GLUCOSE, ELECTROLYTE, AND FLUID ABSORPTION FROM THE SMALL INTESTINE OF GNOTOBIOTIC PIGS INFECTED WITH ESCHERICHIA COLI

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
MARIANNE EILEEN SHIRK
1971

ABSTRACT

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By

Marianne Eileen Shirk

Eighteen 2-week-old hysterotomy-derived gnotobiotic pigs from 2 litters were used to study the effects of colibacillary diarrhea on the absorption of d-glucose, sodium, chloride, potassium, and fluid. The animals were paired and 1 pig from each pair was exposed *per os* to 3×10^6 *Escherichia coli* 0138:K81:NM organisms. Twenty-four hours after infection, isolated jejunal loops were prepared in each animal for *in vivo* measurement of nutrient absorption. These loops were continuously perfused for a period of 4 hours with a solution of 26 mM glucose in lactated Ringer's solution.

Glucose, sodium, chloride, and fluid absorption was not impaired in the *E. coli*-infected loops. These monocontaminated loops consistently absorbed more glucose and produced less fluid than the germfree control loops. Mean glucose absorption (μM/cm/4 hr) was 55.1 in the infected animals but only 28.4 in the controls. Fluid production in the germfree control loops averaged 0.46 ml/cm/4 hr while the infected loops actually absorbed 0.07 ml/cm/4 hr. Sodium movement directly correlated with fluid production ($P<.001$). Chloride movement generally followed a similar

pattern. The germfree intestinal loop secreted 68 μEq/cm of sodium and 41 μEq/cm of chloride during the 4-hour period. The *E. coli*-contaminated loops absorbed 10 and 7 μEq/cm/4 hr of sodium and chloride, respectively. Differences in potassium secretion were not significant.

The most consistent hematologic finding in the pigs exposed to *E. coli* was a marked leukocytosis with a regenerative shift to the left. Some evidence of dehydration was present in infected animals.

The histologic appearance of the duodenum, the intestine anterior and posterior to the loop, and the ileum did not differ between control and infected animals. Varying degrees of epithelial vacuolization, submucosal edema, and cellular infiltration were noted. Similar changes were also seen in the isolated loops. The severity of the lesions did not correlate with changes in absorption.

The findings in the present investigation did not support the theory that malabsorption is a prominent feature in the pathogenesis of colibacillary diarrhea.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Pathology

1971

To my mother and father

Eleanor and Wesley Lickfeldt

ACKNOWLEDGEMENTS

I wish to express my gratitude and appreciation to my major professor, Dr. Glenn L. Waxler, for his expert guidance and encouragement during the course of this research project.

I would also like to thank Dr. C. C. Morrill, Chairman of the Department of Pathology, Michigan State University, for his support and confidence in continuing my education. In addition, I would like to thank Drs. Allan Trapp and David Ellis for serving on my guidance committee.

I am indebted to the following persons for their invaluable technical assistance: Drs. D. T. Drees and B. R. Christie for aiding me in the procurement of the gnotobiotic pigs used in this research work; Mr. James Southern, animal caretaker at the Veterinary Research Farm; Mrs. Dottie Fenner, medical technologist; and Mrs. Mae Sunderlin and Mrs. Frances Whipple, histologic technicians.

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INTRODUCTION

Neonatal colibacillary diarrhea or white scours is one of the most perplexing problems facing the swine industry today. Large economic losses have resulted from high mortality rates in baby pigs during the first week of life. It has been estimated that over 40% of all deaths in neonatal pigs are due to *Escherichia coli* infections (Kenworthy and Allen, 1966a).

Extensive research has been conducted on the pathogenesis of this disease, which is characterized by sudden onset, profuse diarrhea, rapid dehydration and death. The mechanism which causes the fluid accumulation in the intestinal tract is still not clearly understood. Does the diarrhea result from increased intestinal secretion? Does malabsorption become apparent as in transmissible gastroenteritis? Or are both of these factors involved simultaneously?

An *in vivo* system using the perfused intestinal loop technique was adapted to gnotobiotic pigs. The absorption of d-glucose, and the net movement of sodium, potassium, chloride, and fluid was determined in *E. coli*-infected and germfree animals. It was hoped that data obtained from this study would support or contradict current theories on the pathogenesis of colibacillary diarrhea.

LITERATURE REVIEW

The Gnotobiotic Pig

Although the use of the gnotobiotic pig as an important research animal has steadily increased since the early 1960s, many characteristics of this germfree animal have not yet been investigated (Alexander *et al.*, 1969). Meyer *et al.* (1964) stated that the pig was a very good experimental model animal because of its similarities to man in the areas of nutrition, hematology, anatomy of the vascular system, skin, eyes, and gastrointestinal tract. Alexander *et al.* (1969), Betts *et al.* (1960), and Lecce and Reep (1962) reported that, because of its size and ability for early immunologic competence, the gnotobiotic pig would become an excellent model system for the study of immunity and disease. Being colostrum-deprived, the germfree pig does not receive naturally produced maternal antibodies, since swine have epithelial-chorial placentas across which antibodies do not pass. In the study of a particular disease, the germfree animal is free from interfering antibodies, natural resistance, and intercurrent infections. With no immune substances present, the germfree animal is quite uniformly susceptible to infectious agents (Waxler, 1970).

Research Model for Colibacillosis. Kohler and Bohl (1966) pointed out some advantages of using gnotobiotic pigs to study colibacillosis. Gnotobiotic pigs at birth are free from demonstrable antibody and from other infectious agents that may alter the course of the disease. Also,

there are no extraneous strains of *E. coli* present that could interfere with production of the disease experimentally. Lastly, adequate control animals are available. Kohler and Bohl (1966) infected gnotobiotic pigs with *E. coli* and found that the clinical signs appeared to be identical to those seen in the naturally occurring disease caused by organisms of the same serotype. Saunders *et al.* (1963) produced a clinical syndrome comparable to field outbreaks of colibacillosis by exposing gnotobiotic pigs to certain serotypes of *E. coli*. Kenworthy and Allen (1966a) and Miniats (1970) encountered difficulties when they attempted to reproduce neonatal scours or edema disease in conventionally reared pigs. This problem has been attributed to a failure of *E. coli* organisms to establish a significant population in the conventional host following artificial infection. *Escherichia coli* will, however, readily colonize and proliferate in the gnotobiotic gut.

Procurement. Waxler *et al.* (1966) and Waxler (1970) described 2 techniques--hysterectomy and hysterotomy--which have been successfully used in obtaining germfree pigs. The hysterotomy technique was preferred for several important reasons:

1) Individual attention can be given to the newborn to stimulate breathing and increase survival rate.

2) Surgery is performed in a completely closed environment.

3) The sow can be saved for future litters if so desired.

Meyer *et al.* (1964) and Alexander *et al.* (1969), in contrast, favored the hysterectomy procedure because of the facility of time and the ease of anesthetizing the sow. The pigs procured in this manner were free of demonstrable fungi, bacteria, PPLO, ascarids, and viruses.

Colibacillosis in Neonatal Pigs

Colibacillosis, white scours, or diarrhea neonatorum is an acute, sometimes highly fatal, enteritis or gastroenteritis of suckling pigs (Dunne and Bennett, 1970). Kenworthy and Allen (1966a) reported that a 1960 survey in England indicated 40% of the pigs that died at 4 months of age or younger had enteritis associated with *Escherichia coli*. In addition, Kohler and Bohl (1966) referred to a survey in England in which *E. coli* organisms were the pathogens most frequently linked to the presence of disease in pigs less than 3 weeks of age. White scours in pigs and calves has been compared to diarrhea in human infants where *E. coli* has been isolated as an infective agent (Saunders *et al.*, 1960).

Bacterial Characteristics. *Escherichia coli* is a common inhabitor of stables and pens and causes diarrhea in neonatal farm animals (Barnum *et al.*, 1967). Infection is dependent upon the pathogenicity of the bacterium—i.e., the ability to produce toxin--the resistance of the host, and the number of infecting organisms (Dunne and Bennett, 1970).

Many serotypes of *E. coli* have been identified by classification of O, H, and K antigens. Somatic or O antigens are lipopolysaccharides. One hundred forty-seven O antigens have been typed. There are 49 H or flagellar antigens and 89 K or sheath (envelope) antigens. All O antigens have been isolated from colibacillary diarrhea with the exception of 017, 018, 019, and 055. Over 81% of enteropathogenic strains of *E. coli* are hemolytic. This is not, however, important in the pathogenesis of this disease (Kenworthy and Allen, 1966a). Serotype 0138:K81 has been incriminated as a pathogen in both baby pig enteritis and edema disease (Dunne and Bennett, 1970; Gossling and Rhoades, 1966; Hagen and Haugen, 1963; Nielsen *et al.*, 1968). Hagen and Haugen (1963) studied

the enterotoxemic effects of several serotypes of *E. coli* including 0138 isolated from infected pigs. Cell-free extracts of the fecal material from infected animals caused rapid death when injected intravenously into pigs or mice. Similar preparations from "healthy control animals" had no effect when injected. Kohler (1968) concluded that the enterotoxic activity of *E. coli* may be the cause of diarrhea in young pigs. Inoculations with cell-free filtrates in conventional piglets that had received colostrum produced severe diarrhea in 1.5 to 4.5 hours. In addition, comparable findings have been made in germfree pigs (Kohler and Cross, 1969). Moon *et al.* (1966a) claimed that the ability of a strain of *E. coli* to cause distention in a ligated loop system was an indication of its enteropathogenicity and not its general pathogenicity. Nielsen and Sautter (1968) reported that serotype 0138 was enteropathogenic as indicated by the ligated loop technique. Results have been variable when researchers tried to reproduce distention in ligated loops with various enteropathogenic strains of *E. coli* in young pigs (Truszczynski and Pilaszek, 1969). Smith and Halls (1967) were successful, however, in correlating pathogenicity with loop dilation.

Kenworthy and Crabb (1963) reported that the intestinal tract of the healthy piglet is sterile at birth. During colibacillosis, pathogens are capable of almost completely replacing the resident intestinal flora, especially in the jejunum. Saunders *et al.* (1963) stated that the number of organisms had little effect on the severity of infection. Host resistance played a more important role. Six-day-old pigs were much more resistant than 12-hour-old pigs.

Clinical Signs. Colibacillosis occurs in pigs 1 to 8 days of age. Some may become ill within 12 hours following birth and die of an acute

septicemia in 48 hours with or without clinical signs of diarrhea. More commonly, however, pigs exhibit a yellowish-white watery diarrhea, dehydration, and rough hair coats. Tails may become necrotic and slough. Mortality is highest during the first week of life when it may reach 70% or more (Dunne and Bennett, 1970). Saunders *et al.* (1960) found that rapid death of most of a litter occurred within the first 48 hours of life. Colibacillosis occurs most frequently without bacteremia or morphologic evidence of enteritis (Moon *et al.*, 1966b).

Hematologic Aspects. Alexander *et al.* (1969) reported that the gross morphology of internal organs and quantitative measurements of formed blood elements were very similar between germfree and conventional pigs. Meyer *et al.* (1963) stated that the leukocyte and erythrocyte counts and hemoglobin levels of germfree pigs were within normal limits of conventional values. Serum proteins, however, were low. Britt (1967) found no evidence of anemia, hemoconcentration, or serum electrolyte (sodium and potassium) derangements in germfree pigs infected with *E. coli*. Infected pigs responded with either leukocytosis or leukopenia. No significant difference was noted between infected and control groups in regard to hemoglobin and packed cell volume.

