# EXPERIMENTAL ENTERIC COLIBACILLOSIS IN GNOTOBIOTIC SWINE UTILIZING THE LIGATED LOOP TECHNIQUE

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
JAMES P. DAVIDSON
1974

ABSTRACT

EXPERIMENTAL ENTERIC COLIBACILLOSIS IN GNOTOBIOTIC SWINE UTILIZING THE LIGATED LOOP TECHNIQUE

By

James P. Davidson

The ligated loop technique was evaluated in 23 gnotobiotic pigs, 3 to 4 weeks of age, in an attempt to define the nature of false positive loops. Five serotypes, encompassing 6 strains of *Escherichia coli*, were utilized in this experiment. The 6 strains were divided into 3 subgroups: 1) strains enteropathogenic for pigs regardless of age, 2) strains enteropathogenic for pigs under 2 weeks of age and nonenteropathogenic for pigs over 6 weeks of age, and 3) nonenteropathogenic strains. Each experimental loop was inoculated with a single strain, noninjected interloops separated each inoculated loop, and all 6 strains were tested in each of 15 experimental animals.

Strain enteropathogenicity in gnotobiotic pigs, based upon significant visual loop distention, compared favorably with results obtained utilizing the same technique and strains of *E. coli* in conventional animals with the exception that the gnotobiotic jejunal loop may be somewhat less sensitive to certain strains. In addition, postoperative mortality rates among gnotobiotic pigs were higher than reported in conventional pigs.

Light microscopic intestinal lesions in these gnotobiotic pigs were similar to those reported in the conventional pig. Mucosal

erosions and ulcerations were often seen in maximally distended loops.

Two false positive loops occurred during the experiment. Both were in sacs inoculated with strain 115. False positive loops were not seen in other sacs, whether inoculated or not. Results of these experiments support the hypothesis that false positive loops in conventional pigs arise from spontaneous infection.

# EXPERIMENTAL ENTERIC COLIBACILLOSIS IN GNOTOBIOTIC SWINE UTILIZING THE LIGATED LOOP TECHNIQUE

By

James P. Davidson

A THESIS

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

MASTER OF SCIENCE

Department of Pathology

1974

To my wife Ann and our son Marc

## ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to his major professor, Dr. Glenn L. Waxler, for his expert and meaningful guidance, encouragement and enduring patience throughout the course of this investigation.

My sincere thanks go to my academic committee: Drs. S. D. Sleight and V. L. Sanger of the Department of Pathology, for their friendly help and advice in understanding the complexities of experimental pathology as they related to this research problem; Dr. D. J. Ellis of the Department of Large Animal Surgery and Medicine, for his valuable counsel and suggestions within the realm of clinical and practical aspects of the disease; and Dr. C. C. Morrill, former Chairman of the Department of Pathology, for providing the facilities and supplies for this research.

I am also indebted to the following persons for their expert technical assistance: Mr. James Southern, animal caretaker at the Veterinary Research Farm; Mrs. Dottie Fenner, Mr. Charles Bares and Mr. Warren Taylor, medical technologists; and Mrs. Nina Miller, Mrs. Mae Sunderlin and Mrs. Frances Whipple, histopathologic technicians.

This research was supported, in part, by a grant from the Diamond Shamrock Corporation, Cleveland, Ohio.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# INTRODUCTION

Despite major medical advances during the last half century, disease characterized by diarrhea remains as a leading cause of death throughout many of our domestic species and among the peoples of the developing nations. The etiologic implication of *Escherichia coli* in certain of these syndromes has led to greater scientific efforts to understand the ubiquitous role of this organism, a normal constituent of the intestinal flora of vertebrates. Reliable procedures are now available to differentiate the enteropathogenic from the nonenteropathogenic strains of *E. coli*; among these is the ligated loop technique.

The utilization of the gnotobiotic pig in the study of colibacillosis has provided important information toward the understanding of this complex enteric syndrome by allowing the study of a single pathogenic strain in the absence of antibody and commensal intestinal flora.

In our experiments the ligated loop technique was utilized in the gnotobiotic animal in an attempt to define:

1. The comparative aspects of the ligated loop technique between the gnotobiotic and conventional pig.

2. The etiologic nature of false positive reactions encountered in the ligated loop technique.

# LITERATURE REVIEW

Enteric disease associated with the enteropathogenic forms of *Escherichia coli* affects a broad spectrum of animal life including man. The original correlation associating the occurrence of disease, a diarrheal syndrome in calves, with the presence of *E. coli* came in 1891 with the work of Jensen (Sojka, 1965). Since that time the organism has been identified and implicated in neonatal diarrhea of lambs, goats, pigs, poultry and human beings (Barnum *et al.*, 1967). Comparisons have been drawn between enteric colibacillosis in pigs and calves and the disease syndrome found in human infants (Saunders *et al.*, 1960).

## Colibacillosis in Neonatal Pigs

The term colibacillosis applies to a group of diseases caused by *Escherichia coli*. The enteric form of the disease occurs in three distinct age groups of pigs: neonatal (to 4 days of age), pre-weaning at 3 weeks, and at weaning (Stevens, 1963a,b). Enteric colibacillosis in suckling pigs is most commonly manifested as an acute enteritis or gastroenteritis, clinically, and the syndrome has therefore been referred to as baby pig scours, coliform enteritis, diarrhea neonatorum or neonatal colibacillary diarrhea (Leman, 1970).

Stevens (1963a,b) estimated that *E. coli* infections were associated with approximately 75% of all baby pig diarrheas. An English survey in 1960 indicated that of those pigs dying at 4 months of age

or younger, 40% had enteritis associated with *E. coli* (Kenworthy and Allen, 1966a). The morbidity of the disease is highly variable. Not all litters during a farrowing season will become infected, nor will all the pigs within an infected litter show signs of the disease (Barnum *et al.*, 1967). Once an enzootic has begun in an establishment, the severity increases as more sows farrow (Barnum, 1971).

Clinical signs. Enteric colibacillosis is most often diagnosed in pigs 1 to 8 days of age. Onset of the disease may be recognized as early as 12 hours postnatally in a few pigs in which a bacteremia and septicemia have occurred. Although diarrhea is an inconsistent sign in pigs so affected, death usually occurs within 48 hours. More typically, signs in infected pigs will begin with a whitish-yellow, watery diarrhea with ensuing dehydration, emaciation and a roughened hair coat. The tail and perineum commonly become pasted with fluid or semifluid feces, and may also become inflamed. In some cases the tip of the tail becomes necrotic and eventually sloughs. Mortality rates are usually higher in younger pigs. Pigs acquiring the disease within the first 3 days postnatally have a mortality rate close to 70% (Dunne and Bennett, 1970); however, the mortality rate is variable and seldom reaches 100% (Leman, 1970).

Postmortem findings. Gross lesions of enteric colibacillosis have been inconsistent in pigs infected with known enteropathogenic strains. Kohler and Bohl (1966), using a group of experimentally infected gnotobiotic pigs, demonstrated that only about 50% of the necropsied animals had gross abnormalities. The lesions consisted of petechial hemorrhages on the adrenals, mesenteric lymph nodes and in the mesentery, with no evidence of inflammation or congestion in either the

small or large intestine. Intestinal contents were watery and yellow to pale green. Barnum *et al.* (1967) confirmed the absence of inflammatory lesions and observed evidence of dehydration, a full stomach and intestines filled with fluid and undigested food.

Histopathologic observations

The germfree intestine. Morphologically, certain organ systems of the germfree animal deviate significantly from those found in conventional animals. In the intestinal tract Dubos (1966) observed that the germfree animal had shorter crypts, longer villi and a paucity of lymphoid and inflammatory tissue in the epithelium and lamina propria. Staley *et al.* (1968) reported that the jejunal epithelium of the newborn germfree pig was composed of simple columnar epithelium which was highly vacuolate on the distal two-thirds of long, slender villi. Cross and Kohler (1969) compared the autolytic changes in the digestive systems of germfree, monocontaminated and conventional baby pigs. They observed an increasing degree of cellularity in the lamina propria and submucosa which was directly proportional to the diversity of the microbial population of the gut. Neutrophils, lymphocytes and macrophages were increased in number and, for this reason, they applied the term "physiologic inflammation" to the microscopic appearance of a non-germfree intestine. The most striking microscopic changes seen in the intestine were found in the terminal ileum and colon. They reported the villi of germfree pigs to be longer and thinner when compared to *E. coli* monocontaminated and conventional pigs. No significant microscopic alterations were found by Alexander *et al.* (1969) in the duodenal sections from germfree pigs, except that eosinophils were numerous in the lamina propria and Peyer's patches of the jejunum and ileum.

In a study of gnotobiotic and germfree pigs, Kenworthy and Allen (1966b) found a direct relationship between the morphological characteristics of the intestine and the degree and variety of bacterial contamination. The villi of a gut contaminated with a single nonpathogenic strain of bacteria were long, slender and uniform, with little cellular infiltration, and closely resembled the villi of the germfree gut. Dual contamination of the gut with a nonenteropathogenic and an enteropathogenic strain of *E. coli* altered the structure of the gut by producing villi that lacked uniformity and were more conical with flattened tips. In histological sections taken from intestines of pigs reared in the conventinal environment, the villi were shortened and many were found in a leaf-like configuration. Cellular infiltration into the lamina propria was extensive.

Alexander *et al.* (1969) also examined microscopic tissue sections taken from the gnotobiotic pig. They reported numerous neutrophils and eosinophils in the medullary regions of the mesenteric lymph nodes and a sparse population of lymphocytes and reticuloendothelial cells in the same area.

Colibacillosis. There are conflicting reports concerning the histologic appearance of intestinal sections taken from either gnotobiotic or conventional pigs afflicted with colibacillosis. Histopathologic changes were inconsistently seen in conventionally reared animals afflicted with a diarrheal syndrome attributed to *E. coli* (Moon *et al.*, 1970; Barnum *et al.*, 1967). Occasionally villous atrophy or desquamated epithelium was found in the anterior aspect of the intestine and occurred only in pigs in which diarrhea had been observed (Moon *et al.*, 1970). Barnum *et al.* (1967) stated that catarrhal enteritis or villous

atrophy may be more frequent in pigs with a history of prolonged diarrhea.

Dunne and Bennett (1970) considered the microscopic changes of the gut taken from newborn piglets with colibacillosis to be similar to the reaction associated with low-grade irritants. Mucosal alterations consisted of enlarged goblet cells and distended, vacuolated absorptive cells. Gilka (1968) reported that colibacillosis produced an acute serous enteritis in the conventional newborn piglet. The edema was localized in the lamina propria and was accompanied by a neutrophilic infiltration into the villi. A mild neutrophilic infiltration was observed by Kohler (1967) in the villous lamina propria of pigs infected with a single strain of enteropathogenic *E. coli*.

Smith and Jones (1963) found no inflammatory changes in newborn piglets with colibacillosis and concluded that there was no histopathologic difference between these animals and their healthy littermates during the first week of life.

Intestinal sections collected from neonatal colostrum-deprived pigs afflicted with colibacillosis were examined ultramicroscopically by Staley and co-workers (1969). In subclinical cases, fine structural alterations of the absorptive cell were observed primarily in the distal jejunum and ileum and were associated with the presence of the organism within the cell. The bacterium gained entrance into the absorptive cell by phagocytosis; this feature of the infection was thought to relate to the absence of lesions observable with the light microscope.

The histopathologic changes seen in the gnotobiotic pig also vary considerably. Kenworthy (1970) found an acute inflammatory response of the intestine following the inoculation of *E. coli*-0141.

Absorptive epithelium was shrunken, vesiculated and vacuolated. The distal aspect of these cells was often surrounded by neutrophils and was occasionally separated from the basement membrane. An acute exudative response was observed in the lamina propria both in animals whose death was attributable to the disease, as well as surviving animals in which clinical signs could no longer be detected. Necrotic and degenerative changes in the tunica media of the arteries and arterioles of the submucosa immediately below the involved areas of the absorptive epithelium suggested that mucosal lesions could be ascribed to ischemia.

Only minimal lesions were seen in gnotobiotic pigs infected with an enteropathogenic strain of *E. coli* (Drees and Waxler, 1970). These changes, consisting of edema of the lamina propria, dilation of the central lacteal, and an infrequent polymorphonuclear cellular infiltration, were most prominent in the terminal jejunum and ileum.

No inflammatory lesions were found in either the small or large intestine of gnotobiotic pigs infected with enteropathogenic *E. coli* (Kohler and Bohl, 1966). In an additional study by Kohler and Cross (1969), utilizing a cell-free filtrate of *E. coli* cultures, no histopathologic lesions were observed.

Diagnosis. Because no single sign, lesion or laboratory test may be used alone to diagnose colibacillosis, it is necessary to evaluate all available epidemiological, clinical and laboratory data to arrive at a final diagnosis (Sojka, 1971).

Many workers (Moon *et al.*, 1970; Grun *et al.*, 1967; Muylle and Oyaert, 1965) have studied the alterations in the blood stream of pigs afflicted with colibacillosis. However, these findings have not been as widely used diagnostically as those involving the characterization

of the organism. A presumptive diagnosis is frequently based upon the isolation of *E. coli*, in pure culture, from gastric and duodenal contents taken from animals with diarrhea prior to death (Leman, 1970).

Enteric colibacillosis of preweanling pigs must be differentiated from transmissible gastroenteritis (TGE), enterotoxemias due to *Clostridium perfringens* type C, hog cholera, salmonellosis, strongyloidosis and noninfectious digestive disturbances (Barnum *et al.*, 1967; Leman, 1970).

Bacteriological considerations. *Escherichia coli* is a normal inhabitant of the intestine of vertebrates (Leman, 1970) and a common inhabitor of the environment in which animals are found. On farms, it may be commonly isolated from stables and pens and is known to cause diarrhea in neonates (Barnum *et al.*, 1967).

