.86 ± .09	0	1.43 ± .45	.96 ± .17
15	1.14 ± .18	.79 ± .08	2	2.75 ± .45	.96 ± .17
30	.88 ± .12	.82 ± .08	5	3.55 ± .45	1.53 ± .17
			10	4.38 ± .45	1.86 ± .17
			30	4.12 ± .45	1.54 ± .17

<sup>a</sup> picomoles/mg wet weight mucosa.

Table B6. Cyclic GMP concentrations (fmoles/mg mucosa)<sup>a</sup> in normal and infected colonic mucosa exposed to mannitol (20 mM) or theophylline (20 mM)

Time (minutes)	Mannitol (20 mM)		Time	Theophylline (20 mM)	
	Normal	Infected		Normal	Infected
0	172.5 ± 32.5	252.1 ± 27.2	0	261.7 ± 70.1	311.7 ± 52.3
15	213.9 ± 30.3	186.8 ± 27.2	2	271.8 ± 81.6	450.1 ± 50.9
30	220.8 ± 32.5	197.9 ± 27.2	5	631.6 ± 72.3	518.6 ± 48.4
			10	574.3 ± 75.0	569.8 ± 50.9
			30	504.8 ± 65.8	267.2 ± 49.6

<sup>a</sup> femtomoles/mg wet weight mucosa.

Table B7. Experiment IV. Effects of extracts of colonic mucosa and colonic contents of normal and dysentery affected pigs on normal colonic transport

Infected Material	Na <sup>+</sup> <sup>a</sup>	Cl <sup>-</sup> <sup>a</sup>	HCO <sub>3</sub> <sup>-</sup> <sup>a</sup>	TS <sup>b</sup>	H <sub>2</sub> O <sup>c</sup>
N (n = 6) <sup>d</sup>	1.20 ± .11	0.73 ± .22	-0.04 <sup>e</sup> ± .05	1.65 ± .32	4.42 ± 1.04
O (n = 6)	.68 ± .20	1.04 ± .43	-0.69 ± .20	0.83 ± .42	2.31 ± 1.31
P (n = 5)	1.09 ± .30	0.56 ± .47	-0.10 ± .08	1.96 ± .67	5.43 ± 2.08
Q (n = 6)	1.13 ± .31	1.50 ± .34	-0.05 ± .17	2.22 ± .63	4.66 ± 1.57
R (n = 5)	1.31 ± .31	1.65 ± .48	-0.30 ± .27	2.56 ± .96	6.21 ± 2.89
S (n = 4)	1.36 ± .55	1.38 ± .38	0.17 ± .17	1.70 ± .59	3.67 ± 1.76

<sup>a</sup>Na<sup>+</sup>, Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup> are expressed as mEq/g mucosa/hr ± s.e.

<sup>b</sup>TS is expressed as mosm/g mucosa/hr ± s.e.

<sup>c</sup>H<sub>2</sub>O is expressed as ml/g mucosa/hr ± s.e.

<sup>d</sup>n = Number of loops.

<sup>e</sup>Negative values indicate net secretion, positive net absorption.