Histopathologic Observations. Cross and Kohler (1969) studied the autolytic changes in the intestinal tract of germfree, *E. coli*-monocontaminated, and conventional pigs. The most striking differences were found in the ileum and colon. The colonic submucosa in the germfree gut was thinner and had shorter, broader villi than either the *E. coli*-monocontaminated or conventional gut. The conventional and monocontaminated intestines had increased cellularity compared with the germfree animals. The greatest numbers of neutrophils, lymphocytes, macrophages and histiocytes were

found in the conventional animals; least numbers were found in the germfree pigs; and the *E. coli*-monocontaminated gut was intermediate in this respect. Cross and Kohler (1969) described the contaminated gut as being in a state of "physiological inflammation." Dubos *et al.* (1963) described the germfree intestine in the mouse as rather prenatal and undifferentiated. There was an absence or near absence of inflammatory cells in the lamina propria. The crypts were shallow and the villi were delicate. Distended goblet cells were evident. This condition of the intestine was rapidly changed with the addition of one or more microbial species. Staley *et al.* (1968) studied the microscopic appearance of the newborn jejunum of the pig. They reported that the simple columnar epithelium contained few goblet cells but that vacuoles were very prominent in the upper 2/3 of the cells lining the long, slender villi. Alexander *et al.* (1969) reported similar findings in germfree pigs. The epithelial cells of the jejunum and ileum were extremely vacuolate, especially at the tips of the villi, until 7 weeks of age. The presence of cellular elements varied considerably among germfree pigs of the same age group. Plasma cells, eosinophils, and reticuloendothelial cells were found in both the lamina propria and the submucosa. This cellularity, however, was much less than in conventionally reared pigs of the same age.

Gilka (1969) described the histopathologic lesions of the jejunum in newborn piglets with *E. coli* gastroenteritis. He found inflammatory edema in the lamina propria with cellular infiltration, primarily neutrophils, in the villi. Thirteen of 17 pigs had no glycogen in the hepatic cells. No specific changes were seen in the kidney. Dunne and Bennett (1970) reported that the intestinal reaction of newborn pigs with colibacillosis was similar to the reaction to many low-grade irritants. Grossly, the intestines were filled with a yellowish-gray mucous material.

Microscopically, there was a marked distention and vacuolization of the intestinal epithelial cells with enlarged mucus-producing goblet cells. Kohler (1967a) reported the infiltration of neutrophils in the upper small intestine of both *E. coli*-infected and control pigs. No epithelial desquamation or villous atrophy was seen in either group. Moon *et al.* (1966) occasionally found denudation of the epithelium, neutrophilic infiltration of the lamina propria and distention of the lamina propria with nonprotein fluid in the jejunum. Inconsistent changes were reported in other body organs. Moon *et al.* (1970a) described similar lesions but noted that these changes were inconsistent from animal to animal. They found no lesions in 7 of 18 newborn pigs infected with *E. coli*. Seven of 18 exhibited loss of the intestinal epithelium, congestion and some hemorrhage with thrombosis in the submucosal vessels. In 4 of the 18 animals studied, villous atrophy was present in the anterior intestinal tract while the remainder of the gut appeared normal. Smith and Jones (1963) reported no difference between baby pigs with *E. coli* diarrhea and their normal healthy litter mates during the first week of life. No evidence of inflammation was present. Kenworthy and Allen (1966b) described the monocontaminated gut as having long, slender, uniform villi with little cellular infiltration. They concluded that the morphology of the small intestinal epithelium was related to a biochemical interaction between bacteria and diet. Whether these bacteria were antagonistic or symbiotic was important. Kohler and Bohl (1966), in studying lesions of colibacillosis in gnotobiotic pigs, saw no inflammatory reaction in either the small or large intestine in control and infected animals. Kohler and Cross (1969) found no histopathologic lesions in gnotobiotic pigs with diarrhea produced by cell-free filtrates of *E. coli* cultures.

Pathogenesis. Moon *et al.* (1970b) and Nielsen *et al.* (1968) concluded that the pathogenesis of cholera (*Vibrio cholerae*) in man and colibacillosis in animals was similar. Because of these similarities, references to the pathogenesis of colibacillosis in this thesis will be supplemented with research material on *Vibrio cholerae* infection in man and in other experimental animal model systems.

Nielsen *et al.* (1968) divided the pathogenesis of colibacillary diarrhea into 4 important categories: (1) infection, (2) proliferation, (3) production of enterotoxin and (4) production of lesions.

Infection. Infection was considered to be dose-dependent: i.e., the greater the number of organisms to which the animal was exposed, the greater the possibility of infection. Dunne and Bennett (1970) stated that a septicemia develops in the newborn, especially if the infective dose was given prior to the ingestion of colostrum. Kenworthy and Allen (1966a) argued that the amount of fluid accumulation was independent of the number of infecting organisms.

Proliferation. Proliferation of *E. coli* organisms is due to several physiologic factors. First, baby pigs are achlorhydric for a period lasting from 1 to 14 days after birth. The proliferation of organisms is therefore not retarded by the low gastric pH normally present in older pigs (Dunne and Bennett, 1970; Barnum *et al.*, 1967). Smith and Jones (1963) found that, even if the gastric pH decreased soon after birth, *E. coli* organisms had the ability to proliferate in great numbers in the anterior small intestine where the population was normally small. Secondly, the mucus-producing cells of the gut do not actively secrete until after the first meal. The bacteria are therefore able to cling to the mucosa and increase in large numbers in the anterior small intestine

(Nielsen *et al.*, 1968). In addition, the newborn pig's intestine is sterile at birth. No established flora or nutrient competition is present (Barnum *et al.*, 1967). Kohler and Cross (1969) concluded that colibacillary diarrhea could not be explained in terms of growth rate or distribution between pathogenic and nonpathogenic strains.

Production of enterotoxin. *Escherichia coli* organisms are capable of producing an enterotoxin that causes dilation in ligated gut loops. The reaction is similar to that seen with *Vibrio cholerae* organisms. This cell-free filtrate causes diarrhea without grossly altering the mucosal epithelium (Nielsen *et al.*, 1968). Moon *et al.* (1970) produced comparable experimental results with *E. coli* and *V. cholerae* enterotoxins in the rabbit ileum. Kohler and Cross (1969) were able to reproduce clinical signs of colibacillosis with cell-free filtrates in gnotobiotic pigs. Burrows and Musteikis (1966) concluded that vibriosis was essentially a toxemia. Its effect in the gut could not be blocked by antibiotics, nor was it a result of increased bacterial population.

Production of lesions. Many theories have been proposed to explain the pathogenesis of the fluid accumulation seen in colibacillosis:

1) Capillary permeability is altered causing the escape of large osmotically active particles into the intestinal lumen. There is also an increase in hydrostatic pressure (Barnum *et al.*, 1969; De and Chatterje, 1953; Fordtran, 1967; Norris and Majno, 1968). Moon *et al.* (1966a) reported, however, that there was no difference in the osmolarity of the luminal contents between control and *E. coli*-infected ligated loops. They concluded that fluid accumulation occurred through an intact membrane before inflammation occurred. Norris and Majno (1968) found capillary endothelium and basement membranes of the intestinal epithelial cells

intact both under the light and electron microscope. In addition, no intravenous Evans blue dye appeared either in the intestinal wall or in the efflux fluid (Norris and Majno, 1968; Leitch *et al.*, 1966). Moreover, the protein content of control and infected loops was very low (Norris and Majno, 1968; Nielsen and Sautter, 1968). It is impossible to explain fluid production on the basis of hydrostatic pressure alone. The escape of osmotically active particles does not occur because intraluminal fluid during infection remains either isosmotic with plasma or hypotonic (Nielsen *et al.*, 1968).

2) Absorption of osmotically active particles is blocked--i.e., by inhibition of the sodium pump (Fordtran, 1967; Norris and Majno, 1968). Nielsen and Sautter (1968) and Leitch *et al.* (1967) found no disturbance in the sodium-absorptive mechanism. Norris *et al.* (1967) and Norris and Majno (1967) had similar results using radioactive sodium. Moon *et al.* (1966a) found that the sodium content per unit volume was the same in *E. coli* and control ligated loops.

3) Bacteria produce osmotically active metabolites; however, enterotoxin does not increase osmotic pressure of luminal contents (Barnum *et al.*, 1967).

4) Changes in the absorptive surface (epithelial alteration) occurs causing a decrease in efficiency to absorb particles--i.e., malabsorption (Fordtran, 1967; Kenworthy and Allen, 1966a; Dunne and Bennett, 1970; Barnum *et al.*, 1967; Norris and Majno, 1968). Kohler (1967b) concluded that colibacillary diarrhea was not explainable by morphologic changes in the digestive tract. Norris and Majno (1968) found the electrolyte content of cholera ligated loops to be very close to normal values of intestinal fluid. Torres-Pinedo *et al.* (1966) found impaired jejunal absorption of glucose, sodium chloride and fluid during *E. coli* diarrhea

in human infants. Bywater (1970) found that glucose absorption was not altered using *E. coli* enterotoxin in calves. Carpenter *et al.* (1968), using *Vibrio cholerae* enterotoxin in dogs, found glucose absorption unaffected. Moreover, intraluminal glucose actually enhanced isotonic fluid absorption from the ileum and jejunum. Fordtran (1967) reported that glucose absorption during cholera decreased sodium and fluid losses. Serebro *et al.* (1968) found equal or slightly increased glucose absorption in rabbits affected with vibriosis when compared to control animals.

5) Direct stimulatory effect on the epithelial cell by the bacteria or their products (Barnum *et al*, 1967; Norris and Majno, 1968). Nielsen *et al.* (1968) concluded that there was an irritant stimulation to the mucosal cells to secrete fluid. This intestinal fluid was equal in composition to normal intestinal secretions. This secretory effect could be rapidly reversed by using Diamox (Norris and Majno, 1968; Leitch and Burrows, 1968). This theory is rapidly gaining popularity as one of the most logical explanations of the pathogenesis of colibacillary diarrhea (Barnum *et al.*, 1967).

Additional Clinicopathologic Alterations. Medway *et al.* (1969) reported that death in neonatal diarrhea in calves was due to severe metabolic acidosis from loss of bicarbonate ions. Plasma potassium and chloride levels were not altered. Plasma sodium did not decrease until 4 days after infection. Fisher (1965) and Barnum *et al.* (1967) concluded that neonatal death from colibacillosis in pigs and calves was due to progressive dehydration with decreased plasma sodium levels and increased potassium levels leading to cardiac failure. Moon *et al.* (1970) reported similar findings in 1-day-old baby pigs. Benyajata (1966) reported no alterations in serum electrolyte values in adult human experimental cholera produced by choleragen.

Glucose and Electrolyte Absorption in the Normal State

According to Fordtran (1967) the driving force for sodium absorption in the duodenum and jejunum is the active transport of nonelectrolyte substances like glucose and amino acids. Simultaneous fluid absorption also occurs. These active transport systems operate either within the cell membrane by actual penetration of solutes or by passage through aqueous pores or channels. Due to differences in pore sizes in various areas of the intestine, the intraluminal fluid composition of solutes is therefore related to the level of intestine being studied.

Crane (1960) stated that water absorption was necessary for glucose absorption. Newey (1967) concluded that sodium was essential for glucose absorption. In addition, Newey supported the carrier mechanism hypothesis in which the sodium-glucose carrier complex moves in response to sodium ion concentration. Further, maximum glucose absorption occurs in the upper small intestine. Even in the presence of high concentrations of glucose, the absorptive capacity of the gut does not become saturated.