Because of the ubiquitous nature of the organism, and its seeming inconsistency to produce diarrhea, certain means of detecting enteropathogenic strains of *E. coli* were developed to aid in the accurate diagnosis of the disease. The definition of enteropathogenicity among strains of *E. coli* evolved as techniques were developed to establish their identification. Moon and Whipp (1971) reserved the term "enteropathogenic *Escherichia coli*" (EEC) for those strains that could colonize the jejunum in numbers equal to their population in the colon. In addition, an abundant net movement of water and electrolytes across intact intestinal epithelium into the lumen must occur while the EEC are confined to the intestine. Gyles and Barnum (1967) stated that the two requirements for an organism's enteropathogenicity were epidemiological association and the ability to produce a positive gut loop reaction.

Hemolytic strains are commonly enteropathogenic; however, Sojka (1965) reported that this characteristic cannot be used as the sole criterion of pathogenicity. Hemolytic strains of *E. coli* may be non-enteropathogenic and not all enteropathogenic strains of the organism are concomitantly hemolytic.

*Escherichia coli* may be further divided into serotypes based upon the demonstration of 3 of its many antigenic components. The somatic, "O", antigens are lipopolysaccharide and comprise 146 subgroups. The "K" antigens, associated with polysaccharides of the envelope or capsule, are of 3 types: L, B and A, and 91 have been recognized. However, recent evidence by Ørskov *et al.* (1971) questioned the validity of the subclassification of L, B and A, and the very existence of the "K" antigen itself. These workers suggested that the "K" antigen may eventually be included in the larger somatic, "O", antigen grouping. Forty-nine flagellar or "H" antigens have been identified and are thought to be protein (Sojka, 1971).

A limited number of serotypes of *E. coli* have been demonstrated to be enteropathogenic in the ligated loop test. The inoculum may contain organisms or be in the form of a cell-free supernatant from such cultures (Sojka, 1971; Barnum *et al.*, 1967; Kohler and Bohl, 1966; Kohler, 1968).

Because the serotyping technique has been widely employed, enteropathogenic strains of *E. coli* have been identified throughout the world wherever swine are produced (Kohler, 1972).

Pathogenesis. Although many phases of the pathogenesis of colibacillosis have been defined in recent years, our understanding of this complex disease remains incomplete.

Currently, the pathogenesis of colibacillosis is commonly accepted to involve

> "...a susceptible pig infected with an enteropathogenic strain of *E. coli* which has the capacity to proliferate in the proximal small intestine and to produce and release enterotoxins in adequate amounts to cause an alteration in the normal fluid and electrolyte transport functions of the intestine, with resultant diarrhea, dehydration and death." (Kohler, 1972)

Environmental factors, reflecting conditions within the farrowing house, play an important part in the incidence of colibacillosis among susceptible piglets (Kohler, 1972). Arbuckle (1968) reported that the intestines of young pigs appear to support the proliferation of enteropathogenic strains of *E. coli* more readily than does the digestive tract of adult swine, and this may account for the abundance of enteropathogenic strains of the organism in farrowing houses utilized on a continuous basis. The importance of an adequate infective dose of the organism was demonstrated by Kramer and Nderito (1967).

Neonatal animals acquire intestinal *E. coli* as a result of ingestion. This portal of entry is also utilized by the enteropathogenic strains as the primary route of infection (Leman, 1970). Reports indicate that entry may be gained via the blood stream as well. Christie (1967) demonstrated the production of diarrhea in gnotobiotic pigs by subcutaneous inoculation of an enteropathogenic strain of *E. coli* into the umbilical stump. Onset of diarrhea was delayed until the organisms had established themselves in the intestinal tract, 24 hours later. In the study of naturally occurring cases of colibacillosis in neonatal pigs, the constant site of infection has been the intestinal tract. Invasion and proliferation of enteropathogenic strains of *E. coli* in various organs outside of the gastrointestinal tract have been

observed in the terminal stages of the disease in colostrum-fed pigs (Stevens, 1963a).

The ability of enteropathogenic strains to establish infection within the host is related to the susceptibility, or resistance, of the pig. Numerous host factors influence this susceptibility. Dietary stress is highly significant. The gestational diet of the dam not only influences the size, vigor and immediate postnatal health of her offspring, but also affects the quantity and quality of the milk with which the newborn will be fed (Kohler, 1972). Smith and Jones (1963) reported that the high gastric pH of the newborn created an environment conducive to the overwhelming propagation of ingested enteropathogenic as well as benign strains of *E. coli* in the proximal small intestine. The relationship between the age of the host at the time of exposure and the ability of the organism to establish infection has also been stressed by many authors (Moon and Whipp, 1970; Waxler *et al.*, 1971). Colostrum consumption is probably the single most important factor in determining or altering the susceptibility of the newborn pig to enteropathogenic strains of *E. coli*. Although the antibodies provided in the colostrum are eventually absorbed from the intestine, and aid in the prevention of polyserositis and endotoxemic shock (Barnum *et al.*, 1967), their primary protective function rests upon the direct intraluminal action upon the organism. Therefore, the quantity and adequacy of appropriate antibody within the intestinal lumen during the first day of neonatal life affords greater protection in the prevention of colibacillosis than does the antibody present in the serum (Kohler, 1967, 1969; Miniats, 1970).

Although the aforementioned conditions are primal to the onset of colibacillosis, other factors contribute to the overall

pathogenesis of the disease. The influence of gastrointestinal stasis has yet to be fully explored. Nielsen and Sautter (1968) presented indirect evidence that lack of intestinal motility, and confinement of the organism in the ligated loop technique, were of importance in the expression of enteropathogenicity. White *et al.* (1969), in a radiographic study of specific pathogen-free (SPF) and conventional pigs, demonstrated that gastric stasis preceded diarrhea in suckling pigs.

Enterotoxins and their relationship to the onset of colibacillosis have received extensive attention in recent years. Cell-free filtrates from enteropathogenic cultures of *E. coli* caused distention of ligated intestinal loops in a manner similar to that observed when viable organisms of the same strain were utilized (Smith and Halls, 1967a). These workers also reported that extracellular concentrations of the enterotoxins significantly exceeded the intracellular levels, and that the highest levels of the toxin were detected after 6 hours of initial incubation. Endotoxins from the same enteropathogenic strains produced no distention of ligated intestinal loops (Smith and Halls, 1967b).

Initially the enterotoxin was characterized as heat stable (Smith and Halls, 1967b). However, Smith and Gyles (1970) suggested that, in addition, a heat labile enterotoxin was also elaborated by certain enteropathogenic *E. coli*. The production of both enterotoxins was demonstrated to be controlled by a plasmid (Smith and Gyles, 1969). This extranuclear genetic information could be transmitted to non-enteropathogenic *E. coli*, transforming them into enterotoxin producing (enteropathogenic) organisms (Smith and Halls, 1968; Smith and Gyles, 1969).

The means by which enteropathogenic *E. coli* initiate diarrhea are not known. Kohler (1972) has stated that the heat-labile enterotoxin

of *E. coli* and the enterotoxin elaborated by *Vibrio cholerae* share a similar, if not identical, mode of action upon intestinal cells in the production of diarrhea. However, he suggested that extensive research remains to be done to confirm these comparisons. The enterotoxin of *V. cholerae* acts on the luminal surface of gut mucosal cells, affecting water and electrolyte transport (Pierce *et al.*, 1971). The importance of increased concentrations of 3'5' adenosine monophosphate (cAMP) mediated through the action of *V. cholerae* enterotoxin has been stressed in several recent studies (Shafer *et al.*, 1970; Kimberg *et al.*, 1971; Sharp and Hynie, 1971). Increased concentrations of cAMP were demonstrated by Pierce *et al.* (1971) to result in changes in ion transport in gut mucosal cells, most notably the inhibition of active sodium absorption and stimulation of active chloride secretion. These ionic alterations resulted in net fluid accumulation within the intestinal lumen.

The ensuing diarrhea results in dehydration, hemoconcentration and eventual acidosis. Passive congestion of various viscera has also been reported. The sequelae to colibacillosis is recovery or death with recovery being dependent upon the ability of the individual to respond positively and overcome the pathophysiologic stresses that have resulted from the disease (Kohler, 1972).

Immunological considerations. Since the primal role of colostrum has been recognized in the prevention of colibacillosis, various studies have characterized the specific antibody(ies) contained within the mammary secretion during the initial postpartum period.

Wilson (1972) reported that protective antibodies responsible for the prevention of colibacillosis come from colostrum and milk. The

colostrum secretion of the immediate 24-hour postpartum period provides an initial surge of the immunoglobulins IgM and IgA, the latter being synthesized in the mammary gland itself (Porter, 1969a). Following this first supply of antibody, IgG and IgA are subsequently secreted and continue to be present in the milk for a longer period of time than the first group of immunoglobulins (Curtis and Bourne, 1971; Porter, 1969b). These antibodies remain within the lumen of the alimentary tract and act at the site of bacterial proliferation. Ultimately, they are digested or eliminated (Wilson, 1972). Wilson and Svendsen (1971) demonstrated that milk obtained from sows vaccinated with live, formalin-treated cultures of enteropathogenic *E. coli* afforded significant protection against colibacillosis when compared with a similar milk diet obtained from unvaccinated sows. Oral administration of IgG isolated from milk obtained from vaccinated sows also provided protection against colibacillosis through the action of multiplication-inhibition and anti-enterotoxin factors (Wilson, 1972). Three hundred sows, kept under field conditions, were utilized in a well controlled immunization study conducted by Wilson. Results of this preliminary study indicated that immunization, and subsequent resistance of their offspring to colibacillosis following suckling, could be readily obtained through the utilization of homologous, autologous or heterologous vaccines of live, formalin-treated cultures of *E. coli*.

## The Ligated Loop Technique

A convenient animal model for the study of human cholera was described by De and Chatterje (1953) in which sacs, created in the small intestine of rabbits, became distended with fluid when inoculated with vibrio organisms isolated from human patients. The loop technique was later adapted to study the effects of *E. coli* isolated from cases

of acute and chronic enteritis in man (De *et al.*, 1956). Taylor *et al.* (1961) stated that the enteropathogenicity of *E. coli* could be determined by a positive loop reaction (distention), and that enteropathogenicity was not always related to general pathogenicity of the organism. Smith and Halls (1967a) reported that rabbit intestinal loops inconsistently distended with fluid when known enteropathogenic *E. coli*, isolated from other species, were inoculated intraluminally. They reported, however, that positive loop reactions were almost always observed in the host test animal for which the organism was enteropathogenic. The intestine of conventionally raised swine had been previously utilized, first by Namioka and Murata (1962) and subsequently by Moon *et al.* (1966) and Nielsen and Sautter (1968) to study the enteropathogenicity of *E. coli* isolated from pigs. As evidenced in a recent review article by Sojka (1971), the ligated loop technique has gained general acceptance as an effective research tool.

Positive loop reactions are confined within the limits of their sac, with adjacent, noninoculated loops remaining negative. Therefore, an advantage is realized in which several serotypes or strains may be simultaneously tested within the same animal. Problems of colonization relating to gastric acidity and peristalsis, which tends to "wash out" the intestine, are eliminated. When compared to the oral route of exposure, the ligated loop technique has the advantage of a significantly higher rate of reproducibility in response to enteropathogens (Moon and Whipp, 1971).

However, certain limiting factors of the technique need to be considered when conducting the test. Moon *et al.* (1966) reported that loops made in the jejunal section of the small intestine are of greater enterosorptive sensitivity than those of the ileum. Enteropathogenic

*E. coli* do not cause positive responses 100% of the time, and a few pigs appear to be completely refractory (Moon and Whipp, 1972). Age of the host alters the responsiveness of intestinal loops in much the same manner that it alters the observed susceptibility of pigs to natural enzootics (Moon and Whipp, 1970).

The pathologic alterations observed in the ligated loop inoculated with enteropathogenic *E. coli* have done little to abate the confusion surrounding this disease.

Gross lesions have been attributed to both the induced trauma of the procedure and the degree of enteropathogenicity of the organisms evaluated by this procedure. Moon *et al.* (1966) reported a serofibrinous peritonitis, with a fibrinous mesh encasing the ligated loops. Varying degrees of loop distention were observed which ranged from a negative response to maximal distention with an occasional ruptured loop. The flaccidity of the intestinal wall of the ligated loop was directly proportional to the degree of fluid accumulation and subsequent distention. The fluid was characterized as being cloudy and of yellow, white or red color, and containing particulate matter of food and mucus. Nielsen and Sautter (1968) reported finding occasional areas of edema in the mesentery associated with the ligated loops. They also described gastric lesions, limited to the esophageal-cardial region, which were directly related to the ligation procedure.

Nielsen and Sautter (1968) reported that there were no observable differences in the microscopic appearance of positive and negative loops, except in a group of 4 cesarian-derived pigs which had received an oral exposure to the enteropathogenic *E. coli* 0138:K81:H14 approximately 1 month prior to exposure to the same organism via the ligated loop technique. The characteristic lesions in this special group

consisted of edema and a subintimal hyaline fibrinoid deposition in the small arteries and veins throughout the small intestine. The consistent lesion seen by Moon *et al.* (1966) was a fibrinopurulent serositis on all intestinal loops. Other lesions appeared to be directly related to the degree of loop distention. Interloops and negative experimental loops were normal. In moderately distended loops, alterations ranged from simple architectural distortion to more significant changes. These changes included occasional subepithelial bullae, desquamation of apical villous epithelium and congestion. In addition, cellular infiltrations of polymorphonuclear and mononuclear cells in the lamina propria and submucosa were seen. Occasionally short villi, covered with basophilic cuboidal epithelium, were observed. Loops which were either maximally distended or had ruptured had the most severe changes. The intestinal wall from such loops consisted of a thin muscularis, flattened crypts and denuded, congested and hemorrhagic remnants of villi.

Certain limitations of the ligated loop technique have been previously mentioned. However, the most troublesome by far is the appearance of false positive loops, first described in pigs by Moon *et al.* (1966). Such loops, usually the noninoculated interloops, become distended with fluid and are indistinguishable from those experimental loops harboring known enteropathogens. The culturing of contents from such distended, noninoculated loops has not demonstrated the presence of any of the serotypes being tested in the experimental animal.