A Perfusion Technique for Absorption Studies

Jacobs and Luper (1957) first reported the use of an *in vivo* perfusion system in rats to study intestinal absorption. The main advantages of this system were an approximation of the normal living state of the animal and, secondly, the perfusion fluid could be sampled at any time during the experiment. They found this to be a very reliable method of measuring the absorption of nutrients. Serebro *et al.* (1968) adapted this technique to measure the absorption of d-glucose and fluid production in the jejunum of rabbits with toxin-induced cholera.

MATERIALS AND METHODS

General Plan

The pathogenesis of diarrhea produced by *Escherichia coli* is not clearly understood. This research project was proposed to determine if intestinal malabsorption and/or increased intestinal secretion by the *E. coli*-infected gut may be responsible for the diarrhea. Germfree pigs derived by hysterotomy were the experimental animals. Using the isolated loop technique described by Serebro *et al.* (1968), net absorption of glucose and the net absorption or production of fluid and electrolytes--namely sodium, potassium, and chloride--were measured during a 4-hour perfusion period. Animals were paired according to weight and sex. One pig from each pair was infected *per os* with *E. coli* serotype 0138:K81:NM. A total of 18 jejunal loops was studied.

Experimental Animals

Procurement. Using the technique described by Waxler *et al.* (1966), 18 germfree pigs were delivered by hysterotomy into sterile plastic isolators. Yorkshire gilts in their 112th day of gestation were anesthetized with 20 to 25 ml of 2.5% procaine hydrochloride* injected into the epidural space at the lumbosacral articulation. This was followed by presurgical tranquilization with promazine hydrochloride.** The gilts were killed

*Epidural, Haver-Lockhart, Kansas City, Mo.

**Sparine, Wyeth Laboratories, Cleveland, Ohio.

upon completion of delivery. Litter 1 contained 8 animals. Ten live pigs and 1 stillborn pig were obtained from Litter 2. The newborn germfree pigs were then transferred to the rearing room where they remained for approximately 10 days until absorption studies were begun.

Rearing. In the rearing area, the newborn pigs were placed in isolators so that they could be paired according to sex and weight as closely as possible. No more than 4 pigs were placed in an isolator. These experimental animals were maintained on a sterile semisynthetic diet.* In Litter 1, pigs were fed 3 ounces 3 times daily (t.i.d.) for 3.5 days, 4 ounces t.i.d. for 3.5 days, and 5 ounces t.i.d. for the remainder of the experiment. With Litter 2, however, dietary intake was increased in an attempt to increase pig size to facilitate surgical technique. Three ounces t.i.d. on Day 1 were followed by 4 ounces t.i.d. for the next 4.5 days and the 5 ounces t.i.d. as before.

Bacteriologic Procedures

Preparation of Infective Agent. *Escherichia coli* serotype 0138:K81:NM was used in this research project to produce colibacillosis in the previously germfree animals. This organism had been cultured, preserved by lyophilization in glass ampules, and then stored at -20 C until needed (Christie, 1967). Three days prior to infection of the first experimental animal, an ampule was opened under sterile conditions and the contents were inoculated into semisolid BHI** medium (0.15 gm agar/100 ml). This culture was maintained at 37 C. Blood agar plates*** were streaked at the

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*SPF Lac, Borden Company, New York, N.Y.

**Bacto Brain Heart Infusion, Difco Laboratories, Detroit 1, Mich.

***Defibrinated Sheep Cells, Colorado Serum Co., Denver, Colorado. Tryptose Blood Agar, Difco Laboratories, Detroit 1, Mich.

same time and incubated for 24 hours to insure the presence of a pure culture of *E. coli*. Eighteen hours prior to the intended exposure time, *E. coli* organisms were transferred from the BHI medium to liquid thioglycollate* medium and incubated at 37 C. (For Litter 2, nutrient tryptose agar slants** were used in place of the semisolid BHI medium.)

A series of standard density (fixed number of particles/ml) nephelometer tubes was prepared according to the method of McFarland (Hepler, 1968). In addition, a fixed amount of thioglycollate medium equal to the concentration in the culture tubes was placed into each nephelometer tube. In this way, the density of the culture tubes could be compared to the standard with a higher degree of accuracy. After estimation of the initial density of the culture vial, serial dilutions were made with sterile physiological saline solution to obtain a final concentration of approximately 3×10^6 organisms/ml. The usual dilution procedure was as follows: 0.2 ml of the 18-hour thioglycollate culture was pipetted into 10 ml of sterile saline. One milliliter of this solution was transferred into 9 ml of sterile saline, resulting in a final concentration of approximately 3×10^6 organisms/ml (dilution factor of 1:500).

Method of Exposure. Approximately 24 hours before perfusion studies were begun, 1 ml of inoculum containing 3×10^6 organisms/ml was passed into the isolator reserved for infection. The pig intended for exposure was also transferred into this isolator. The screw-capped vial containing the culture was opened within the unit, mixed with the semisynthetic diet and fed to the animal.

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*Fluid Thioglycollate Medium, Difco Laboratories, Detroit 1, Mich.

**Tryptose Agar, Difco Laboratories, Detroit 1, Mich.

Determination of Sterility of Environment. When the pigs were 5 and 14 days of age, swabs from rectal and cage areas were removed from the isolators and both inoculated into BHI and on blood agar plates. They were then incubated at 37 C and examined at routine intervals for evidence of growth.

Hematology

At 12 days of age, 3-ml blood samples were obtained from each pig in Litter 1. The initial preinfection samples were collected in ethylenediaminetetraacetic acid (EDTA)-treated vials. Postinfection sampling was done just prior to preparation of the isolated loops. The same procedure was carried out on Litter 2 with the exception that the preinfection samples were taken at 8 days of age.

Total leukocyte counts, differential leukocyte counts, microhematocrit packed cell volume, and hemoglobin determination by the cyanmethemoglobin technique were conducted according to Benjamin (1961).

Experimental Procedure

Preparation of Animals. Approximately 24 hours following oral exposure to *E. coli*, the animal pair (1 control and 1 infected) was removed from the rearing room and transferred to the experimental surgical area. Food had been withheld 5 hours prior to scheduled surgery time. Each infected animal was observed for clinical signs of colibacillosis. The pigs were weighed and the results recorded.

Preparation of Materials. Sterile technique was practiced during the experimental surgery whenever possible. Instruments, gloves, perfusion tubing, and venous catheters were sterilized either by chemical means or

by autoclaving. The perfusion solution contained 26 mM* glucose (468 mg/100 ml) and 0.3 mM phenolsulfonphthalein (PSP)** in lactated Ringer's solution.*** Flasks (250 ml capacity) containing 100 ml of the solution were autoclaved and then frozen until required.

Surgical Technique. Pigs were anesthetized with sodium pentobarbital.† The initial dosage was estimated at 0.5 ml/kg and given to effect via the anterior vena cava.

When surgical anesthesia was reached, the jugular vein of each pig was canulated with polyethylene tubing.†† The catheter was then connected to an intravenous fluid administration outfit.††† Lactated Ringer's solution was the fluid used to maintain hydration at the rate of 60 ml/hr or 1 drop every 3 seconds. Additional anesthesia could be administered via this route. Hematocrit readings were taken from the ear vein every hour to monitor the state of hydration. All surgical manipulations were performed on the germfree control animals first.

Following surgical preparation of the area, a 6-inch midline abdominal incision was made beginning just posterior to the umbilicus. The ileo-cecal junction was located and brought up through the incision. Using the approximation that 1/2 foot equals 7 to 10 vascular arcades, a point

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*mM = millimoles.

**Phenol Red, Hartman-Leddon Co., Philadelphia, Pa.

***Lactated Ringer's Injection, USP, Abbott Laboratories, North Chicago, Ill. 60064.

†Pentobarbital Sodium Injection, Haver-Lockhart, Kansas City, Mo.

††Intramedic, Clay Adams Division of Becton-Dickinson and Co., Parsippany, N.J. 07054.

†††Veno-pak, Abbott Laboratories, North Chicago, Ill. 60064.

3 feet anterior to the ileocecal junction was identified and an intestinal clamp applied. A second point 20 cm anterior to the first was determined and clamped off. The anterior and posterior ends of the loop were established. The intestinal contents in the loop had been removed by gentle massage prior to clamping.

The posterior segment of the loop was canulated first with 1/4 inch diameter tygon tubing* (Figure 1). The walls of that portion of the tubing inside the loops were perforated with several holes to insure adequate drainage of the loop. The anterior segment was canulated with unperforated tubing (Figure 2). Caution was exercised to make sure that the loop had been securely fastened to the tubing because of the slippery surfaces of the intestine and of the canula. The intestine anterior and posterior to the loop was ligated during preparation of the loop to prevent spillage into the abdominal cavity. The loop was then replaced into the abdomen. The incision was held together with towel clamps leaving only the tubing exposed. This method of closure facilitated periodic observations of the loop to make sure overdistention of the loop had not occurred. The total time required to complete the surgery was 45 to 60 minutes.

Perfusion. When preparation of the jejunal isolated loop had been completed in each animal, the perfusion pump** was started. The solution was pumped at the rate of 2 ml/minute from the reservoir flask through the loop and back into the reservoir flask. The perfusing fluid was

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*Tygon, U.S. Stoneware, Akron, Ohio.

**Sigmamotor Model T-8, Middleport, N.Y.

Figure 1. Canulation of the perfused loop. The posterior segment (A) has been canulated. The tubing to be placed in the anterior portion of the loop is shown (B).

Figure 2. The completed perfused loop. The anterior canula (A) and the posterior canula (B) are in place. The loop is ready to be returned to the abdominal cavity.