## The Gnotobiotic Pig

Certain physiological and anatomical similarities between the pig and man have made the animal an ideal model for the comparative study of gastrointestinal disease (Meyer *et al.*, 1964). Because no immune

substances are transferred *in utero*, and colostrum is withheld from the germfree pig, interfering antibody is absent (Kim *et al.*, 1966). These animals are therefore more uniformly susceptible to infectious agents than are their conventionally reared counterparts (Waxler *et al.*, 1971).

Utilization of the gnotobiotic pig in studies of colibacillosis affords additional advantages over the conventional pig kept under field conditions. Since the occurrence of *E. coli* is widely spread throughout the environment in which pigs are maintained, the gnotobiotic state eliminates all but those desired serotypes and strains. Without the presence of normal intestinal flora, the disease may progress under minimal restrictions and interference. All of these advantages result in the elimination of many of the experimental variables present under field conditions (Kohler and Bohl, 1966). The data obtained from *E. coli*-infected gnotobiotic pigs do not significantly differ from those recorded in enzootics of the disease (Saunders *et al.*, 1963; Kohler and Bohl, 1966).

## MATERIALS AND METHODS

### Experimental Animals

Procurement. Twenty-three germfree Yorkshire pigs, from 4 litters, were delivered by hysterotomy into sterile, plastic isolators, using the technique described by Waxler *et al.* (1966). Epidural anesthesia was administered to the gilts on the 112th day of their gestation. Twenty to twenty-five milliliters of 2.5% procaine hydrochloride* was injected into the epidural space at the lumbosacral articulation. Presurgical tranquilization with promazine hydrochloride** was required in 3 of the 4 gilts. Each gilt was euthanatized following the surgical delivery of all piglets. Approximately 4 hours after delivery, the germfree pigs were transferred into the rearing isolators where they remained for the next 3 weeks. Table 1 lists information concerning both experimental and control animals and includes age, sex and number of animals contained in each group.

Rearing. In the rearing room the newborn pigs were randomly placed into individual cages within sterile, plastic isolators, so that each isolator contained no more than 4 pigs. The experimental animals in Litters 1, 2 and 4 were maintained on the same sterile, semisynthetic

---

*Epidural, Haver-Lockhart Co., Kansas City, Missouri.

**Sparine, Wyeth Laboratories, Cleveland, Ohio.

Table 1. Age, sex and number of *E. coli*-inoculated and gnotobiotic control pigs

| *E. coli* exposed pigs | | | | Gnotobiotic control pigs | | | |
|---|---|---|---|---|---|---|---|
| Litter number | Sex | Animal number | Age at time of exposure (days) | Sex | Animal number | Type of control | Age at time of surgery (days) |
| 1 | M | 1 | 22 | M | 2 | Anesthetize | 22 |
| | F | 4 | 26 | F | 3 | Ligate & sterile broth | 22 |
| | F | 5 | 26 | | | | |
| | F | 6 | 28 | | | | |
| | M | 7 | 28 | | | | |
| | F | 8 | 28 | | | | |
| 2 | F | 9 | 21 | | | | |
| | F | * | 21 | | | | |
| | M | * | 21 | | | | |
| | M | 10 | 21 | | | | |
| | M | 11 | 25 | F | 13 | Anesthetize | 25 |
| | M | 12 | 25 | F | 14 | Ligate & sterile broth | 25 |
| | F | 15** | 27 | F | 17** | Anesthetize | 27 |
| | M | 16** | 27 | M | 18** | Ligate & sterile broth | 27 |
| 3 | F | * | 21 | | | | |
| | M | * | 21 | M | 19 | Anesthetize | 21 |
| | M | * | 21 | | | | |
| | F | * | 23 | | | | |
| | M | * | 23 | | | | |
| | F | * | 23 | | | | |
| | F | * | 23 | | | | |
| | F | 20 | 26 | F | 21 | Ligate only | 26 |
| | M | * | 26 | | | | |

Table 1 (cont'd.)

| *E. coli* exposed pigs | | | | Gnotobiotic control pigs | | | |
|---|---|---|---|---|---|---|---|
| Litter number | Sex | Animal number | Age at time of exposure (days) | Sex | Animal number | Type of control | Age at time of surgery (days) |
| 4 | F | 22 | 21 | | | | |
| | M | * | 21 | | | | |
| | F | 23 | 21 | | | | |

*Death occurred during 24-hour postsurgical period.

**_Micrococcus sp._ cultured from isolator housing these 4 pigs.

diet.* Litter 3 was maintained on another variety of semisynthetic diet.** All pigs were fed 90 ml of the liquid diet 3 times daily for the first 2 days. The volume was increased to 120 ml, 3 times daily, for the next 5 days, and then the animals were maintained on 150 ml every 8 hours for the duration of the experiment. The air temperature of the rearing room was 90 F.

Determination of sterility. Composite specimens were collected from the excrement trays beneath each rearing cage and surrounding cage areas with sterile swabs. Three tubes of thioglycollate medium*** and 6 blood agar plates† were utilized per isolator. Thioglycollate medium was incubated at room temperature, 37 C and 55 C. Blood agar plates were incubated aerobically and anaerobically at the same 3 temperatures. Cultures were taken from each isolator at the time the pigs were 5 and 14 days of age.

Surgical manipulation and inoculation. Approximately 18 hours prior to surgery, feed was withheld from the animals to be utilized in the next day's experiment. Water was withheld approximately 5 hours prior to surgery.

The sterile surgical isolator containing the instruments was connected and sealed to the residential isolator. In addition, the ampules of bacteria and sterile trypticase soy broth were included in the pass-through collar during the preparatory procedure to connect

---

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

**Similac with Iron, Ross Laboratories, Columbus, Ohio.

***Bacto Fluid Thioglycollate Medium (Dehydrated), Difco Laboratories, Detroit, Michigan.

†Tryptose Blood Agar Base, Difco Laboratories, Detroit, Michigan. Defibrinated Sheep Red Cells, Colorado Serum Company, Denver, Colorado.

the 2 isolators. Following the appropriate sterilization procedures of the pass-through tunnel, each isolator seal was removed and the entire surgical-residential unit was moved to the surgical area.

The ligated loop procedure was conducted on each experimentally inoculated animal, utilizing a modification of the techniques of Moon *et al.* (1966), Nielsen and Sautter (1968) and Moon (1970).

Anesthesia was induced with sodium pentobarbital,* given intravenously into the anterior vena cava, at an estimated dosage of 0.5 mg/kg, or to effect. Pentylenetetrazol** was routinely utilized intravenously to reestablish normal respiration as needed during the course of the surgical procedure.

A laparotomy was performed through a midline abdominal incision. The cecum and terminal ileum were identified and used as a reference point to orient the preparation of the loops. The first umbilical tape ligature was placed approximately 1 meter cranial to the cecum, with subsequent ligatures being tied at approximate 10-cm intervals, moving in a cranial direction. Six to seven experimental loops were created in each pig. Interloops, serving as uninoculated control loops, separated the experimental (inoculated) loops from one another.

Each experimental loop was inoculated intraluminally with 0.25 ml of a specific serotype of *E. coli*, as a trypticase soy broth culture. A 26-gauge needle was utilized for the inoculation. All 6 strains were tested in each experimental animal.

---

*Pentobarbital Sodium Injection, Haver-Lockhart Co., Kansas City, Missouri.

**Pemepherine Injection, Haver-Lockhart Co., Kansas City, Missouri.

The abdominal wall and peritoneum were closed by a simple continuous stitch using umbilical tape. The pigs received no food or water during the postoperative period.

Control animals. Some pigs from each litter, excepting Litter 4, were utilized to check the separate effects of the surgical procedure. Pigs which were only anesthetized, others which underwent the intestinal ligation procedure only, and still others in which the sterile trypticase broth was inoculated into the intestinal loops served to monitor each individual step of the experimental plan.

On those days in which both control and experimental animals were to be utilized, the control animals were the first to be handled and were then returned to their residential isolator before the remaining animals were inoculated with the 6 strains of *E. coli*. Animals infected with *E. coli* were housed in separate residential isolators from the germfree pigs, whether they were control pigs or pigs to be utilized on subsequent days' experiments.

## Bacteriological Procedures

Preparation of the infective agent. Five serotypes of *Escherichia coli*, encompassing 6 strains,* were tested in all experimentally infected animals. Details* concerning serotype, strain and enteropathogenicity are given in Table 2.

The cultures were originally supplied and subsequently maintained on tryptic soy agar slants.** The cultures were transferred to fresh

---

*Supplied by Dr. H. W. Moon, U.S.D.A., Agricultural Research Service, National Animal Disease Laboratory, Ames, Iowa.

**Bacto Tryptic Soy Agar, Difco, Detroit, Michigan.

Table 2. Serotype, strain number and enteropathogenicity of the experimental organisms

| Serotype | Strain number | Enteropathogenicity |
|---|---|---|
| 08:K87,K88ab:H19 | 263 | Yes |
| 09:KU115(A):NM | 987 | Yes |
| 09:KU115(A):NM | 115 | No* |
| 0101:KU460(A):NM | 431 | Yes** |
| 064:K+:NM | 637 | Yes** |
| 043:K-:H28 | 123 | No |

*Was loop positive when originally isolated; has been consistently loop negative in all recent tests.

**These strains are consistently loop positive in conventional pigs 2 weeks or younger and consistently loop negative in pigs 6 weeks and older.

tryptic soy slants on a monthly basis throughout the experiment. Inoculated tubes were sealed with waxed corks and stored in the dark at room temperature.

Five hours prior to injection of the organisms into the test animals, a small colony was selected from each storage slant and, by sterile transfer, added to individual 20 ml aliquots of sterile trypticase soy broth.* The soy broth was incubated at 37 C for 3 hours. Ten milliliters of the soy broth culture were aseptically transferred to glass ampules and sealed with a flame. The sealed ampules were again incubated at 37 C until they were transferred to the surgical room at the end of the fifth hour of incubation.

The set of 6 sealed ampules were externally cleaned and placed in the pass-through tunnel immediately prior to the uniting of the residential and surgical isolators and subsequent to chemical sterilization of the tunnel.

Culturing of inoculating syringes. Immediately following the day's inoculation procedure the remaining contents of each syringe were cultured on tryptose agar plates** to insure that viable, uncontaminated cultures had been introduced into the experimental jejunal loops. The agar plates were incubated at 37 C and observed for 3 days thereafter. Representative colonies were selected and stained with Gram stain after 24 hours' growth. The stained organisms were examined under the light microscope.

---

*Trypticase Soy Broth, B.B.L., Division of BioQuest, Cockeysville, Maryland.

**Bacto Tryptose Agar Dehydrated, Difco, Detroit, Michigan.

## Necropsy and Laboratory Procedures

Both control and experimental animals were euthanatized by an intracardial injection of sodium pentobarbital approximately 24 hours after inoculation of the intestinal loops. Entry into the peritoneal cavity was initially made through an incision in the left flank. The skin, muscle and ribs were then removed from the entire left lateral aspect of the animal to facilitate manipulation of the abdominal viscera. The ileocecal junction was located. The ileum was clamped and transsected at its point of union with the cecum. Working cranially, the ileum was carefully removed from its mesenteric attachments until all of the intestinal tract incorporating the experimental segment had been removed from the abdominal cavity. Approximately 1 meter cranial to the most cranial ligature, the second clamp was applied and the intestine transsected at that point. That portion of the segment containing the ligated loops was then removed from the abdominal cavity and straightened so that experimental loops and interloops could be measured and visually evaluated in terms of fluid accumulation (distention). All loops were assigned a value ranging from 0 (no distention) to 4+ (maximal distention). To be considered as a positive distention a loop must have had to attain at least a 3+ rating. The length of each experimental loop and the volume of the fluid contained within that loop were recorded for those pigs for Litters 2, 3 and 4, and then utilized to calculate an "index." This "index", based upon the ratio of the volume (ml) of fluid contained per centimeter length of the sac confining the fluid, was utilized by Burrows and Musteikis (1966) and eliminates any differences in sac volume as it relates to variability in sac length. A mean value was calculated from the 15 indices obtained for each strain. Inoculated loops which failed to

distend were assigned an index value of 0 and were included in the mean index. A measurement was made from the caudal clamp, originally located at the ileocecal valve, to the first (most caudal) ligation to insure that the initial experimental loops were not placed in the ileum.

Intestinal loops were cultured for bacterial growth according to the following protocol. The experimental loops were cultured if they were inoculated with a known enteropathogenic strain and yet failed to distend with fluid. Interloops were cultured for bacterial growth if they showed a greater than 2+ distention. All inoculated loops from control animals inoculated with sterile trypticase soy broth were cultured to confirm their sterility.

The sample of intestinal contents to be cultured was collected from the lumen of the experimental loop or interloop using an adaptation of the technique described by Christie (1967) to culture organs of germfree pigs. A small focal area on the serosal surface was arbitrarily selected as the site from which the intraluminal culture would be withdrawn. A wooden handled spatula was heated to red hotness and immediately placed on the selected serosal focus allowing the surface to become singed, thereby sterilizing the site. A flamed bacteriological loop was thrust through the prepared serosal area into the lumen of the sac. The sample was collected some distance away from the seared site and the loop was withdrawn. The sample was immediately streaked onto a tryptose agar plate and incubated for 12 hours at 37 C. Representative colonies were selected and streaked onto E.M.B. agar plates* and incubated at 37 C for 48 hours. Characteristic colonies were selected and examined as a Gram-stained smear on glass slides.

---

*Levine E.M.B. Agar, Difco, Detroit, Michigan.

Histopathologic technique. Tissues were collected for histopathologic examination from each experimental and control animal included in this study. In the experimental animals and those control animals subjected to intestinal ligation, tissues were selected from each loop and interloop, in addition to liver, kidney and mesenteric lymph nodes. Tissue specimens collected from the intestinal tract of those animals serving as the anesthetic controls were taken at 20 cm intervals commencing at 90 cm cranial to the cecum and terminating after 8 sections had been taken. Sections of the liver, kidney and mesenteric lymph nodes were also collected from those control animals.

Tissue sections were fixed in 10% formalin, embedded in paraffin, cut at 6 microns and stained with hematoxylin and eosin.