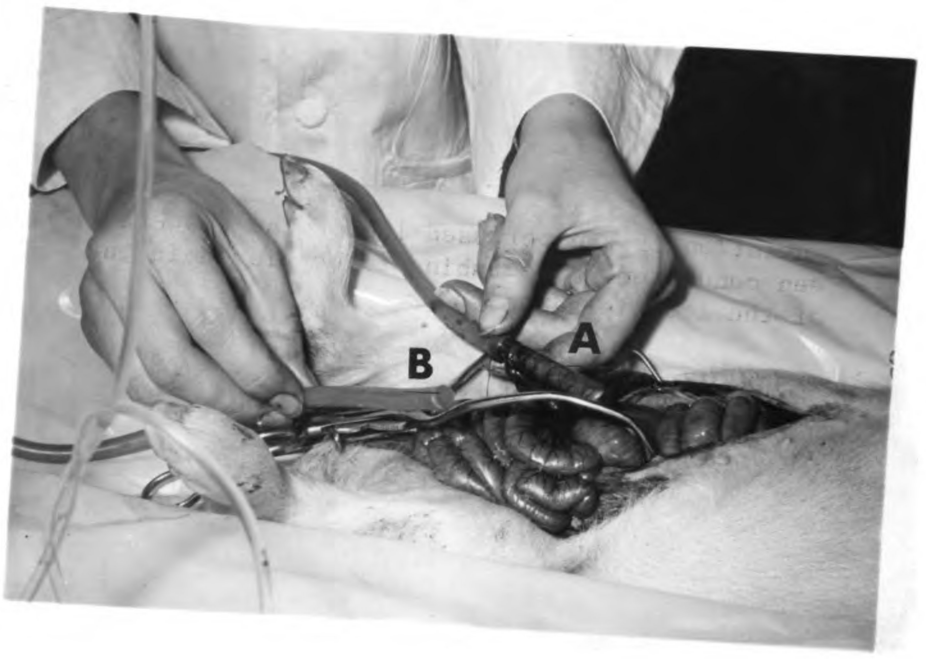

Figure 1

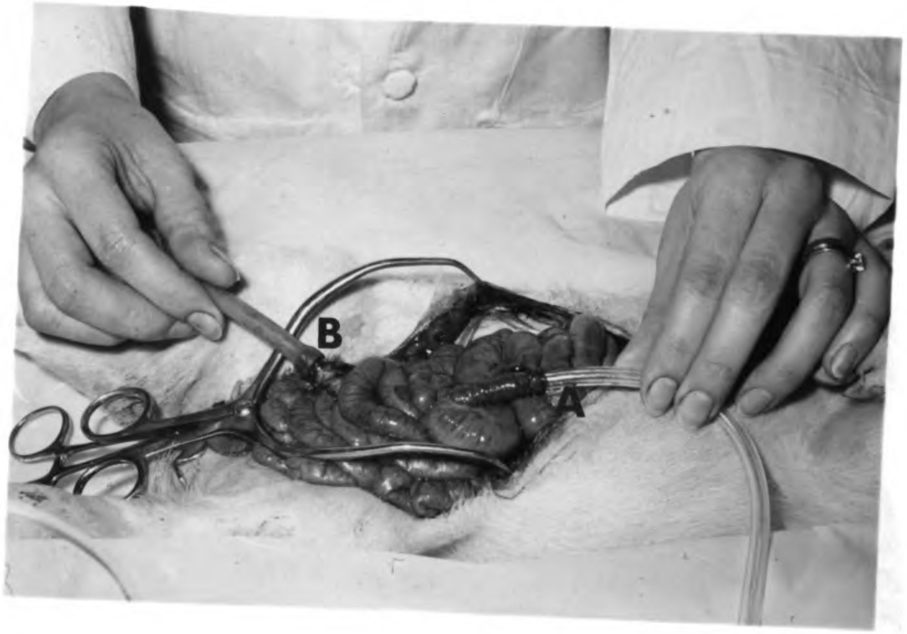

Figure 2

continually circulated in this manner for 4 hours. The reservoir flasks were held at 40 C by the use of a water bath* (Figure 3).

During the 4-hour experimental period, body temperature was monitored and kept within the normal range (100 to 102 F) with the aid of 2 heat lamps. In Litter 2, plastic-coated heating pads (placed under the pigs) were used in place of the heat lamps.

Three-milliliter samples were taken from each reservoir at 0, 1, 2, 3, and 4 hours. These samples were placed into stoppered vials and frozen for analysis of glucose, sodium, potassium, and chloride concentrations. At the termination of the experiment any fluid remaining in the loop was squeezed into the reservoir.

Necropsy and Histopathology. The pigs were euthanatized at the end of the 4-hour period with a lethal dose of sodium pentobarbital. The pH of the duodenum, jejunum (anterior to the loop), and ileum was measured with pH papers.**

The length of the loop, distance of the distal end of the loop from the ileocecal valve, and the length of the entire small intestine (pylorus to cecum) were measured. Tissues were taken for histopathologic examination from the duodenum, 2 inches anterior to the loop, midloop, 2 inches posterior to the loop, the ileum (4 inches anterior to the ileocecal valve), liver, and kidney. These samples were preserved in 10% buffered formalin and later sectioned at 6 μ and stained with hematoxylin and eosin.

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*#DES 117720 Precision Scientific Co., Chicago, Ill.

**pHydrion Papers, Micro Essential Laboratory, Brooklyn, N.Y.

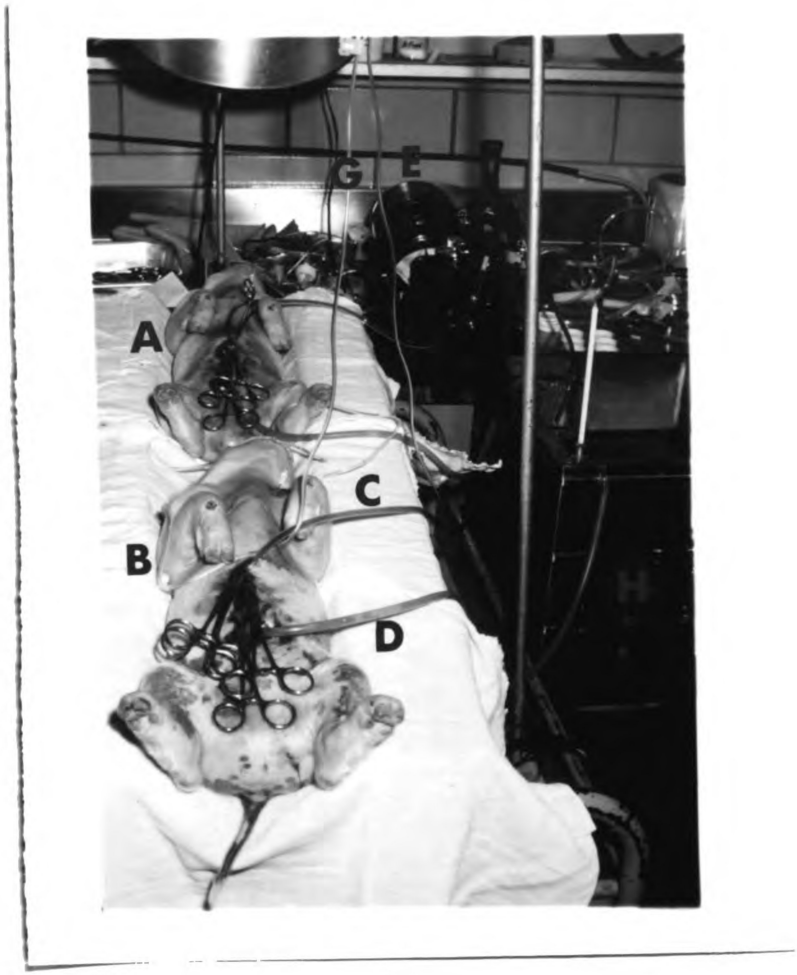

Figure 3. Laboratory setup. Following surgical procedures, the perfusion was begun. Control pig (A), infected pig (B), influx tube from reservoir (C), efflux tube from reservoir (D), pump (E), reservoir (F), intravenous tubing (G), water bath (H).

Chemical Determinations

Each 3-ml sample was thawed and analyzed for glucose, sodium, potassium, and chloride concentrations. Glucose content was measured by the ortho-toluidine method (Feteris, 1965). Sodium and potassium ions were determined by flame photometery* according to the method described by Gradwohl (1956). Chloride ion concentration was estimated using the method of Schales and Schales (1941). The volume of fluid remaining in the reservoir was measured in a graduated cylinder. Fifteen milliliters--i.e., the amount taken out during sampling--was added to this figure to obtain the total volume of fluid remaining in the reservoir.

Calculations

In the present study, absorption is defined as the disappearance of a particular nutrient from the perfusion fluid. Production is considered an increase in the amount of a particular nutrient in the perfusion fluid.

The amount of glucose absorbed by the isolated loop during the 4-hour experiment was determined in the following manner:

1) Glucose absorbed (mg) = initial glucose in reservoir (mg) - glucose (mg) removed in sampling - glucose (mg) in reservoir after the 4-hour perfusion.

2) The initial glucose in reservoir (mg) = glucose concentration of sample I (mg/ml) x 100 ml.

3) Glucose (mg) removed in sampling = [concentration in Sample I (mg/ml) x 3 ml (amount in each sample) + Sample II x 3 ml.....)].

4) Glucose (mg) left in the reservoir = concentration in Sample V (mg/ml) x the final volume (ml) in the reservoir.

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*Coleman Model 21 Flame Photometer, Coleman Instruments, Inc., 42 Madison Street, Maywood, Ill.

The final results were converted into the units mM/cm/4 hr:

glucose (mg) absorbed ÷ length of loop (cm) ÷ 180 mg glucose mM.

Absorption or production of sodium, potassium, and chloride ions was determined in a similar manner except that the final expression was calculated in μEq/cm/4 hr. Fluid movement was estimated in this manner:

Initial volume (100 ml) - [final volume (ml) + 15 ml (removed in sampling)] = fluid absorbed. A negative number indicates production. Final figures were expressed in ml/cm/4 hr = fluid movement (ml) ÷ length of loop (cm). Statistical analysis of the data between pairs of animals was carried out by the *t* test method (Goulden, 1952).

RESULTS

Procurement and Rearing Procedures

Survival rate was 100% in each litter of germfree pigs delivered by hysterotomy. These baby pigs adapted readily to the artificial environment. They quickly learned to drink from the feeding pans within the stainless steel cages.

Bacteriologic Findings

None of the swabs taken from rectal and cage areas within the isolated environment produced bacterial growth either on blood agar or in BHI media. *E. coli* was recovered in pure culture from each experimentally infected animal 24 hours after exposure. All the control pigs remained germfree until they were removed from the isolators for surgery.

Clinical and Surgical Observations

Profuse watery diarrhea with an inflamed perineal area was the most consistent clinical sign of colibacillosis in the monocontaminated animals 24 hours after exposure. Appetites of the infected animals remained normal.

Anesthesia. Sodium pentobarbital was a very satisfactory anesthetic agent. It was seldom necessary to administer additional amounts during the 4-hour experimental period.

Hydration. Although individual variation occurred, in general, intravenous fluid supplementation was adequate to maintain hydration during the perfusion study using a flow rate of 1 drop/3 seconds. Hematocrit readings are recorded in Table 1.

Measurement of the Intestine. At necropsy, considerable variation existed in the placement of the perfused loop--i.e., the distance of the posterior segment of the loop from the ileocecal valve. This was noted especially in Pairs 5 and 9. The length of the loop as determined at necropsy was consistent with the estimations made during surgery. Differences between loop lengths in infected and control animals were not statistically significant. One loop, Pig 196, was observed to be overdistended (Table 2).

Animal Data. The body weight, sex, and length of small intestine are listed in Table 3. The average weight was 1.72 kg. The mean intestinal length from the pylorus to the ileocecal valve was 463.2 cm (15.25 ft). Increase in body weight correlated roughly with an increase in intestinal length.

Hematology

The hematologic findings from preinfection samples from Litters 1 and 2 are shown in Table 4. The arithmetic means of these values are summarized in Table 5. The 12-day-old pigs had consistently higher hemoglobin and packed cell volume values. In addition, the ratio of neutrophils to lymphocytes was greater in the younger pigs. No obvious difference was noted between the other parameters.

Table 1. Results of hematocrit determinations (%)

Pair Number	Infected Time (hr) 0	2	4	Control Time (hr) 0	2	4
1	37	38.2	46	32	27	29.5
2	34	35.5	43	30	29	28.2
3	39	43.5	43	30	30.5	35.2
4	42	45	39	24.5	28	34
5	24	27	34	25	27	27
6	35	36	38	26.5	31	30
7	37.5	36	37	28	32	36
8	38	35	35	21.5	35	35
9	30	33	32	27	27	27
Mean	35.2	36.6	38.6	27.2	29.6	31.3

Table 2. Length, placement, and distention of perfused loops

Pair Number	Length of Loop (cm)		Placement of Loop[†] (cm)		Distention	
	I*	C**	I	C	I	C
1	23.5	22.5	N.D.[††]	81.3	-	-
2	22.0	22.0	106.7	91.4	-	-
3	20.0	21.0	68.6	85.1	-	-
4	20.0	20.0	66.0	63.5	+	-
5	27.9	24.1	76.2	142.2	-	-
6	30.5	38.1	99.1	108.0	-	-
7	29.2	27.9	104.1	105.4	-	-
8	35.6	31.7	100.3	119.4	-	-
9	25.4	25.4	91.4	121.9	-	-
Mean	26.0	25.8	89.0	104.6		

*I = Infected.

**C = Controls.

[†]Distance of posterior end of loop from ileocecal valve.

[††]N.D. = Not Determined.

Table 3. Comparison of body weight, sex, age, and length of small intestine

Animal Number	Age (days)	Sex	Body Weight (kg)	Length of Small Intestine (cm)
190*	12	F	1.18	411.5
191	12	M	1.70	823.4
192	13	M	1.52	411.5
193	13	F	1.52	411.5
194	14	F	1.28	398.8
195	14	M	1.52	398.8
196	15	M	1.42	325.1
197	15	M	1.38	396.2
283	11	M	1.90	452.1
284	11	M	1.75	457.2
285	12	M	2.08	487.7
286	12	F	1.55	449.6
287	13	F	2.08	508.0
288	13	M	2.18	510.6
289	14	M	2.00	434.3
290	14	M	1.80	449.6
291	15	F	2.50	561.3
292	15	F	1.60	483.0
Mean			1.72	463.2

*Even numbers - infected animals.

Table 4. Preinfection hematologic findings

Pig Number	Age (days)	Hemoglobin (gm/100 ml)	PCV (%)	Corrected Total WBC/mm^3	Differential Leukocyte Count (%)						
					Neutrophils						
					Seg*	NS	L	M	B	E	NRBC
190	12	9.7	36	7,010	52	6	40	2	0	0	2
191	12	11.0	38	10,762	45	6	47	2	0	0	5
192	12	10.3	35	7,250	33	0	66	1	0	0	0
193	12	11.0	41	7,304	25	5	69	1	0	0	2
194	12	10.8	40	7,950	39	3	57	1	0	0	0
195	12	9.5	35	8,366	37	4	58	1	0	0	1
196	12	11.7	40	6,154	31	0	69	0	0	0	4
197	12	9.5	34	6,510	39	3	57	1	0	0	6
283	8	9.1	28.3	8,235	44	7	44	4	0	1	2
284	8	9.4	27.8	8,150	54	0	45	1	0	0	0
285	8	8.9	26.8	10,000	46	1	52	1	0	0	2
286	8	8.7	26.0	6,732	52	3	41	3	1	0	1
287	8	8.9	27.0	11,500	51	5	42	1	0	1	1
288	8	9.9	30.0	6,286	52	5	40	2	0	1	5
289	8	8.5	25.8	5,340	54	0	44	2	0	0	3
290	8	9.2	27.8	9,223	53	3	40	3	1	0	0
291	8	9.7	28.8	10,680	39	0	58	0	2	1	0
292	8	8.6	27.7	8,614	54	2	43	0	0	1	1

*Seg. = Segmented neutrophil, NS = nonsegmented neutrophil, L = lymphocyte, M = monocyte, B = basophil, E = eosinophil, NRBC = nucleated red blood cell.

Table 5. Mean values of preinfection hematologic findings

Determination	Litter 1	Litter 2
Hemoglobin (gm/100 ml)	10.4	9.09
PCV (%)	37.4	27.5
Total corrected leukocyte count (cells/mm^3)	7,663	8,476
Neutrophils		
Segmented (%)	37.6	49.9
Nonsegmented (%)	3.4	2.6
Lymphocytes (%)	57.9	44.9
Monocytes (%)	1.1	1.7
Basophils (%)	0.0	0.4
Eosinophils (%)	0.0	0.5
NRBC (/100 leukocytes)	2.5	1.5

The hemograms from samples taken 24 hours following exposure of the monocontaminated pigs to *E. coli* are presented in Table 6. Mean values from control and infected pigs were combined and compared in Table 7.

All infected animals, except Pig 284, had leukocytosis, relative and absolute neutrophilia, relative lymphopenia, and a regenerative left shift. Moderate monocytosis also occurred. In addition, the monocontaminated pigs had consistently higher hemoglobin and packed cell volume values than the germfree controls. Pig 284 had a relative neutrophilia and lymphopenia with a left shift. However, total numbers of leukocytes were not increased.

Table 6. Postinfection hematologic findings

Pig Number	Age (days)	Hours After Infection	Hemoglobin (gm/100 ml)	PCV (%)	Corrected Total WBC/mm^3	Differential Leukocyte Count (%)						
						Neutrophils						
						Seg**	NS	L	M	B	E	NRBC
191*	12	---	10.7	32	6,905	35	7	56	2	0	0	5
193*	13	---	9.2	28	3,037	30	3	67	1	0	0	7
195*	14	---	9.9	29	5,150	38	4	57	1	0	0	0
197*	15	---	7.6	23.5	4,951	60	0	35	5	0	0	2
190	12	26	12.1	37	16,882	66	16	16	2	0	0	1
192	13	25	10.8	33	15,941	70	15	10	5	0	0	1
194	14	25	11.6	38	18,158	63	28	8	1	0	0	1
196	15	22	13.2	41	14,909	64	17	12	7	0	0	9
283*	11	---	7.8	25	6,833	36	4	58	1	1	0	2
285*	12	---	8.3	25	6,475	35	7	55	3	0	0	1
287*	13	---	8.8	27	4,822	41	3	54	2	0	0	1
289*	14	---	7.0	21.5	4,099	35	4	59	1	1	0	1
291*	15	---	8.6	25.3	3,850	32	1	66	0	1	0	0
284	11	22	8.6	24	6,340	61	10	24	5	0	0	0
286	12	25	11.5	35	12,618	62	12	24	2	0	0	0
288	13	24	11.2	35	17,780	50	25	21	4	0	0	0
290	14	24	11.5	36	18,600	59	15	24	2	0	0	0
292	15	25	9.3	28	9,550	59	12	21	8	0	0	0

*Controls; **Seg = Segmented neutrophils, NS = nonsegmented neutrophil, L = lymphocyte, M = monocyte, B = basophil, E = eosinophil, NRBC = nucleated red blood cell.

Table 7. Mean values of postinfection hematologic findings

Determination	Control	Infected
Hemoglobin (gm/100 ml)	8.72	11.1
PCV (%)	26.5	34.4
Total corrected leukocyte count ($cells/mm^3$)	5,113	14,725
Neutrophils		
Segmented (%)	38.3	60.7
Nonsegmented (%)	3.65	16.9
Lymphocytes (%)	56.1	17.1
Monocytes (%)	1.95	3.95
Basophils (%)	0.3	0.0
Eosinophils (%)	0.0	0.0
NRBC (/100 leukocytes)	2.2	1.8

Analysis of Perfusion Fluid

pH Determinations. Determinations of pH were made at the termination of the perfusion period from the duodenum, isolated loop, and terminal ileum (Table 8). None of the differences between pairs of animals was statistically significant when the t test was applied.

Glucose and Fluid Movement. The amount of glucose absorbed (μM/cm/4 hr) and net fluid movement (ml/cm/4 hr) in *E. coli*-infected and germfree loops are shown in Table 9. It is apparent that the infected animals consistently absorbed more glucose ($P<.01$) and produced less fluid ($P<.01$) than did the control pigs. In Figure 4, net fluid movement is plotted against glucose absorption.

Electrolyte Movement. The results of the net sodium, potassium, and chloride ion exchange across the intestinal mucosa are listed in Table 10. The variation in absorption of sodium ions between germfree and monocontaminated loops was highly statistically significant ($P<.001$). Increased sodium ion secretion directly correlated with excessive fluid production in control pigs ($P<.001$). Sodium was absorbed by most of the monocontaminated loops (Figure 5). There was no statistical difference in potassium and chloride movement when infected and control data were compared.

Histopathology

Duodenum. Little difference was noted between the microscopic structure of the duodenum in the infected and control animals. Both the germfree and monocontaminated intestine had long, slender villi with varying degrees of vacuolization of the epithelial cells and dilatation of the subepithelial lymphatic channels (Figure 6). Some hyperemia was seen in the submucosal vessels of both control and monocontaminated animals. Inflammatory cells, including neutrophils, lymphocytes, plasma cells, and reticuloendothelial cells, could be seen scattered through the tissue of both groups (Figure 7).

Small Intestine 5 cm Anterior to the Perfused Loop. At this level of intestine, the epithelial cells appeared vacuolate. In some instances this distention was extreme. Lacteals were dilated. Noninflammatory submucosal edema was evident in almost every section examined (Figure 8). Varying degrees of cellular infiltration with neutrophils were evident. Reticuloendothelial cells, plasma cells, and lymphocytes could be identified. The severity of the submucosal edema correlated with the amount

Table 8. Comparison of pH of intestinal contents in infected and control pigs

Pair Number	Duodenum		Perfused Loop		Ileum	
	I*	C**	I	C	I	C
2	7	7	7.5	7	8	8
3	6	7	7.5	8	8.5	8
4	7	8	8	7.5	6	7.5
5	6	7	7	7.5	7	8
6	6.5	7	7	6.5	7	7
7	6.5	7	6	7.5	7.5	7.5
8	6.5	5.5	7	7.5	7	7.5
9	6.5	N.D.†	7	7.5	7.5	7.5
Average	6.4	6.9	7.1	7.3	7.4	7.6

*Infected.

**Control.

†N.D. = Not Determined.

Table 9. Fluid movement and d-glucose absorption in *E. coli*-infected and control loops

Pair Number	Net Fluid Movement (ml/cm/4 hr)		Glucose absorbed (μM/cm/4 hr)	
	I*	C	I	C
1	-0.19**	-0.33	+48.3	+10.5
2	+0.11	-0.91	+48.1	+20.1
3	-0.10	-0.48	+18.2	+24.1
4	+0.38	-0.40	+20.7	+21.3
5	+0.03	+0.08	+56.9	+33.2
6	+0.07	-0.03	+66.7	+36.7
7	+0.27	-0.61	+77.2	+41.0
8	0.00	-0.88	+66.9	+37.8
9	+0.08	-0.55	+92.9	+30.5
Mean	+0.07	-0.46	+55.1	+28.4

*I = infected, C = control.

**+ indicates absorption, - indicates production.

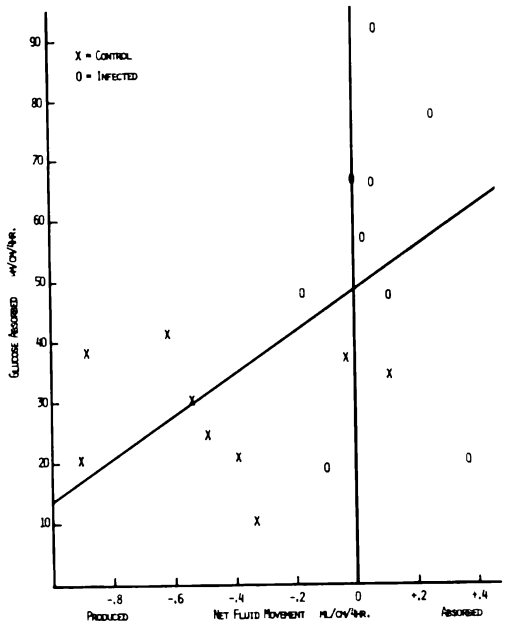

Figure 4. Glucose absorption and net fluid movement in *E. coli*-infected and control loops.

Table 10. Electrolyte movement in *E. coli*-infected and control loops

Pair Number	Sodium (μEq/cm/4 hr)		Potassium (μEq/cm/4 hr)		Chloride (μEq/cm/4 hr)	
	I*	C	I	C	I	C
1	+13.0**	-49.0**	- 2.80	-4.10	-11.0	- 8.00
2	+14.0	-114.0	- 3.30	-7.30	- 5.80	-18.0
3	+ 2.00	- 71.0	- 2.30	-5.80	+18.0	-27.0
4	+42.0	- 56.0	-12.6	-5.80	+50.0	-20.0
5	+86.0	+ 8.00	- 0.10	-6.00	+60.0	-69.0
6	-89.0	- 29.0	- 6.90	-3.70	-67.0	-28.0
7	+55.0	-108.0	- 2.30	-6.10	+39.0	-72.0
8	-17.0	-110.0	- 5.30	-6.30	+28.0	-72.0
9	-20.0	- 87.0	- 6.30	-5.10	- 3.00	-55.0
Mean	+10.0	- 68.0	- 4.60	-5.60	+ 7.00	-41.0

*I - infected; C - control.