## System for Statistical Evaluation of Results

A system was devised whereby the effect of gross and microscopic lesions observed within an inoculated loop could be quantitated in the form of a single number. These numbers would then be used as an evaluation of the enteropathogenicity of each strain tested during the experiment. Information from control animal loops was utilized as the baseline against which all experimentally inoculated loops were judged. Observable lesions in experimental loops were subsequently assigned a numerical weight of 1 (mild), 5 (moderate) or 10 (marked), based upon the degree of deviation from the normal, and are summarized in Table 3. Each inoculated sac was then numerically evaluated and a total score tallied. Fifteen scores were generated for each strain with the exception of 431, which was inoculated into 20 loops. A mean score and its standard error were calculated for each strain.

Table 3. Criteria utilized in statistical evaluation of strain enteropathogenicity

| Criterion | Frequency in control animals | Assigned value (severity) |
|---|---|---|
| Mucosa | | |
| Cuboidal absorptive epithelial cell | 0 | 5 |
| Mucosal erosion | 0 | 10 |
| Mucosal ulceration | 0 | 10 |
| Depression of mitotic activity in cells of glands of Lieberkühn | 23.2% | 1 |
| Mucosal surface flattened and stretched | 9.8% | 1 |
| Lamina propria | | |
| Neutrophils - occasional | 0 | 0 |
| - moderate | 0 | 1 |
| - heavy numbers | 0 | 5 |
| Congestion - mild | 12.2% | 0 |
| - moderate | 0 | 0 |
| - severe | 0 | 5 |
| Hemorrhage | 0 | 10 |
| Necrosis | 0 | 10 |
| Submucosa | | |
| Edema - mild | 14.6% | 0 |
| - moderate | 11.0% | 1 |
| - severe | 2.4% | 5 |
| Eosinophils present | 2.4% | 0 |
| Neutrophils - occasional | 15.9% | 1 |
| - moderate to heavy numbers | 0 | 5 |
| Congestion - mild | 24.4% | 0 |
| - moderate | 0 | 1 |
| - severe | 0 | 5 |
| Hemorrhage | 0 | 10 |
| Necrosis | 0 | 10 |
| Muscularis | | |
| Neutrophils - moderate to heavy numbers | 48.8% | 0 |
| Congestion - mild | 29.3% | 0 |
| - moderate | 13.4% | 1 |
| - severe | 2.4% | 5 |
| Necrosis | 0 | 10 |
| Serosa | | |
| Normal | All anesthetized controls | 0 |
| Inflammatory reaction (mild to moderate) | All other controls | 0 |

Table 3 (cont'd.)

| Criterion | Frequency in control animals | | Assigned value (severity) |
|---|---|---|---|
| | non-injected | broth injected | |
| **Luminal contents** | | | |
| Mucus in lumen | 3.8% | 2.3% | 1 |
| RBCs present - few | 3.8% | 20.5% | 0 |
| - heavy | 3.8% | 9.1% | 5 |
| **Distention** | | | |
| 0 } | | | |
| 1+ } | | 100% | |
| 2+ } | | | |
| 3+ | | 0 | 5 |
| 4+ | | 0 | 10 |

# RESULTS

## Presurgical Observations

All animals in the first 3 litters of gnotobiotic pigs survived. In the fourth litter the survival rate was 25% and deaths were attributable to the stress of reduced environmental temperatures within the room used to house the residential isolators.

Samples taken from the excrement trays and miscellaneous areas within the isolators produced no bacterial growth, with the exception of 1 isolator housing 4 pigs (Animals 15, 16, 17 and 18). From this isolator, a *Micrococcus sp.* was recovered and identified.

## Surgical Observations

Surgical anesthesia was adequate with sodium pentobarbital. However, it was not uncommon for the pig to undergo respiratory failure once intestinal ligation had begun. The intravenous administration of a respiratory stimulant was often necessary to reestablish normal breathing.

## Clinical and Gross Findings

The mortality rate resulting from intestinal ligation, and subsequent inoculation of the 6 strains of *E. coli*, was slightly more than 40%. In this group, the experimental animals failed to survive for the duration of the 24-hour postsurgical period. No deaths occurred in the control animals (Table 1).

The postsurgical condition of the experimental and control animals, immediately prior to euthanasia, varied. Those controls which were anesthetized only were bright and alert and appeared essentially normal. Animals which had undergone jejunal ligation, whether inoculated with *E. coli* or not, were less active and demonstrated differing degrees of abdominal discomfort. A few animals were recumbent and semicomatose.

The appearance of the peritoneal cavity immediately after euthanasia varied according to the surgical and inoculative procedures performed 24 hours previously.

Peritonitis was a consistent observation in both the experimental and control animals that had undergone jejunal ligation. The significant findings included fibrinous tags which were commonly associated with the intestinal ligatures and a variable increase in the amount of peritoneal fluid.

Distention of the jejunal loops was observed in the experimental animals only. Intraluminal fluid accumulation did not occur within the loops of control animals. However, a consistent finding in both experimental and control animals was the accumulation of fluid in the intestinal segment cranial to the jejunal segment containing the loops. Occasionally interloops located between *E. coli* inoculated experimental loops became distended with fluid. These distended interloops were cultured for bacterial growth, and in each case *E. coli* could be isolated. The other interloops within the same pig were not distended and were negative for bacterial growth when randomly sampled.

A summary of the observed distentions in experimental loops caused by the serotype of the *E. coli* tested is listed in Tables 4 and 5.

Loop reactions noted with strains 115 and 123 rarely deviated from what was observed in control pigs and the control interloops of

Table 4. Reactions of serotypes of *E. coli* in experimental jejunal loops

| Serotype | Pig number | Observed distention† | Index†† | Sac location |
|---|---|---|---|---|
| 09:KU115(A):NM Strain 115 | 1 | 0 | * | 1 |
| | 4 | 0 | * | 1 |
| | 5 | 1+ | * | 4 |
| | 6 | 0 | * | 3 |
| | 7 | 1+ | * | 2 |
| | 8 | 0 | * | 4 |
| | 9 | 0 | * | 2 |
| | 10 | 0 | * | 3 |
| | 11 | 1+ | * | 3 |
| | 12 | 1+ | * | 7 |
| | 15 | 0 | * | 5 |
| | 16 | 0 | * | 3 |
| | 20 | 0 | * | 5 |
| | 22 | Ruptured - 4+ | ** | 3 |
| | 23 | 0 | * | 1 |
| 043:K-:H28 Strain 123 | 1 | 1+ | * | 7 |
| | 4 | 0 | * | 4 |
| | 5 | 0 | * | 1 |
| | 6 | 0 | * | 2 |
| | 7 | 2+ | * | 4 |
| | 8 | 0 | * | 5 |
| | 9 | 0 | * | 1 |
| | 10 | 0 | * | 4 |
| | 11 | 1+ | * | 4 |
| | 12 | 0 | * | 1 |
| | 15 | 0 | * | 4 |
| | 16 | 0 | * | 6 |
| | 20 | 0 | * | 3 |
| | 22 | 0 | * | 6 |
| | 23 | 0 | * | 3 |

Table 4 (cont'd.)

| Serotype | Pig number | Observed distention † | Index †† | Sac location |
|---|---|---|---|---|
| 064:K+:NM Strain 637 | 1 | 3+ | *** | 2 |
| | 4 | 1+ | * | 5 |
| | 5 | 0 | * | 2 |
| | 6 | 1+ | * | 6 |
| | 7 | 0 | * | 6 |
| | 8 | 0 | * | 2 |
| | 9 | 2+ | 0.8 | 5 |
| | 10 | 2+ | 0.2 | 6 |
| | 11 | 0 | * | 7 |
| | 12 | 0 | * | 4 |
| | 15 | 1+ | * | 1 |
| | 16 | 1+ | * | 4 |
| | 20 | 0 | * | 1 |
| | 22 | 3+ | 0.7 | 1 |
| | 23 | 0 | * | 2 |
| 0101:KU460(A):NM Strain 431 | 1 | 4+ | *** | 5 |
| | 4 | 0 | * | 2 |
| | 5 | 4+ | *** | 5 |
| | 6 | 2+ | * | 5 |
| | 7 | 0 | * | 1 |
| | 8 | 0 | * | 3 |
| | 9 | 2+/1+ | 0.7/* | 6/3 |
| Smooth/Rough | 10 | 4+/4+ | 2.0/1.6 | 1/2 |
| | 11 | 0/0 | * | 1/2 |
| | 12 | 3+/0 | 1.7/* | 5/6 |
| | 15 | 1+/0 | */* | 7/6 |
| | 16 | 1+/0 | */* | 5/2 |
| | 20 | 0 | * | 2 |
| | 22 | 0 | * | 2 |
| | 23 | 0 | * | 4 |

Table 4 (cont'd.)

| Serotype | Pig number | Observed distention† | Index†† | Sac location |
|---|---|---|---|---|
| 08:K87,K88ab:H19 Strain 263 | 1 | 1+ | * | 6 |
| | 4 | 1+ | * | 3 |
| | 5 | 4+ | *** | 6 |
| | 6 | 4+ | *** | 4 |
| | 7 | 3+ | *** | 5 |
| | 8 | 1+ | * | 1 |
| | 9 | 4+ | 3.0 | 7 |
| | 10 | 0 | * | 5 |
| | 11 | 2+ | 0.8 | 5 |
| | 12 | 1+ | * | 2 |
| | 15 | 1+ | * | 3 |
| | 16 | 1+ | * | 1 |
| | 20 | 4+ | 2.3 | 6 |
| | 22 | 3+ | 1.7 | 4 |
| | 23 | 0 | * | 5 |
| 09:KU115(A):NM Strain 987 | 1 | 4+ | *** | 4 |
| | 4 | 4+ | *** | 6 |
| | 5 | 4+ | *** | 3 |
| | 6 | 3+ | *** | 1 |
| | 7 | 4+ | *** | 3 |
| | 8 | 4+ | *** | 6 |
| | 9 | 4+ | 2.9 | 4 |
| | 10 | 4+ | 3.2 | 7 |
| | 11 | 4+ | 3.1 | 6 |
| | 12 | 4+ | 2.4 | 3 |
| | 15 | 3+ | 1.5 | 2 |
| | 16 | 4+ | 3.0 | 7 |
| | 20 | 0 | * | 4 |
| | 22 | 4+ | 3.0 | 5 |
| | 23 | 0 | * | 6 |

Table 4 (cont'd.)

*Loop contents too thick to determine volume without causing damage to mucosa.

**Loop contents in peritoneal cavity.

***Loop contents were measurable but not performed in early stages of the experiment.

†Distentions were evaluated from 0 (no fluid accumulation) to 4+ (maximum fluid accumulation).

$$\text{††Index} = \frac{\text{Volume of fluid contained within the sac (ml)}}{\text{Length of sac (cm)}}$$

Table 5. Summary of reactions of serotypes of *E. coli* tested in experimental jejunal loops

| Serotype | (Strain) | Percentage of loops with significant enterosorption | Mean "Index" | (range) |
|---|---|---|---|---|
| 09:KU115(A):NM | (987) | 86.7 | 2.7 | (0-3.2) |
| 08:K87,K88ab:H19 | (263) | 40.0 | 0.87 | (0-3.0) |
| 0101:KU460(A):NM | (431) | 42.0 | 0.375 | (0-2.0) |
| 064:K+:NM | (637) | 13.3 | 0.17 | (0-0.8) |
| 09:KU115(A):NM | (115) | 6.0 | * | (0-*) |
| 043:K-:H28 | (123) | 0 | 0 | |

*Ruptured sac, volume undeterminable.

experimental pigs. A single exception is noted in Loop 3 of Pig 22, which received an inoculum of Strain 115. This loop became maximally distended and subsequently ruptured prior to euthanasia 24 hours following surgery.

Increased variability was observed in the loop reactions of Strains 431 and 637. Thirteen of the fifteen loops inoculated with Strain 637 were characterized as having reactions of 2+ and below. Strain 431 produced reactions of 3+ and greater in only 5 of 21 loops. Of these positive reactions, 4 of the 5 loops were maximally (4+) distended; no 4+ distentions were recorded for Strain 637.

Loop reactions to Strain 263 consisted of 6 of 15 evaluations being rated at 3+ or greater, of which 4 were maximally distended. Thirteen of the fifteen loops inoculated with Strain 987 had reactions which were characterized as being 3+ and greater. Of the positive loop responses, 11 loops were maximally (4+) distended.

The gross character of the fluid or contents was relatively consistent with the degree of distention. Negative loop reactions and distentions of up to 2+ contained viscid material of normal color. Loop reactions characterized by distentions of 2+ and greater contained material of increasing fluidity, which was highly variable in color and turbidity. Contents from maximally distended loops, containing either Strain 263 or 987, were translucent, pale yellow or white, and occasionally tinged with blood.

The muscular tone of the intestinal wall was directly proportional to the degree of its distention. Mural flaccidity was evaluated following the draining of the contents. Loops of 3+ distention and greater were more flaccid and increasingly had less tone when compared to control

interloops. The walls of maximally distended loops were extremely thin and semitransparent when observed prior to draining. Following draining, the collapsed walls from such loops were flaccid and extremely stretched.

## Bacteriological Monitoring

Culturing of the remaining contents in syringes used to inoculate sacs confirmed, in every case, that viable organisms had been introduced into each ligated loop. Microscopic appearance of Gram-stained smears was consistent with that of *E. coli*.

The contents of all noninoculated interloops with distentions of 3+ and greater were cultured to differentiate between distentions due to the presence of enteropathogenic *E. coli* and fluid accumulations possibly due to normal secretory activity of the intestine. Gram-negative rods, resembling *E. coli*, were consistently isolated from distended interloops.

In addition, loops inoculated with known enteropathogens, Strains 431, 637, 263 and 987, which failed to cause accumulations of intraluminal fluid, were monitored for bacterial growth. In no case were these loops found to be germfree. The organism isolated from such loops was a Gram-negative rod which grew on media selective for the growth of enteric microorganisms and was assumed to be *E. coli*.