**+ indicates absorption; - indicates production.

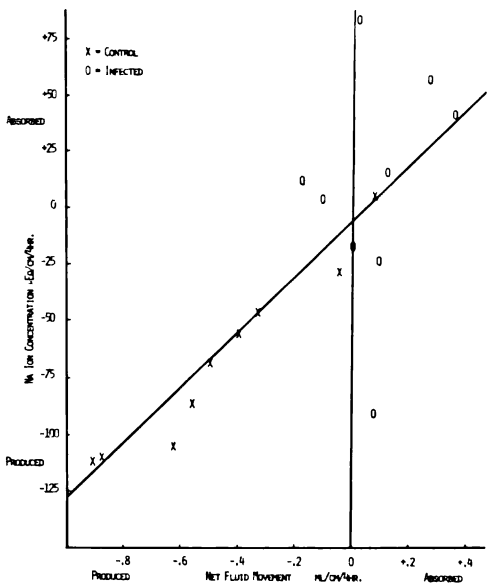

Figure 5. Sodium ion and net fluid movement in *E. coli*-infected and control loops.

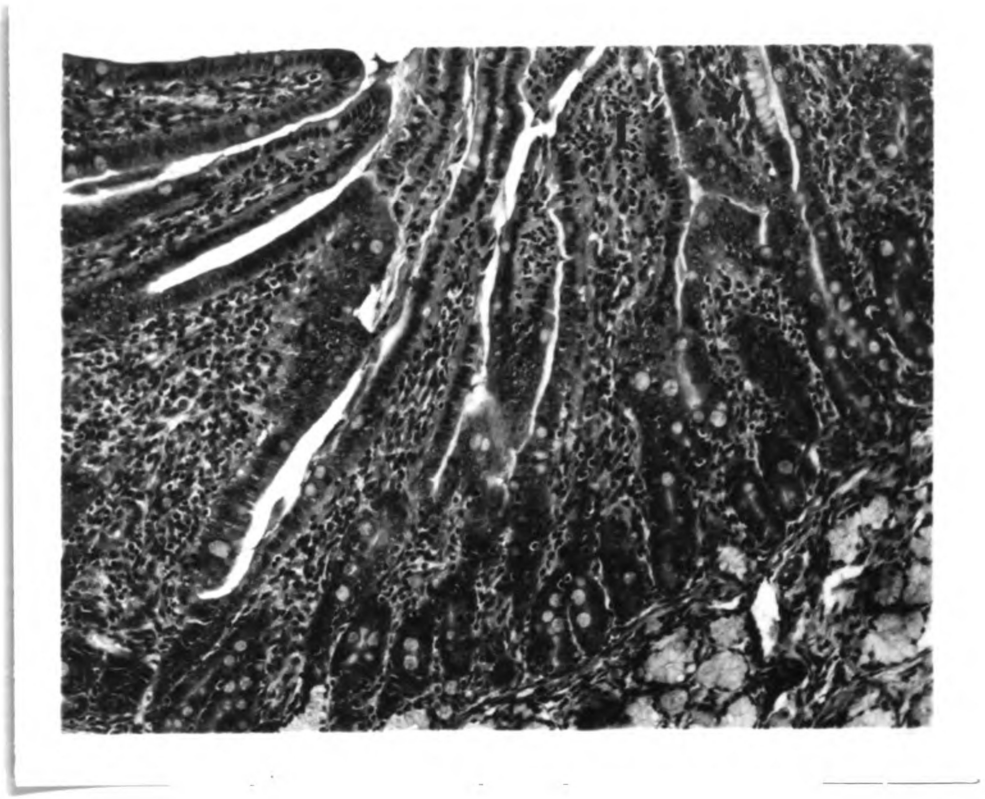

Figure 6. Pig 284 (infected). Duodenum 28 hours after exposure. Only mild epithelial vacuolization (V) is present accompanied by cellular infiltration into the lamina propria (I). Note the abundance of goblet cells (G). The villi are long and slender with little branching. Hematoxylin and eosin; x 150.

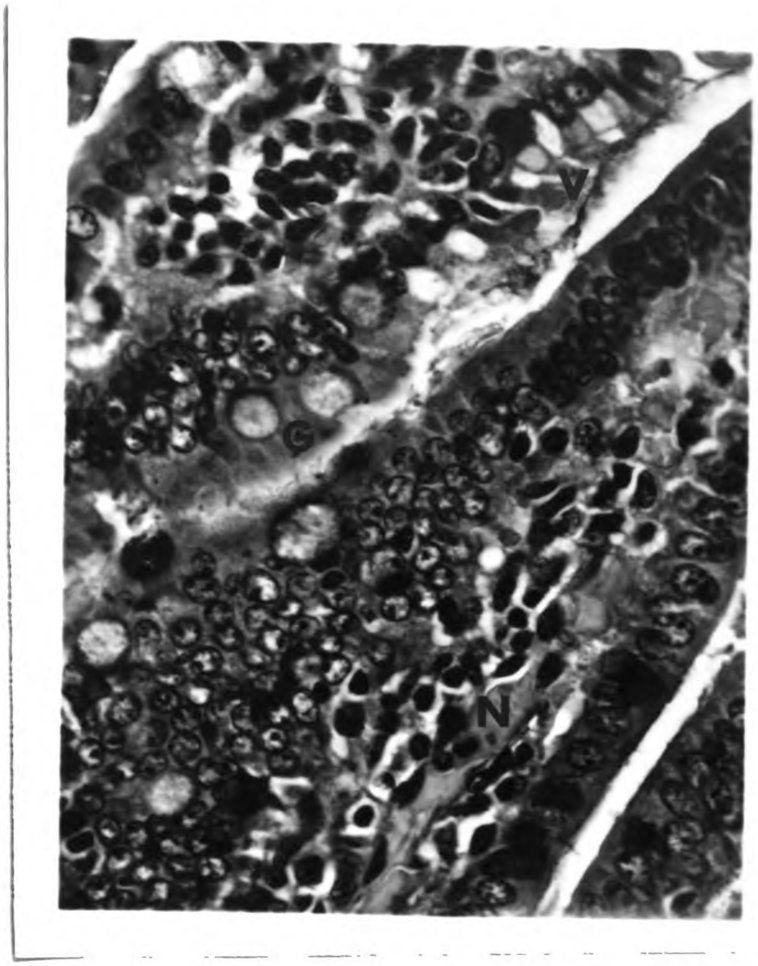

Figure 7. Pig 284 (infected). Field similar to that in Figure 6. Note goblet cells (G) and vacuolate epithelium (V). Neutrophils (N) are invading the lamina propria. Hematoxylin and eosin; x 600.

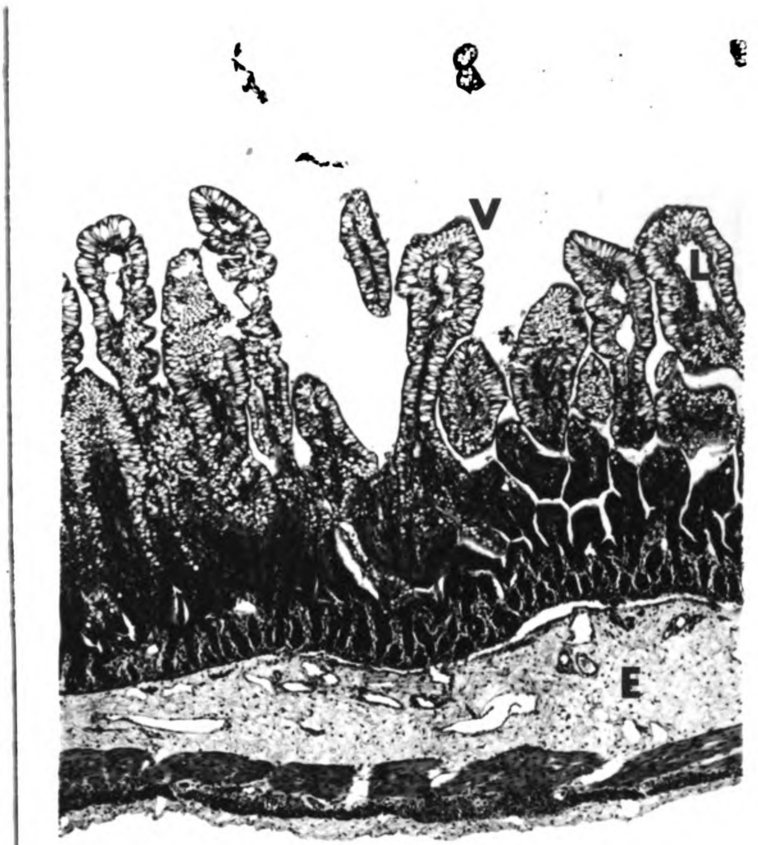

Figure 8. Pig 285 (control). Small intestine 5 cm anterior to loop. Extreme epithelial vacuolization (V) is evident with dilatation of the lacteals (L). Noninflammatory edema (E) is present in the submucosa. Note that the epithelium is intact. Hematoxylin and eosin; x 60.

of hyperemia and cellularity. The above changes were recorded in both control and infected animals. The monocontaminated pigs tended to have a greater number of goblet cells, less epithelial vacuolization, and a larger number of infiltrating cells than did the germfree intestine. The contrast, however, was not striking. In 1 infected animal (Pig 288), an acute necrotic enteritis with denudation of the epithelium, extensive cellular invasion, and marked submucosal edema was observed.

Perfused Loop. This area of the intestine was the site of the absorption study. The following lesions were recorded in 10 animals (4 controls and 6 infected). Six of these 10 animals were members of Pairs 6, 7, and 9--i.e., Pigs 285 and 286, 287 and 288, and 291 and 292. Moderate to extreme vacuolization of the epithelium was noted. The epithelium was, however, intact in almost every section examined. Marked hyperemia of the lamina propria and submucosa and marked edema of the submucosa were evident (Figure 9). Inflammatory cells were increased in number when compared to sections taken anterior to the loop. Neutrophils were predominant (Figure 10). Mild to severe fibrinous serositis was present in each section (Figure 11).

The lesions recorded in the remaining 8 loops were mild. Of the 8 loops (5 controls and 3 infected) 4 were from animal Pairs 3 (194 and 195) and 5 (283 and 284). Little or no edema or cellular invasion was noted. The epithelium in both control and infected loops was markedly vacuolate (Figure 12).

Small Intestine 5 cm Posterior to Perfused Loop. The microscopic appearance of the intestine at this level was similar to that of the sections 5 cm anterior to the loop. In the control animals, the epithelium was markedly vacuolate but intact. The most consistent finding was moderate

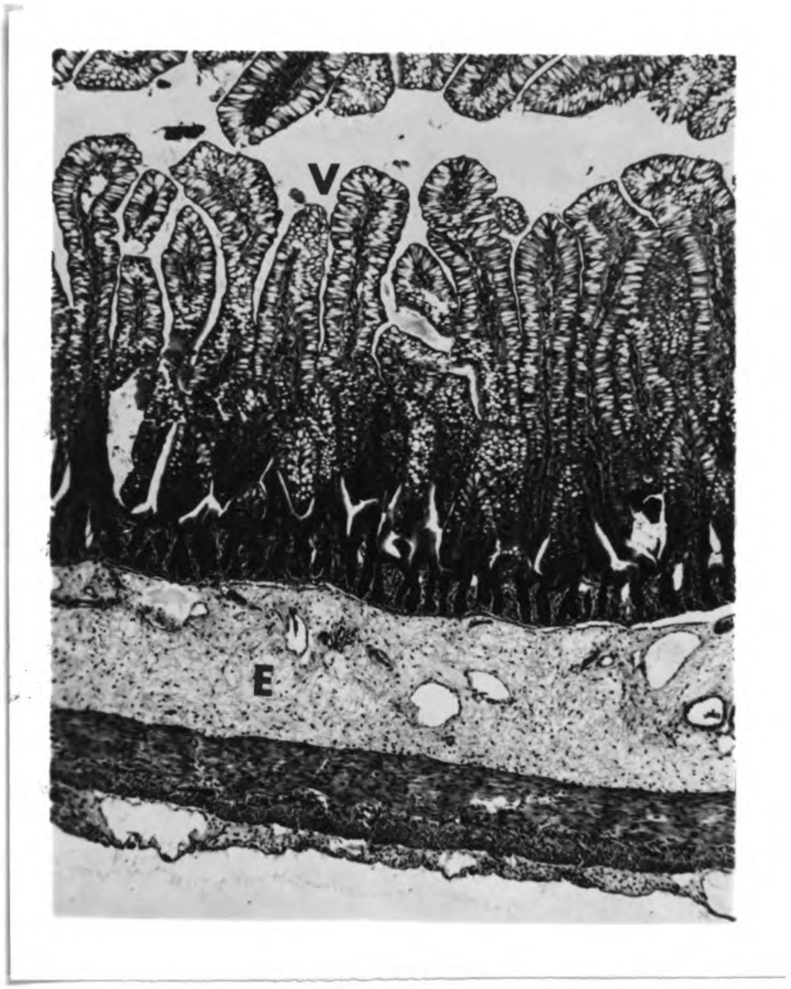

Figure 9. Pig 287 (control). Perfused loop. Note the marked epithelial vacuolization (V) and submucosal edema (E). There is increased cellularity of the submucosa. The epithelium is intact. The villi are long and slender. No blunting or shortening is evident. Hematoxylin and eosin; x 60.

Figure 10. Pig 286 (infected). Perfused loop. Vacuolate epithelium is seen. Note the infiltration of the lamina propria with neutrophils (N). Hematoxylin and eosin; x 600.

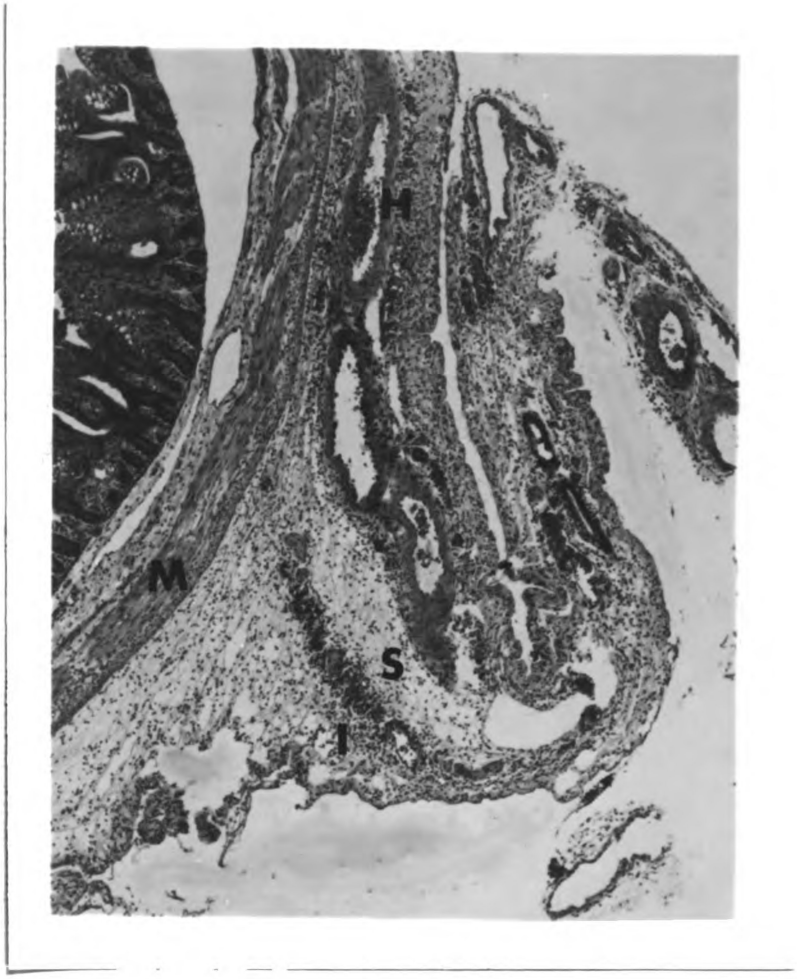

Figure 11. Pig 287 (control). Perfused loop. Marked fibrinous serositis is present (S). Hyperemia (H) and cellular infiltration are apparent (I). Muscularis mucosa is designated (M). Hematoxylin and eosin; x 60.

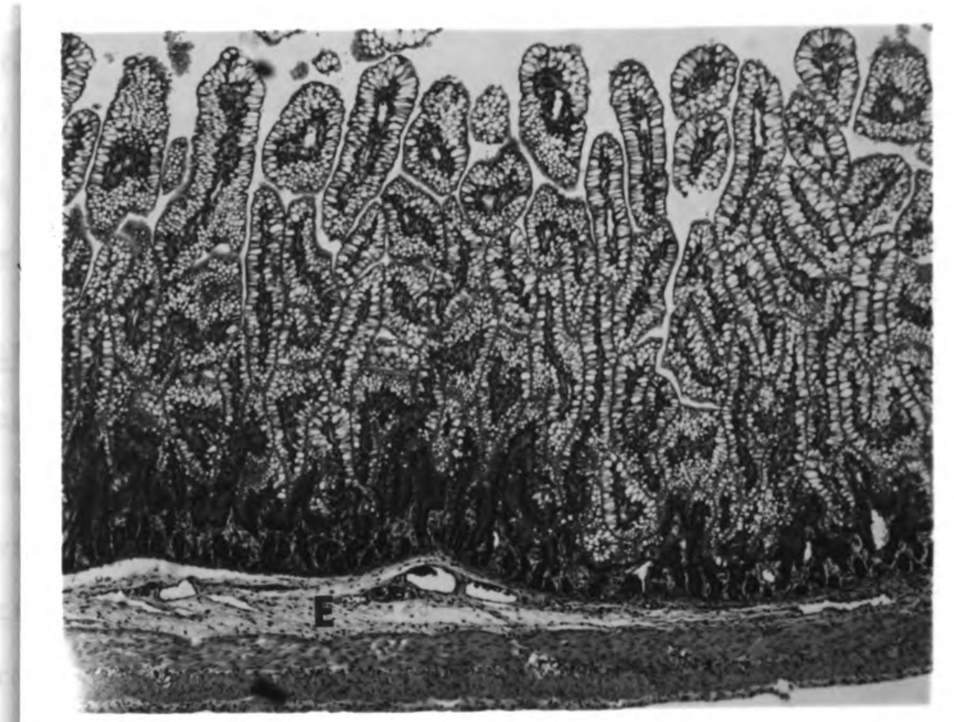

Figure 12. Pig 197 (infected). Perfused loop. Although epithelial vacuolization is still extreme, only slight submucosal edema is present (E). Note that the villi are intact. Hematoxylin and eosin; x 60.

to marked submucosal edema. Cellular infiltration, predominantly neutrophils with lymphocytes and plasma cells, was noted in most of the control animals. In 5 of the controls, mild fibrinous serositis was present at the mesenteric attachment.

The above histologic observations were also made in the infected intestines. Even though the changes were somewhat more pronounced, the difference again was not striking. One of the infected segments (Pig 196) had undergone extensive change. There was acute necrotic enteritis with neutrophilic exudation, hemorrhage, and marked submucosal inflammatory edema. The epithelium had been severely damaged.

Small Intestine 5 cm Anterior to the Ileocecal Valve. Similar histologic changes were noted between infected and control animals. Villi were long and slender. Epithelial vacuolization varied from moderate to extreme. Varying degrees of submucosal edema and cellularity were present. Two sections (Pigs 289 and 196) had extensive lesions. There was acute necrotic enteritis with nearly complete destruction of the epithelium. Hemorrhage and inflammatory exudate were present in the intestinal lumen (Figure 13). In Pig 194, discrete areas of necrosis were found in the mucosa.

Liver. The only consistent change seen in the liver was the presence of moderate to severe centrolobular vacuolization of the hepatic parenchymal cells. These vacuoles were negative for both fat and glycogen when stained with special stains. Extramedullary hematopoiesis was noted in several tissue sections. The above observations were made in both the germfree and *E. coli*-infected animals.

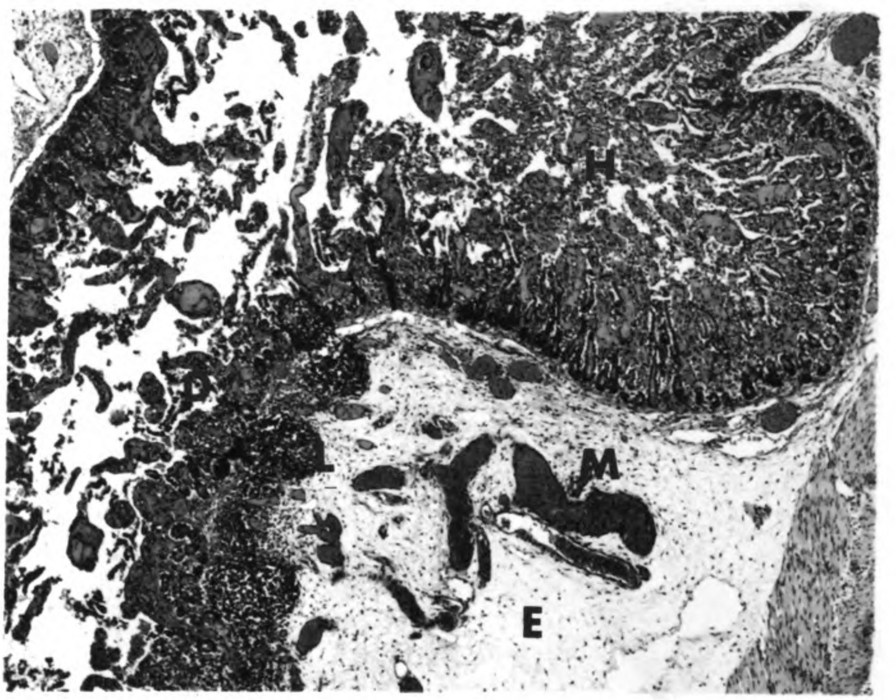

Figure 13. Pig 289 (control). Small intestine 5 cm anterior to the ileocecal valve. Acute necrotic enteritis is evident, with marked hemorrhage (H) and destruction of the epithelium (D). The submucosa is edematous (E), with marked hyperemia (M). Note the presence of lymphoid tissue (L). Hematoxylin and eosin; x 60.

Kidney. No lesions were found in the renal cortex of either experimental group. In 3 instances, hydropic changes in the collecting tubules were noted in monocontaminated pigs only.