## Histopathologic Findings

The small intestine, liver, kidney and mesenteric lymph nodes were evaluated histologically. The jejunal lesions were highly variable and appeared to be related to the enteropathogenicity, and subsequent fluid accumulation, of each strain of *E. coli* tested.

The small intestine. Sections were routinely taken from the duodenum and jejunum. Jejunal sections were collected from those segments immediately cranial and caudal to the experimental area, in addition to each test loop and interloop contained within this segment. In those control animals not subjected to intestinal ligation, comparable areas from the intestinal mass were evaluated.

The following observations were consistent in control and experimental animals. Exceptions where found are listed. Vacuolation of absorptive epithelial cells was directly related to the level of the jejunum being examined. The degree of vacuolation of mucosal epithelium increased gradually from the most cranially located sacs to a highly vacuolated epithelium found in the most caudally located loops. The lamina propria consistently contained a randomly dispersed population of eosinophils, individualized lymphocytes, mesenchymal cells and an occasional neutrophil. The submucosa and muscularis routinely harbored small numbers of neutrophils. Submucosal neutrophils tended to be dispersed and few in number. Extravascular neutrophils present in the tunica muscularis were arranged in small aggregates near vessels separating the longitudinal and circular layers of this smooth muscle mass. In those animals subjected to intestinal ligation, an acute neutrophilic and serofibrinous peritonitis was consistently observed on the serosal surface of the small intestine. In the majority of cases the condition was mild. Moderate to severe peritonitis was occasionally observed and was always in pigs which had received the 6 strains of *E. coli*. The degree of inflammatory response on the peritoneal surface was most severe on those loops which were maximally distended and which also contained the greatest degree of histopathologic changes in the tissue strata beneath their serous surfaces.

Goblet cell discharge was commonly observed in sacs which became significantly distended, while the mucosa of the interloops on either side of such sacs contained normal numbers and distributions of goblet cells (Figures 1 and 2).

The inoculation of serotype 043:K-:H28 (Strain 123) resulted in very few histopathologic alterations (Figure 3). The microscopic appearance of the majority of sections collected from these sacs closely resembled those taken from control animals (Figure 4), or the noninoculated interloops. However, in 3 inoculated sacs a moderate to severe submucosal edema was observed. The extent of the edema was variable, being focal in Animal 6 and generalized in Animals 1 and 16. These variations were unrelated to sac location within the jejunal segment in all 3 animals.

With the exception of 2 animals, Numbers 1 and 22, serotype 09:KU115(A):NM (Strain 115) produced nonalterative and noninflammatory reactions similar to those observed with Strain 123. Significant submucosal edema was found in only 2 of 13 animals, Numbers 10 and 22, being generalized and focally distributed, respectively. However, 2 severe sac responses occurred following the inoculation of this organism. In Animal 1, 4 to 5 small focal areas of mucosal ulceration with congestion, hemorrhage and necrosis within the supporting lamina propria were observed. An associated increase in tissue neutrophils in the involved areas was also seen. The inflammatory response did not progress beyond the muscularis mucosa, with the exception of occasional areas of hyperemia in the submucosa and tunica muscularis. The microscopic mucosal lesions observed in Pig 22 were vaguely similar to those reported for Animal 1, but of a more severe intensity. Approximately 40% of the luminal surface was involved, with areas previously occupied

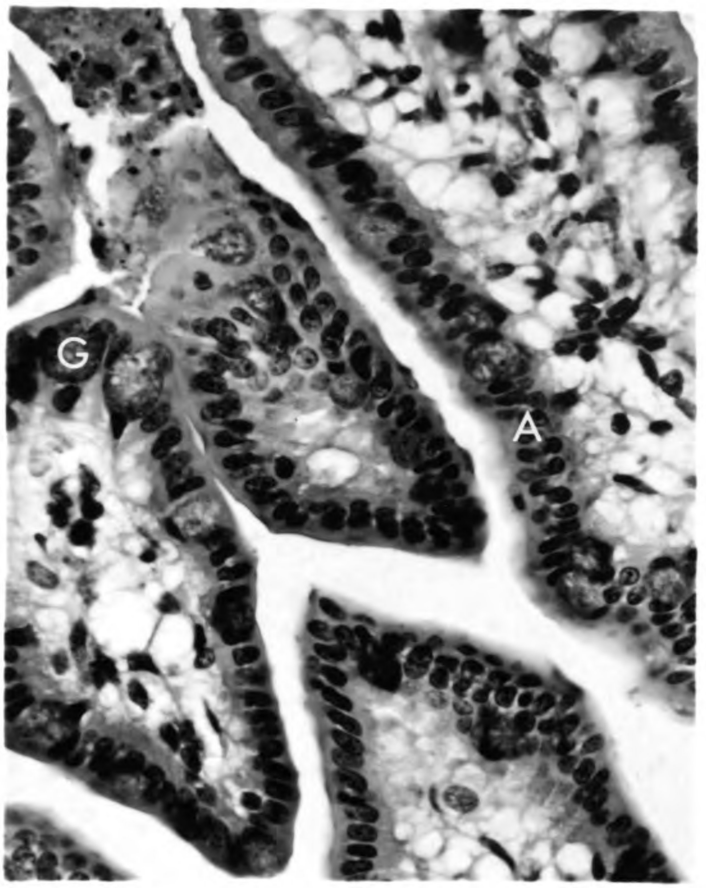


**Figure 1.** Pig 9, interloop 3 (noninoculated). Jejunum, 24 hours postinoculation. Notice the presence and abundance of goblet cells (G) interspersed among the absorptive epithelial cells (A) of the mucosa. Hematoxylin and eosin; x 600.

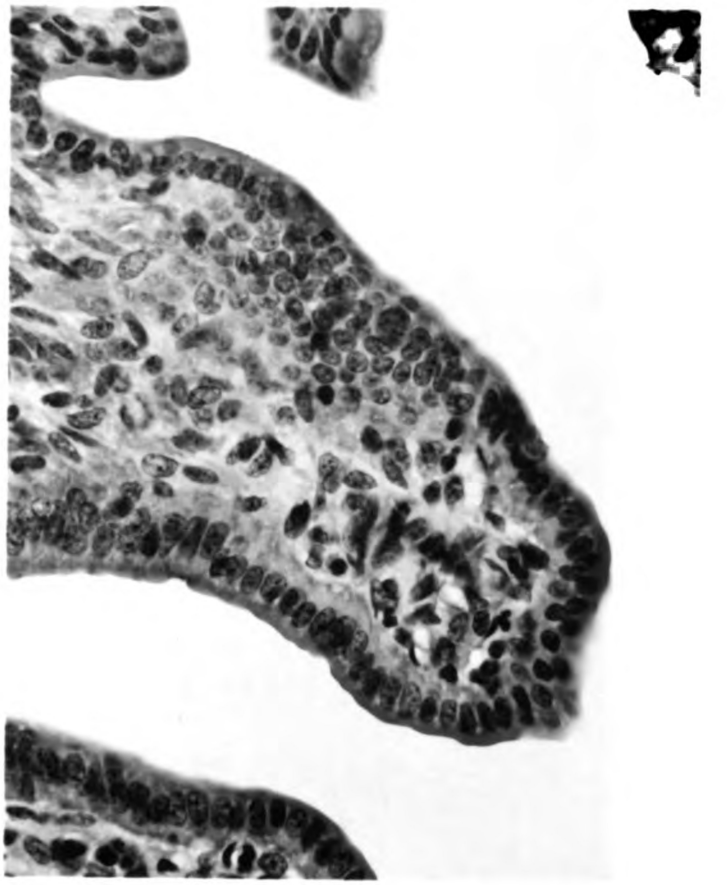

Figure 2. Pig 9, loop 4 (Strain 987). Jejunum, 24 hours postinoculation. Field similar to Figure 1. Notice the absence of goblet cells in the mucosa. Hematoxylin and eosin; x 600.

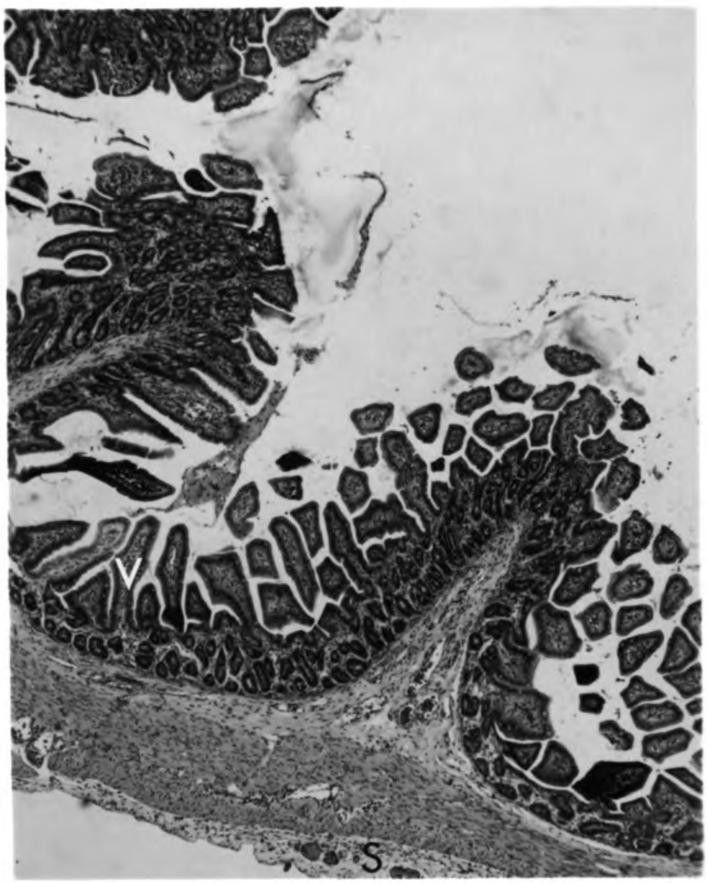


Figure 3. Pig 11, loop 4 (Strain 123). Jejunum, 24 hours postinoculation. Notice the essentially normal configuration of the section, with the villi (V) being long, fingerlike projections of the mucosa. A mild peritonitis is seen on the serosal surface (S). Hematoxylin and eosin; x 60.

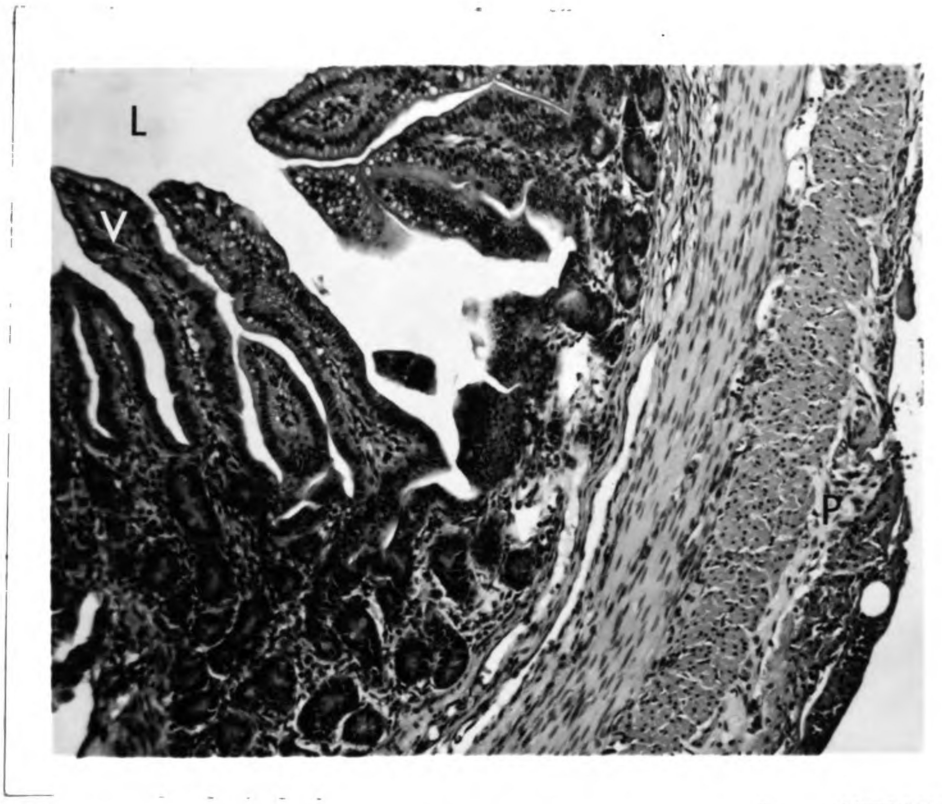


Figure 4. Pig 14 (control), loop 4, inoculated with sterile broth. Jejunum, 24 hours postinoculation. Villi (V) are slender, fingerlike projections of the mucosa, extending into the lumen (L). Notice the moderate peritonitis (P) on serosal surface. Hematoxylin and eosin; x 150.

by normal mucosal and submucosal tissue being replaced by a mass of necrotic and inflammatory cells (Figure 5). Mucosal areas which were not ulcerated or eroded bore short, clublike villi covered with low columnar to cuboidal epithelium and were relatively devoid of goblet cells. Marked acute neutrophilic and hemorrhagic myositis was observed in the region of the tunica muscularis subjacent to the ulcerated mucosal area.

Serotype 064:K+:NM (Strain 637) produced only 1 incidence of mucosal ulceration (Animal 1). Hyperemia and hemorrhage were the only alterations observed in the submucosa with little involvement of the associated tunica muscularis. All lesions were confined to a single locus, occupying approximately one-third of the absorptive surface. Villi near the area of mucosal ulceration were shortened, clubbed and occasionally fused. Mitotic activity was depressed in the crypts of Lieberkühn, and mild localized areas of edema were scattered throughout the submucosa. The remainder of the section was essentially normal. A significant distention was also observed in Animal 22. In this instance there was no evidence of mucosal erosion or ulceration. However, the villi were shortened, and mitotic activity of the cells within the crypts of Lieberkühn was greatly reduced. Although the intestine was distended, the general histologic appearance of the section was essentially normal. Mild submucosal edema was observed in 2 additional sections (Animals 5 and 6) and was generalized in each instance. Moderate to severe submucosal edema was found in 3 other sections. Its distribution was generalized in Animals 11 and 16 and focal in Animal 9.

Severe histopathologic alterations, characterized by mucosal ulcerations and associated changes in the submucosa and muscularis

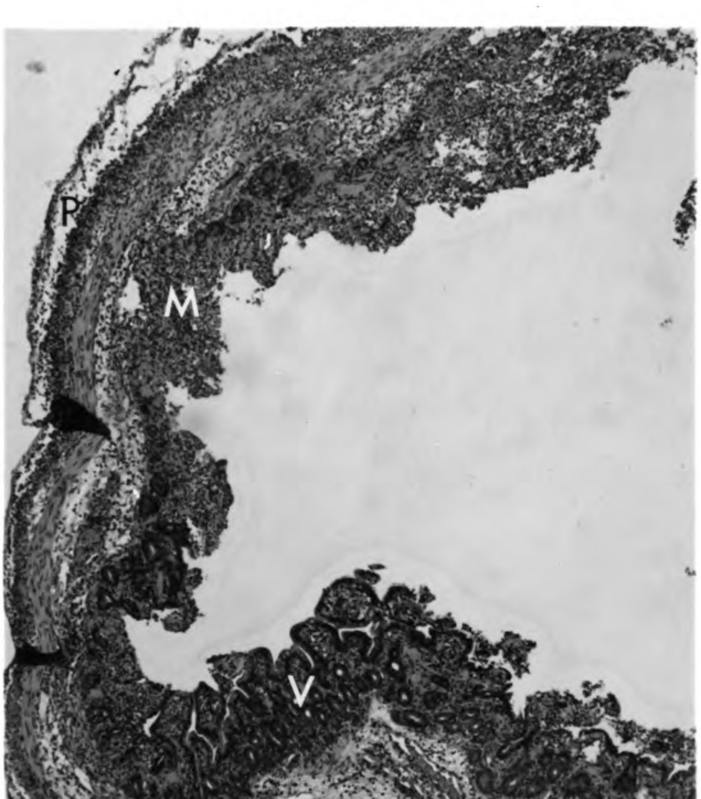


Figure 5. Pig 22, loop 3 (Strain 115). Jejunum, 24 hours postinoculation. Notice the extensive mucosal ulceration and erosion (M), shortened villi (V), and peritonitis (P) on the serosal surface. Hematoxylin and eosin; x 60.

occurred in only 1 of 19 loops (Animal 5) inoculated with the serotype 0101:KU460(A):NM (Strain 431). The appearance of this section varied little from what had been observed for other serotypes which caused ulcerative lesions, with the exception of the presence of a severe, localized submucosal edema. Significant loop distentions, without mucosal ulceration or erosion, were observed in Animals 1, 10 and 12. Strain 431-inoculated loops from these animals contained evidence of reduced mitotic activity within the cells of the crypts of Lieberkühn, moderate to severe focal submucosal edema and a thinning and stretching of the normal histological arrangement of the sac. Severe focal submucosal edema was also observed in 4 nondistended sacs (Animal 12, Sacs 5 and 6, and Animal 16, Sacs 2 and 5).

The inoculation of serotype 08:K87,K88ab:H19 (Strain 263) into 15 loops resulted in the production of 3 sacs, from Animals 5, 9 and 20, which were characterized by the typical ulcerative and necrotic lesions previously described but without the occasionally accompanying submucosal edema. Mucosal lesions occupied approximately 30% of the luminal surface of each section with the exception of the loop from Animal 20, in which 60% involvement was seen. Ulcerative lesions and extensive inflammatory reactions were not observed in 3 additional loops which had also distended significantly (Animals 6, 7 and 22). However, in these latter instances a general stretching of the microscopic configuration and shortening of the mucosal epithelium to a more cuboidal shape were observed. Extensive submucosal edema was observed in only 1 loop (Animal 11) inoculated with this strain and occurred in a sac which failed to attain a significant degree of distention. The suppression of mitotic activity of the cells with the crypts of Lieberkühn was found in loops which were significantly distended.

Eleven of the fifteen loops inoculated with the serotype 09:KU115(A):NM (Strain 987) became significantly distended. Necrotic and ulcerative lesions as typified by Figure 6 were observed in 6 of the 11 distended loops (Animals 1, 5, 7, 10, 11 and 22). Erosive lesions of the mucosa with an accompanying moderate inflammatory response in the deeper strata of the section were seen in the test loop from Animal 16, which also had significant fluid accumulation. In all 6 cases above, the extent of mucosal involvement was usually greater than 30% of the total luminal surface but never exceeded more than 50%. Moderate focal submucosal edema was observed in 3 loops (Animals 1, 10 and 11). The remaining significantly distended loops contained less severe histopathologic lesions, primarily characterized as circulatory changes commonly associated with inflammation. Mild neutrophilic infiltrations of the lamina propria, submucosa and tunica muscularis were occasionally observed in this latter instance. Mitotic activity of the cells within the crypts of Lieberkühn was routinely depressed in all inoculated loops which distended significantly.

The liver, kidney and mesenteric lymph nodes. Evaluations were made of the kidney and liver from all experimental animals. In all cases, the liver remained essentially normal during the postinoculation period. The renal cortex consistently underwent mild degenerative changes which were characterized by cloudy swelling and hydropic change in the convoluted tubules. Congestion was also frequently observed in these renal sections.

The mesenteric lymph nodes were collected and evaluated in 4 experimental animals, Numbers 15, 20, 22 and 23, near the termination of the study. In each section, vast numbers of neutrophils occupied the

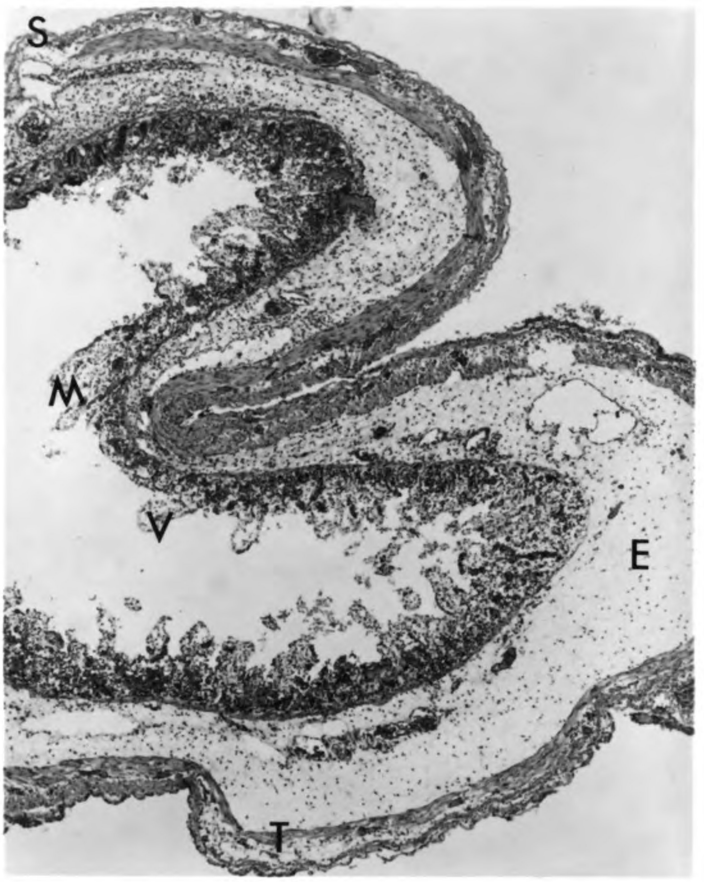


Figure 6. Pig 11, loop 6 (Strain 987). Jejunum, 24 hours postinoculation. Notice the extensive ulceration and erosion of the mucosa (M) with remnants of villi (V) still present, severe edema (E) in the submucosa, and the stretching of the tunica muscularis (T) into a thin layer. Severe areas of peritonitis are seen on the serosal surface (S). Hematoxylin and eosin; x 60.

subcapsular sinuses and the medullary regions of the node. Small numbers of bilobed, metamyelocytic eosinophils were uniformly distributed throughout the collection of sinusoidal neutrophils.

Little or no significant differences were found to exist among the 3 groups of control animals, with the exception that peritonitis was absent in those animals which were only anesthetized. Few alterations were seen in the appearance of the absorptive epithelium, and the mucosa was routinely thrown into folds as is normally reported. Mitotic activity of the cells within the crypts of Lieberkühn was occasionally depressed in isolated loops but was not found to follow any pattern in controls.

The mesenteric lymph node sections from control animals generally were devoid of neutrophils, with the exception of Animal 19, in which a pronounced neutrophilia was seen in the sinuses throughout the node. The mesenteric lymph nodes from Animals 17 and 18 contained moderate to large numbers of bilobed eosinophils. The majority of these cells were located near the periphery of the gland, especially in the subcapsular sinuses. However, an occasional eosinophil was observed within the immature germinal centers of the gland.

Statistical evaluation of results. The results of the statistical evaluation of each strain, based upon the gross and microscopic alterations reported for each organism, are summarized in Table 6 and Figure 7. From Figure 7 it can be seen that 3 essential ranges of responses are present: 1) a low score of less than 2.5, 2) a midrange from 2.6 to 13.5, and 3) an upper range extending to 43.5. Strain 115 spans between the mid- and upper ranges.

Table 6. Individual strain scores, strain means and standard error of the strain means

| Serotype | (Strain) | Sac location | Scores | Strain mean | S.E. of $\bar{x}$ |
|---|---|---|---|---|---|
| 043:K-:H28 | (123) | 1 | 0,5,7 | 2.00 | 0.60 |
| | | 2 | 1 | | |
| | | 3 | 0,1 | | |
| | | 4 | 0,0,1,1,2 | | |
| | | 5 | Not tested | | |
| | | 6 | 0,5 | | |
| | | 7 | 5 | | |
| 09:KU115(A):NM | (115) | 1 | 0,3,43 | 10.28 | 6.35 |
| | | 2 | 0,6 | | |
| | | 3 | 0,1,1,5,83 | | |
| | | 4 | 1 | | |
| | | 5 | 0,1 | | |
| | | 6 | Not tested | | |
| | | 7 | 0 | | |
| 08:K87,K88ab:H19 | (263) | 1 | 0 | 21.07 | 8.34 |
| | | 2 | 1 | | |
| | | 3 | 1,5 | | |
| | | 4 | 11,27 | | |
| | | 5 | 0,0,8,18 | | |
| | | 6 | 1,53,72 | | |
| | | 7 | 98 | | |
| 09:KU115(A):NM | (987) | 1 | 7 | 35.28 | 8.22 |
| | | 2 | 7 | | |
| | | 3 | 14,59,74 | | |
| | | 4 | 10,27,64 | | |
| | | 5 | 48 | | |
| | | 6 | 0,12,42 | | |
| | | 7 | 27,103 | | |
| 064:K+:NM | (637) | 1 | 0,1,6 | 7.07 | 4.47 |
| | | 2 | 0,0,64 | | |
| | | 3 | Not tested | | |
| | | 4 | 0,7 | | |
| | | 5 | 5,11 | | |
| | | 6 | 1,1,2 | | |
| | | 7 | 1 | | |

Table 6 (cont'd.)

| Serotype | (Strain) | Sac location | Scores | Strain mean | S.E. of $\bar{x}$ |
|---|---|---|---|---|---|
| 0101:KU460(A):NM | (431) | 1 | 0,17,18 | 10.95 | 2.72 |
| | | 2 | 0,0,1,1,6,22 | | |
| | | 3 | 0 | | |
| | | 4 | 0 | | |
| | | 5 | 11,18,23,25,44 | | |
| | | 6 | 3,6,22 | | |
| | | 7 | 2 | | |

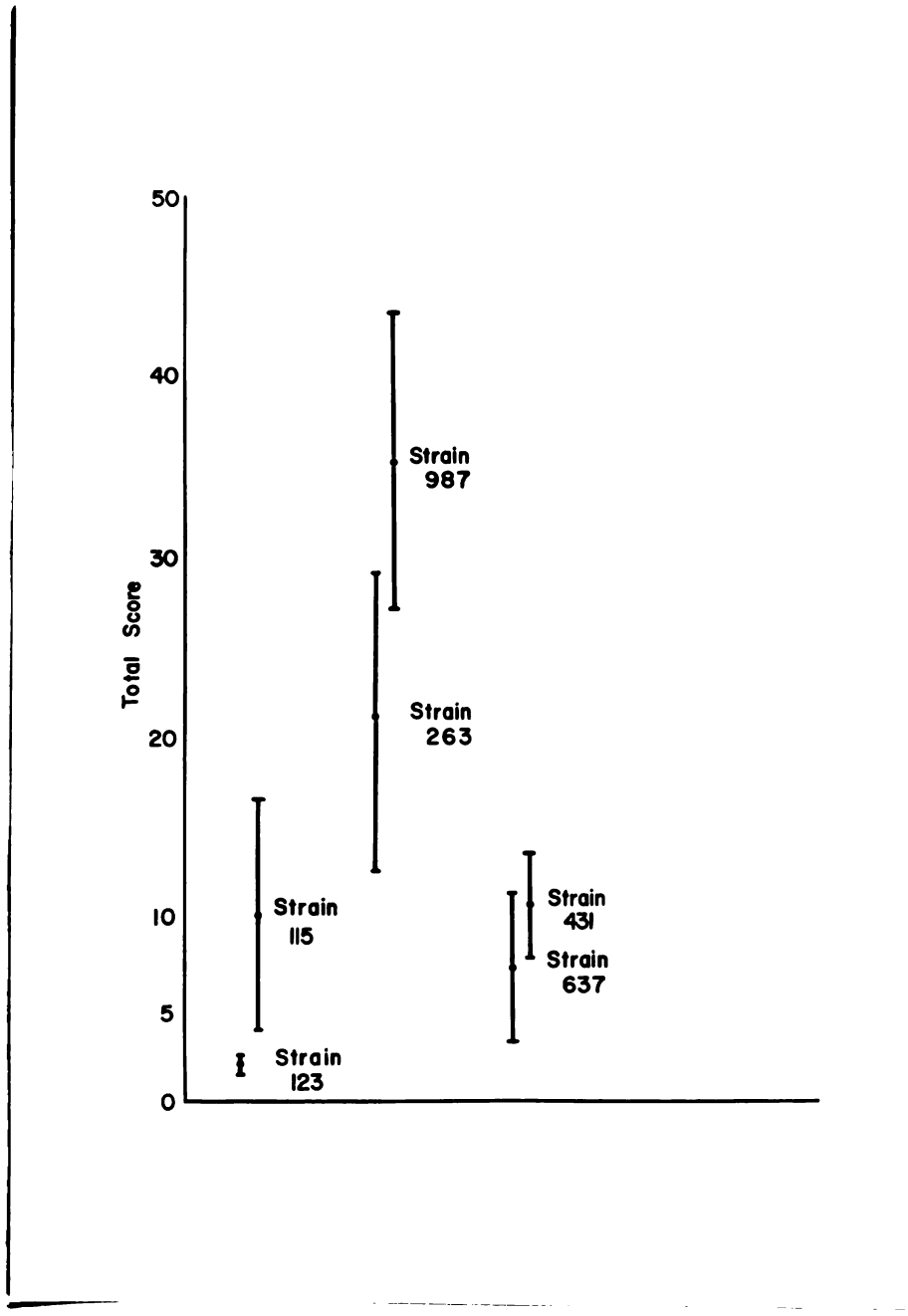


Figure 7. Plot of mean and standard error of the mean for each strain. Taken from Table 6.