DISCUSSION

General Comments

Procurement and Rearing of Gnotobiotic Pigs. The hysterotomy technique used in this research project was quite satisfactory. Survival rate was 100%. Isolation procedures and rearing methods provided and maintained a germfree environment during the experimental period.

Experimental Techniques. The perfusion method of studying intestinal absorption provided a suitable means of measuring the *in vivo* absorption or production of solutes and fluid. However, the actual placement of the loop--i.e., the distance of the posterior end of the loop from the ileocecal valve--was difficult to standardize from pig to pig. It was hoped that, using the measuring technique described by Serebro *et al.* (1968), the loop would be placed in the jejunum approximately 3 feet anterior to the ileocecal valve. The mean distance of the perfused loops from the ileocecal valve in the present research project was approximately 3 feet. However, the range was considerable--2.1 feet to 4.7 feet. The Serebro *et al.* (1968) method of measuring intestinal length was not consistently reliable. In addition, the mean intestinal length was about 15 feet, placing the loop in the last 1/5 of the intestine. According to Newey (1967) maximum glucose absorption occurs in the upper small intestine. Swallow *et al.* (1968) found that the canine proximal jejunum was 10 times as sensitive to cholera toxin as the distal ileum.

Bywater (1970) found no difference between the ileum and jejunum in his studies on toxin-induced colibacillosis in the calf. Nielsen *et al.* (1968) stated that the posterior segment (ileum) of the small intestine was resistant to the effects of enterotoxin because ileal transport processes were more efficient than those in the more cephalad portion of the intestine. Smith and Halls (1967) found the last 1 to 3 meters (3 to 9 feet) unreactive to *E. coli* organisms. They suggested that the last 10 feet and the first 3 feet of the small intestine should not be used for studying the effects of *E. coli*.

The use of heat lamps in Litter 1 to maintain body temperature was inadequate. The skin directly beneath the lamps often became parched while body temperature remained below 99 F. The plastic-coated heating pads placed under the pigs in Litter 2 were much more satisfactory in maintaining body temperature.

Hematology

According to the extensive literature review compiled by Calhoun and Smith (1970) the preinfection hematologic values obtained from the germfree pigs were all within normal ranges for conventional pigs of the same age. The fact that the 12-day-old pigs had consistently higher hemoglobin and packed cell volume values than the 8-day-old pigs was supported by the findings of Britt (1967). None of these animals had evidence of anemia.

A marked leukocytosis with a regenerative left shift was the most characteristic finding in the *E. coli*-infected animals. Some evidence of hemoconcentration was found in the monocontaminated animals when compared to controls.

Intestinal Absorption

Although the intestinal fluid in the infected animals tended to be more acid than in the controls, no statistically significant differences were found in the pH values of the duodenum, isolated loop, and terminal ileum between the monocontaminated and germfree animals. Smith and Jones (1963) found no difference in the pH values of intestinal chyme between healthy and *E. coli*-infected baby pigs raised conventionally. However, Kohler (1968) and Kohler and Cross (1969) reported that fecal diarrheal fluid produced in response to *E. coli* enterotoxin was significantly more alkaline than in controls. Fecal pH was not measured in the present study.

It was originally proposed that PSP would be used as a nonabsorbable marker. Net fluid movement could be calculated from the PSP concentration (Serebro *et al.*, 1968). Due to the extreme turbidity of the perfusion solution, consistent and reliable results could not be obtained. Determination of PSP concentration, therefore, was not done. According to Serebro *et al.* (1968) actual measurement of fluid remaining in the reservoir with a graduated cylinder correlated within 1 to 2 ml of the calculated fluid movement using PSP concentrations.

In the present research project, the absorption of d-glucose in *E. coli*-infected animals was significantly greater ($P<.01$) than in the germfree controls. Serebro *et al.* (1968) found an equal and even slightly increased absorption of d-glucose in rabbits with toxin-induced cholera when compared to controls. Bywater (1970) reported no difference in glucose absorption in Thiry-Vella loops between control calves and calves with toxin-induced colibacillosis. Carpenter *et al.* (1968) had similar results using Thiry-Vella loops in dogs with toxin-induced cholera.

Torres-Pinedo *et al.* (1966), however, found impaired glucose absorption in human infants with colibacillary diarrhea.

Solute movement--i.e., glucose, sodium, and, to a lesser extent, chloride correlated with fluid movement. That is, the control loops consistently absorbed less glucose and produced more fluid, sodium, and chloride than the *E. coli*-infected loops. It had been postulated that the control loops would absorb more glucose and electrolytes and produce less fluid than the infected loops. Bywater (1970) found a marked secretion of sodium, chloride, and fluid into the lumen of Thiry-Vella loops subjected to *E. coli* enterotoxin. Carpenter *et al.* (1968) and Swallow *et al.* (1968) in experiments with toxin-induced cholera in dogs found that copious amounts of isotonic fluid were produced in the infected animals. However, they found that the addition of glucose to the perfusion fluid enhanced the absorption of fluid in both control and infected loops. In other words, the presence of intraluminal glucose markedly decreased the isotonic fluid production in the cholera loops. Serebro *et al.* (1968) reported similar findings. Infected loops, however, still contained more fluid than control loops (Carpenter *et al.*, 1968; Swallow *et al.*, 1968; Serebro *et al.*, 1968). Parsons (1967) concluded that the presence of intraluminal glucose stimulated water absorption. Moreover, water absorption does not occur without simultaneous solute absorption. The fact that potassium movement was inconsistent and not significant was also found by Bywater (1970).

Questions were then raised in interpreting the results. First, why did control animals absorb less glucose than did the infected animals? Secondly, why did the *E. coli*-contaminated loops absorb fluid while the control loops actually produced fluid and electrolytes? The possibility that the *E. coli* organisms themselves utilized glucose from the perfusion

fluid should be considered as one explanation as to why the infected pigs absorbed more glucose than the germfree controls. Perhaps the *E. coli*-infected intestine behaves in a manner that more closely approximates a natural physiological state and the absorptive mechanisms of the germfree gut are rather prenatal, undifferentiated, and not as efficient (Dubos *et al.*, 1963). Therefore, the presence of intraluminal glucose stimulated and enhanced the absorption of fluid and electrolytes to a greater degree in the *E. coli*-infected gut than in the germfree gut. Levin (1967) reported that increased blood flow will cause increased absorption. Substances such as glucose can be stored in or metabolized by the intestinal epithelial cells. Moreover, Dowling (1967) stated that glucose utilization and metabolism increased in intestinal hypertrophy and in devitalized cells. Several other factors may have had an influence on absorption results. Perfusion was carried out under artificial conditions. Perhaps flow rate should have been slower to allow more absorption. The amount of perfusion fluid should have been greater in order to maintain initial solute concentration and pH. That is, the volume of the reservoir should be increased from 100 to 250 or 400 ml. PSP should be removed from the solution. PSP determinations are not feasible and the substance may be irritating especially to the mucosa of the germfree gut. Moreover, the loops should be placed more anterior to the ileocecal valve--at least 6 feet. Also, additional studies should be conducted in which glucose would be omitted from the perfusion fluid in 1 litter of pigs.

Histopathology

The state of inflammation and edema observed in the perfused loops did not correlate with the amount of fluid production or the amount of

glucose absorbed. For example, in animal Pair 3, the histologic lesions seen in both animals were mild with little or no edema or inflammation present. The control pig produced 480 µl/cm/4 hr of fluid and absorbed 24.1 µM/cm/4 hr of glucose. In contrast, the monocontaminated pig produced 100 µl/cm/4 hr of fluid but absorbed only 18.2 µM/cm/4 hr of glucose. In animal Pair 5, which had lesions very similar to Pair 3, different results were obtained. The control loop absorbed 80 µl/cm/4 hr of fluid while the infected loop absorbed 30 µl/cm/4 hr. However, only 33.2 µM/cm/4 hr of glucose were absorbed by the control pig but 56.9 µM/cm/4 hr of glucose were absorbed by the monocontaminated gut.

Severe histologic changes were seen in Pigs 285 and 286 (Pair 6). Marked submucosal edema was present with cellular infiltration and hyperemia. Still, the infected pig absorbed 70 µl/cm of fluid and 66.7 µM/cm of glucose while the control pig produced 30 µl/cm of fluid and absorbed only 36.7 µM/cm of glucose during the 4-hour period. Regardless of whether the histologic changes were severe or mild, the infected animals consistently absorbed more fluid, sodium, and glucose than did the control animals:

	Infected	Control
Mean glucose absorption (µM/cm/4 hr)	55.1	28.4
Mean fluid movement (µl/cm/4 hr)	70.0	-46.0*
Mean sodium absorption (µEq/cm/4 hr)	10.0	-68.0

*Negative number indicates production.

The submucosal edema observed in both germfree and infected intestines may have been related to handling of the intestines during surgery.

Speculations on the Pathogenesis of Colibacillary Diarrhea

The results of this research project suggest that intestinal malabsorption is not a primary feature of colibacillary diarrhea in 2-week-old gnotobiotic pigs. Glucose, sodium, chloride, and fluid absorption in *E. coli*-infected loops was significantly greater than in the germfree controls. Turk and Stephens (1966) found that the absorption rate of zinc was significantly higher in chickens infected with coccidiosis than in control birds, especially during the first day after exposure. They concluded that mild intestinal damage with slight inflammation increased absorption while severe damage and hemorrhage decreased or stopped absorption. In addition, increased cellular permeability to certain substances occurred as a result of inflammation due to infection. It is possible that in the present study intestinal inflammation in the presence of *E. coli* organisms stimulated the absorption of fluid, sodium, and glucose in isolated perfused loops. The germfree gut, however, with similar histologic changes did not have comparable absorptive capacity.

SUMMARY

Eighteen 2-week-old hysterotomy-derived gnotobiotic pigs from 2 litters were used to study the effects of colibacillary diarrhea on the absorption of d-glucose, sodium, chloride, potassium, and fluid. The animals were paired and 1 pig from each pair was exposed *per os* to 3×10^6 *Escherichia coli* 0138:K81:NM organisms. Twenty-four hours after infection, isolated jejunal loops were prepared in each animal for *in vivo* measurement of nutrient absorption. These loops were continuously perfused for a period of 4 hours with a solution of 26 mM glucose in lactated Ringer's solution.

Glucose, sodium, chloride, and fluid absorption was not impaired in the *E. coli*-infected loops. The monocontaminated loops consistently absorbed more glucose and produced less fluid than the germfree control loops. Mean glucose absorption (μM/cm/4 hr) was 55.1 in the infected animals but only 28.4 in the controls. Fluid production in the germfree control loops averaged 0.46 ml/cm/4 hr while the infected loops actually absorbed 0.07 μl/cm/4 hr. Sodium movement directly correlated with fluid production ($P<.001$). Chloride movement generally followed a similar pattern. The germfree intestinal loop secreted 68 μEq/cm of sodium and 41 μEq/cm of chloride during the 4-hour period. The *E. coli*-contaminated loops absorbed 10 and 7 μEq/cm/4 hr of sodium and chloride, respectively. Differences in potassium secretion were not significant.

The most consistent hematologic findings in the pigs exposed to *E. coli* were a marked leukocytosis and regenerative shift to the left. Some evidence of dehydration was present in infected animals.

The histologic appearance of the duodenum, the intestine anterior and posterior to the loop, and the ileum did not differ between control and infected animals. Varying degrees of epithelial vacuolization, submucosal edema, and cellular infiltration were noted. Similar changes were also seen in the isolated loops. The severity of the lesions did not correlate with changes in absorption.

The findings in the present investigation did not support the theory that malabsorption is a prominent feature in the pathogenesis of colibacillary diarrhea.

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