# DISCUSSION

## Postoperative Clinical Features

Considerable physiologic stress resulted from intestinal ligation as evidenced by the general postoperative behavior of the animals. The inoculation of the 6 strains of *E. coli* tended to increase this embarrassment as seen in the higher mortality rates among the experimental group when compared with the ligated controls. All ligated control animals continued to live through the 24-hour postoperative period, while the experimental group experienced a survival rate of only 58%.

Based upon clinical signs, those control animals which were subjected to the intestinal ligation alone, although less active than the other control groups, appeared more nearly normal than the experimental group. The elaboration of enterotoxins, and the subsequent distention of enteropathogen-inoculated loops, appeared to play a direct and decisive role in the postoperative state of the animal not unlike what might be expected in an animal found to have a natural infection of colibacillosis.

These results appear to be in contrast to the work of Smith and Halls (1967a) in which little if any significant differences were observed in the postoperative states of experimental and control animals. Their experiment utilized conventionally reared, 7- to 12-week-old piglets. The fact that the immunological status, age and preoperative environmental considerations substantially differed

between the experimental animals of Smith and Halls and those utilized in our studies might easily account for this apparent discrepancy.

Other authors (Moon *et al.*, 1966; Gyles and Barnum, 1967; Moon *et al.*, 1970; Moon and Whipp, 1970), employing the ligated loop technique in pigs, did not discuss the postoperative status of their animals.

## Gross Findings--The Peritoneal Cavity

As was mentioned above, certain common similarities existed between the group of controls subjected to the intestinal ligation and the experimental animals. In both groups, serofibrinous peritonitis was observed and correlated with the findings of Nielsen and Sautter (1968) and Moon *et al.* (1971). The initiating causes were most likely the presence of an irritating foreign material, the umbilical tape ligatures used to define the sacs, and the trauma resulting from the operative handling of the abdominal viscera during surgery. Peritonitis observed in experimental animals tended to be more serous in composition than that observed in the ligated controls. Microscopic examination of loop cross sections tended to suggest reasons for the differences between the 2 groups. Loops inoculated with highly enteropathogenic strains often resulted in stretching and inflammation of the sac wall. The ability of the wall of such sacs to act as an effective limiting structure is somewhat questionable, and it might be hypothesized that intensification of the serous aspect of the peritonitis may be related to the release of minute quantities of toxin-rich fluids into the peritoneal cavity.

## Reactivity of the Serotypes

Based upon gross observations of loop distentions and the calculated "mean indices" for each serotype tested, the results obtained

compare favorably with previously published data, with few notable exceptions (Table 7). Strain 263 consistently caused a higher percentage of positive loop responses for other investigators than was observed when inoculated into germfree pigs, and for reasons not fully understood. The mean index for each strain was calculated to also reflect the number of nonreactive loops observed. If this figure was adjusted to reflect an 80% positive loop reaction rate, as would be expected from published reports, the mean index should be approximately 1.95 and would fall well within the ranges published for this strain. The apparent discrepancy of positive reaction rates for Strain 431 which exists between the work of Moon *et al.* (1966), Moon and Whipp (1970) and our data relates exclusively to the age of the animals at the time of the experimentation. Moon and Whipp (1970) demonstrated that with certain strains of enteropathogenic *E. coli*, the age of the animal at the time of infection was of maximal importance in relation to the percentage of positive loop responses obtained. In their study, over 93% of the loops inoculated with Strain 431 were significantly distended when tested in pigs less than 1 week of age, in sharp contrast to an 8% response in pigs of similar breed but ranging in age from 3 to 10 weeks. The animals utilized in our study were between the ages of 3 and 4 weeks and, therefore, compare favorably with the expected incidence based upon published reports. The foregoing age-induced resistance factor applies equally to Strain 637, which caused a 100% positive response in pigs less than 1 week of age and only a 20% response in pigs between the ages of 3 and 10 weeks (Moon and Whipp, 1970).

Strain 115, utilized by Moon *et al.* (1966) and found to be enteropathogenic, had originally been isolated from an enzootic of

Table 7. Comparison of experimental and published data for the 6 strains of *E. coli* tested in porcine intestinal loops

| Experimental data | | | Published data | | |
|---|---|---|---|---|---|
| Strain | Positive jejunal loop responses (%) | Mean index | Positive loop responses--% (location) | Mean index | Authors |
| 987 | 86.7 | 2.7 | 75 (jejunal) | 1.69* | Moon and Whipp (1970) |
| 263 | 40.0 | 0.87 | 100 (duodenal) | 1.7 - 2.6 | Gyles and Barnum (1967) |
| | | | 74 (jejunal and ileal) | --- | Moon, Sorensen and Sautter (1966) |
| | | | 91 (jejunal) | 2.0 | Moon and Whipp (1970) |
| 431 | 42.0 | 0.375 | 95 (jejunal and ileal) | --- | Moon, Sorensen and Sautter (1966) |
| | | | 8 (jejunal) | 0.2 | Moon and Whipp (1970) |
| 637 | 13.3 | 0.17 | 100 (jejunal and ileal) | --- | Moon, Sorensen and Sautter (1966) |
| | | | 20 (jejunal) | --- | Moon and Whipp (1970) |
| 115 | 6.0 | ** | 96 (jejunal and ileal) | --- | Moon, Sorensen and Sautter (1966) |
| | | | 0 (jejunal) | 0 | Moon and Whipp (1970) |
| 123 | 0 | 0 | 0 (jejunal and ileal) | 0 | Moon, Sorensen and Sautter (1966) |
| | | | <1 (jejunal) | --- | Moon and Whipp (1970) |

*Mean for 6, class 1, enteropathogenic *E. coli*, including Strains 987 and 263. H. W. Moon, personal communication, 1970.

**Sac ruptured, volume undeterminable.

colibacillosis. Moon and Whipp (1970) utilized this strain again and found that the enteropathogenicity of the organism had been lost. Strain 115 was supplied to us in its nonenteropathogenic state, and our experiences with this strain tended to support these findings up until the terminal experiment. Inoculation of this strain into Pig 22 produced not only a maximally-distended loop but one which ruptured during the 24-hour period following inoculation with the organism. The complete inoculation of this loop involved a second penetration of the needle through the intestinal wall. Although this event did not occur with any other loops during the course of this study, it appears highly unlikely that such an alteration in technique would be responsible for a significant distention. Moon and Whipp (1970) reported the occurrence of a single significant distention in the 52 trials with this strain. Although less likely to occur, our report of 1 significant distention in 15 trial loops is within the limits of probability based upon the experiences of Moon and Whipp (1970) with this strain. This inconsistency does, however, apparently address itself to the problem of falsely positive distended loops reported as a complication of the ligated loop technique when conventional animals were utilized. The problem of false positive loops will be dealt with at some length later in the discussion.

Histopathologically, very little significance can be placed upon the occurrence of ulcerative and erosive lesions commonly associated with 4+ or maximally distended loops. Fresh *et al.* (1964) demonstrated that mucosal lesions, similar to those described above, were directly related to continuously excessive intraluminal hydrostatic pressures present in the ligated loop and had no primary relationship to the presence of enteropathogens or their toxins. Inflammatory changes,

also reported in conjunction with the mucosal destruction, relate to the normal host defense mechanisms associated with such tissue necrosis. Little variation was observed among serotypes concerning the quantitative extent of mucosal surface involvement when ulceration or erosion did occur. The degree of mucosal involvement generally ranged between 33 to 40% of the total absorptive area in each section examined. Those serotypes producing a consistently higher percentage of enterosorption did not correspondingly result in a more extensive degree of histopathologic mucosal change. Therefore, the presence of bacterial toxins appears to be insignificant in exaggerating the mucosal response to increased intraluminal pressures. The remaining changes, consisting of absorptive cell shortening, clubbing and/or the elimination of villus structure, and the thinning of the tunica muscularis all relate to increased intraluminal pressures occurring in association with loop distention and the subsequent stretching of the intestinal layers. It should be stressed, however, that although the presence of microscopic intestinal lesions associated with loop distention are not significant in and of themselves, their occurrence does relate to the enteropathogenicity of the organism and the subsequent ability of the serotype or strain to cause enterosorption. The only histopathologic lesion directly related to the enteropathogenicity of the organism appeared to be the increased discharge of mucus by goblet cells. This lesion was reported by Moon *et al.* (1971).

Examination of histopathologic sections from liver and kidney failed to demonstrate significant lesions. Mesenteric lymph nodes were consistent in appearance with that described by Alexander *et al.* (1969), with the exception of the subcapsular sinusoidal areas which were heavily populated with the same neutrophil and eosinophil mixture reported for the medullary tissue.

The numerical rating system provided an excellent means by which the enteropathogenicity of the various serotypes might be compared. The control groups served to delineate those effects which were attributable to the ligation technique alone. Peritonitis and a mild "physiologic inflammation" of the intestinal mucosa were found to relate to the surgical technique and the presence of microorganisms in the intestinal lumen, respectively. With the exception of Strain 115, the location of the plotted points on the graph relating to total enteropathogenic effects of single serotypes fell within a range predictable and consistent with the information originally supplied with the accompanying cultures by Dr. Moon. From Figure 7 it can be seen that 3 essential ranges of response are present. Strain 123 was unable to produce a total score of more than 2.5 and was supplied as a nonenteropathogenic strain. The group of organisms represented by Strains 263 and 987 had been previously identified as being consistently loop-positive enteropathogens in pigs irrespective of age at time of inoculation. The mean scores of these 2 strains were substantially above the mean points plotted for either of the 2 other classifications of organisms, clearly ranking Strains 263 and 987 to be of greater enteropathogenic capabilities than the other 4 strains tested.

The age-resistance relationship reported by Moon and Whipp (1970) plays a significant role in the expression of the enteropathogenicity of Strains 637 and 431. These 2 strains are known to be consistently loop positive in conventional pigs under 2 weeks of age and relatively nonenteropathogenic in pigs over 6 weeks of age (Moon and Whipp, 1971). The plots in Figure 7 for these 2 strains are consistent with the above fact in that the mean scores of 2.7 and 4.5, respectively, obtained for 4-week-old pigs fall between the scores of the other 2 major

groups. The rating system also failed to demonstrate any significant differences between the rough and smooth variants of Strain 431 which appeared at midcourse in the experiment. The mean score for 431-S was 9.3, while that for 431-R was 9.5, and both values compared favorably with scores for the strain both prior to the split and following the return to a uniform colony appearance.

The highly aberrant behavior of 2 test loops inoculated with the nonenteropathogen, Strain 115, is of great interest. The resulting scores from these 2 loops were well within the range reported for the highly enteropathogenic Strains 263 and 987 and resulted in an exaggerated shifting of the mean score for the Strain 115 upward to the extent that it fell within the age-resistance class range of enteropathogens. If those 2 remarkable scores were removed from the tally, the resultant mean score for serotype 115 would be 1.5 and safely within the range of nonenteropathogenicity. These 2 unexpected loop responses will be treated in a subsequent section of the discussion dealing with "false" reactions associated with the ligated loop technique.

In general, the rating system clearly substantiated the direct relationship existing between the enteropathogenicity of a strain of *E. coli* and the gross and microscopic alterations observed in the gut following the ligated loop procedure. The total score was highly reflective of the enteropathogenicity and correlated with the serotypic information originally supplied by Moon (1970) at the beginning of the experiment.

The changes seen in the mitotic activity of cells within the crypts of Lieberkühn were closely associated with the degree of loop distention observed in the experimental animals. Since the amount of

fluid accumulation directly relates to enterotoxin elaboration and thereby the enteropathogenicity of the organism, the decrease in cell division occurring in the crypts of Lieberkühn may be in response to either the presence of the toxin, the mechanical effects of distention or a combination of both.

The effects of dual microbial contamination within single sacs were inadvertently evaluated in Animals 15 and 16. These animals had been accidentally contaminated with an apparently nonpathogenic *Micrococcus sp.* early in the neonatal period and were harboring the organism in their digestive tracts at the time of the experiment. No significant differences in loop response to the *E. coli* were found between the experimentally infected animals contaminated with the *Micrococcus sp.* and the larger body of experimentally infected pigs which had remained germfree prior to the inoculation of the test strains.

Edema in the lamina propria and the submucosa was randomly distributed throughout the loops of the experimental animals and in those control animals in which the ligation procedure was performed. Therefore, the occurrence of such alterations appears to relate directly to the surgery and resulting postoperative changes.

Twice during the course of these experiments falsely positive loops were observed. Both occurrences were confined to Strain 115, with 1 example being detected both grossly and microscopically in Sac 3 of Animal 22 and the second being solely detected by the presence of extensive microscopic lesions in Sac 1 of Animal 1.

Aberrant distended loops (false positives) have been reported by numerous authors while utilizing the ligated loop technique in rabbits and pigs (Taylor *et al.*, 1958; De and Ghose, 1959; Moon *et al.*, 1966; Moon and Whipp, 1971).

Mechanisms by which these false positive loops may arise have also been suggested. De and Ghose (1959) attributed their occurrence to rapid injection or large volumes of the media placed within the ligated loop. Both of these points are inapplicable to our experimental system, and our findings tend to support the similar experience of Moon *et al.* (1966). Spontaneous infection of the sacs was thought to result in distention of interloops or sacs inoculated with known non-enteropathogens by Taylor *et al.* (1958) and Moon *et al.* (1966). Moon and Whipp (1971) noted that false positive loops were limited to the caudal sacs of the ileal segment in rabbits, and they implicated the surgical technique and/or the normal secretory activity of this intestinal segment as the underlying cause of aberrant loop distention. Spontaneous infection was ruled out by these workers in that false positive loops also occurred in sacs not inoculated with *E. coli*, as well as in loops treated with specific antibiotics. In our experimental design, the placement of the loops within the ileal segment of the small intestine was avoided by positioning the initial, most caudal, ligature approximately 1 meter cranial to the ileocecal junction. Confirmation of this distance was made following euthanasia and aided in establishing the spatial relationship of the first sac to the ileocecal junction. In Animal 1, the first loop was located 124 cm cranial to the ileocecal valve. The distance in Animal 22 was 105 cm for the first ligature and, therefore, Sac 3 would have been no closer than 145 cm to the beginning of the spiral colon. Additional evidence failing to support the surgical/physiological theory of Moon and Whipp (1971) is that, of the over 200 loops made throughout the trials, only 1 of these sacs became falsely distended. This loop was inoculated with the Strain 115 that also resulted in moderate to severe

histopathologic alterations in an additional loop as well. False positive loops were not observed in any other sacs, whether from experimental or control animals. Enterosorption, which occurred in a few interloops from experimental animals and was attributed to leakage from adjacent significantly distended loops, was not considered to fit the definition of a false positive reaction. Therefore, the dual implication of Strain 115 as a potential enteropathogen appears to be inconsistent with the hypothesis of Moon and Whipp (1971) concerning their conclusions from the data obtained in rabbits and pigs.

The leakage of toxins between loops must also be considered. The distention pattern for each of the 2 animals assists in delineating this effect, and is listed in Table 8. No interloop distentions occurred between the 115 inoculated loop and the next significantly distended loop, going in either direction. This information would apparently eliminate the possible effect of distention due to enterotoxin leakage.

Table 8. Loop distention patterns for animals harboring false positive loops

| | Sac location | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal | Ileum —— | 1 | **I-1 | 2 | I-2 | 3 | I-3 | 4 | I-4 | 5 | I-5 | 6 —— Duodenum |
| 1 | | -* | - | + | - | + | - | + | - | - | - | - |
| 22 | | + | - | - | - | +* | - | + | - | + | - | - |

*Sac receiving Strain 115.

**I = interloop.

Therefore, the most probable explanation for such false positive loop appearances relates to the subject of spontaneous infection. Spontaneous infection, in this context, as it applies to conventional animals, is interpreted to mean the activation of potential enteropathogens present in the "normal" flora of the intestinal tracts of animals utilized for the ligated loop technique. These enteropathogens have remained in a quiescent or nonenteropathogenic state for unknown reasons. The conditions under which the expression of such potential enteropathogenicity is realized must somehow be satisfied, on occasion, by the ligation of the intestine into isolated loops. Such a procedure would tend to negate the effects of peristalsis, the flushing of the tract in general, and most probably other ill-defined physiologic alterations. This appears to be supported by the work of Moon and Whipp (1970) in which the Class III (nonenteropathogenic) strains of *E. coli* resulted in 6 false positive distentions out of 302 loops tested, or approximately 2% incidence. When examined separately by strain, the false positive rates for Strains 123 and 115 were 1.9 and 2.4%, respectively. Frequency of false positive reactions in interloops was not available either from their work or from the experiments of others. Our experiments demonstrated that Strain 115 caused false positive reactions at a rate of 13.3%. If, however, reactivity of the serotypes were judged strictly on gross distentions alone, as occurred in most of the cited work with the exception of Moon *et al.* (1966), the rate for this strain would be reduced to 6.7%. Although considerably higher, this latter figure compares more favorably with the report of Moon and Whipp (1970).

Strain 115 has an interesting history concerning its enteropathogenicity. When initially utilized by Moon *et al.* (1966), the organism

caused a positive loop reaction rate of 96%. By 1970 Moon and Whipp reported that the rate had fallen to 2.4%, and they characterized the organism as belonging to the Class III, nonenteropathogenic, group.

Our experiences support this reclassification of Strain 115. However, it appears that, especially in the germfree pig system, the occasional expression of an enteropathogenic trait in a nonenteropathogen may occur. Therefore Strain 115, and probably additional nonenteropathogens, might be considered to be rarely but conditionally enteropathogenic. These strains might then account for the occurrence of a false positive event in ligated loops.

The incidence of false positive loops was lower in our work when compared to reports in conventional pigs. Thus an advantage is realized when utilizing the gnotobiote in this testing procedure. However, it may be generally concluded that the germfree intestine appears to be less sensitive to the technique.

## SUMMARY

The ligated loop technique was evaluated in 23 gnotobiotic pigs, 3 to 4 weeks of age, in an attempt to define the nature of false positive loops. Five serotypes, encompassing 6 strains of *Escherichia coli*, were utilized in this experiment. The 6 strains were divided into 3 subgroups: 1) strains enteropathogenic for pigs regardless of age, 2) strains enteropathogenic for pigs under 2 weeks of age, and nonenteropathogenic for pigs over 6 weeks of age, and 3) nonenteropathogenic strains. Each experimental loop was inoculated with a single strain, noninjected interloops separated each inoculated loop, and all 6 strains were tested in each of 15 experimental animals.

Strain enteropathogenicity in gnotobiotic pigs, based upon significant visual loop distention, generally compared favorably with results obtained utilizing the same technique and strains of *E. coli* in conventional animals with the exception that the gnotobiotic jejunal loop may be somewhat less sensitive to certain strains. In addition, postoperative mortality rates among gnotobiotic pigs were higher than reported in conventional pigs.

Light microscopic intestinal lesions in these gnotobiotic pigs were similar to those reported in the conventional pig. Mucosal erosions and ulcerations were often seen in maximally distended loops.

Two false positive loops occurred during the experiment. Both were in sacs inoculated with Strain 115. False positive loops were

not seen in other sacs, whether inoculated or not. Results of these experiments support the hypothesis that false positive loops in conventional pigs arise from spontaneous infection.

# BIBLIOGRAPHY

BIBLIOGRAPHY

Alexander, T. J. L., Miniats, O. P., Ingram, D. G., Thompson, R. G., and Thackeray, E. L.: Gnotobiotic pigs: Procurement, microbial flora, serum porteins and lymphatic tissues. Can. Vet. J., 10, (1969): 98-105.

Arbuckle, J. B. R.: The distribution of certain *Escherichia coli* strains in pigs and their environment. Brit. Vet. J., 124, (1968): 152-159.

Barnum, D. A.: The control of neonatal colibacillosis of swine. Ann. N. Y. Acad. Sci., 176, (1971): 385-400.

Barnum, D. A., Glantz, P. J., and Moon, H. W.: Colibacillosis. Ciba Veterinary Monograph Series. Ciba Foundation, Summit, N.J., 1967.

Burrows, W., and Musteikis, G. M.: Cholera infection and toxin in the rabbit ileal loop. J. Inf. Dis., 116, (1966): 183-190.

Christie, B.R.: *Experimental Colibacillosis in Gnotobiotic Pigs.* M.S. Thesis, Michigan State University, East Lansing, Michigan, 1967.

Cross, R. F., and Kohler, E. M.: Autolytic changes in the digestive system of germfree, *Escherichia coli* monocontaminated and conventional baby pigs. Can. J. Comp. Med. Vet., 33, (1969): 108-112.

Curtis, J., and Bourne, F. J.: Immunoglobulin quantitation in sow serum, colostrum and milk and the serum of young pigs. Biochim. et Biophys. Acta, 236, (1971): 319-332.

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

De, S. N., and Chatterje, D. N.: An experimental study of the mechanism of action of *Vibrio cholerae* on the intestinal mucous membrane. J. Path. Bact., 66, (1953): 559-562.

De, S. N., and Ghose, M. L.: False reaction in ligated loop of rabbit intestine. Indian J. Path. Bact., 2, (1959): 121-128.

Drees, D. T., and Waxler, G. L.: Enteric colibacillosis in gnotobiotic swine: A fluorescence microscopic study. Am. J. Vet. Res., 31, (1970): 1147-1157.

Dubos, R.: The microbiota of the gastrointestinal tract. Gastroenterology, 51, (1966): 868-874.

Dunne, H. W., and Bennett, P. C.: Colibacillosis and edema disease, in *Diseases of Swine*, 3rd ed., H. W. Dunne, ed. The Iowa State University Press, Ames, Iowa, (1970).

Fresh, J. W., Versage, P. M., and Reyes, V.: Intestinal morphology in human and experimental cholera. Archives of Pathology, 77, (1964): 529-537.

Gilka, F.: A comparative study on the pathological picture of transmissible gastroenteritis (TGE) and *E. coli* gastritis in newborn piglets with special reference to the gastrointestinal tract. Acta Vet. Acad. Sci. Hung. Tomus, 18, (1968): 39-50. Cited by Leman, A. D., ed.: *Swine Health: Common Diseases Affecting Baby Pigs-A Research Review.* College of Veterinary Medicine, University of Illinois, Urbana-Champaign, Illinois, (1970): 24-25.

Grun, E., Gurtler, H., and Abadjieff, W.: Studies on pigs. XXVIII. Clinical, hematological and biochemical studies after injection of a freeze thawed extract of beta hemolytic *E. coli*. Arch. Exp. Vet. Med., 21, (1967): 1517-1530. Cited by Leman, A. D., ed.: *Swine Health: Common Diseases Affecting Baby Pigs-A Research Review.* College of Veterinary Medicine, University of Illinois, Urbana-Champaign, Illinois, (1970): 25.

Gyles, C. L., and Barnum, D. A.: *E. coli* in ligated segments of pig intestine. J. Path. Bact., 94, (1967): 189-194.

Kenworthy, R.: Effect of *Escherichia coli* on germ-free and gnotobiotic pigs. I. Light and electron microscopy of the small intestine. J. Comp. Path., 80, (1970): 53-63.

Kenworthy, R.,and Allen, W. D.: The significance of *Escherichia coli* to the young pig. J. Comp. Path. Therap., 76, (1966a): 31-44.

Kenworthy, R., and Allen, W. D.: Influence of diet and bacteria on small intestinal morphology, with special reference to early weaning and *Escherichia coli*. J. Comp. Path., 76, (1966b): 291-296.

Kim, Y. B., Bradley, S. G., and Watson, D. W.: Ontogeny of the immune response. I. Development of immunoglobulins in germfree and conventional colostrum-deprived piglets. J. Immunol., 97, (1966): 52-63.

Kimberg, D. V., Field, M., Johnson, J., Henderson, A., and Gershaw, I.: Stimulation of intestinal mucosal adenyl cyclase by cholera enterotoxin and prostaglandins. J. Clin. Invest., 50, (1971): 1218-1230.

Kohler, E. M.: Studies of *Escherichia coli* in gnotobiotic pigs. V. Evaluation of the effects of oral and parenteral administration of immune serum. Can. J. Comp. Med., 31, (1967): 283-289.

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

Kohler, E. M.: Studies on the effects of immune serum on the course of *E. coli* infections in gnotobiotic pigs, in *Germ-Free Biology*. Plenum Press, New York, N.Y., (1969): 219-225.

Kohler, E. M.: Pathogenesis of neonatal enteric colibacillosis of pigs. J.A.V.M.A., 1960, (1972): 574-582.

Kohler, E. M., and Bohl, E. H.: Studies of *Escherichia coli* in gnotobiotic pigs. I. Experimental reproduction of colibacillosis. Can. J. Comp. Med. Vet., 30, (1966): 1294-1297.

Kohler, E. M., and Cross, R. F.: Studies of *Escherichia coli* in gnotobiotic pigs. IV. Effects of feeding bacteria free filtrates of broth cultures. Can. J. Comp. Med. Vet., 33, (1969): 173-177.

Kramer, T. T., and Nderito, P. C.: Experimental *Escherichia coli* diarrhea in hysterectomy-derived, one-day-old fasting pigs. Am. J. Vet. Res., 28, (1967): 959-964.

Leman, A. D., ed.: *Swine Health: Common Diseases Affecting Baby Pigs-A Research Review*. College of Veterinary Medicine, University of Illinois, Urbana-Champaign, Illinois, (1970): 18-33.

Meyer, R. C., Bohl, E. H., and Kohler, E. M.: Procurement and maintenance of germ-free swine for microbiological investigations. Appl. Microbiol., 12, (1964): 295-300.

Miniats, O. P., Mitchell, L., and Barnum, D. A.: Response of gnotobiotic pigs to *Escherichia coli*. Can. J. Comp. Med., 34, (1970): 269-276.

Moon, H. W.: Personal communication, (1970).

Moon, H. W., Nielsen, N. O., and Kramer, T. T.: Experimental enteric colibacillosis of the newborn pig: Histopathology of the small intestine and changes in plasma electrolytes. Am. J. Vet. Res., 31, (1970): 103-112.

Moon, H. W., Sorensen, D. K., and Sautter, J. H.: *Escherichia coli* infection of the ligated intestinal loop of the newborn pig. Am. J. Vet. Res., 27, (1966): 1317-1325.

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

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

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

Muylle, E. and Oyaert, W.: Effect of coli endotoxin on some blood constituents of the pig. Zentralbl. Veterinaermed., 13, (1965): 405-409. Cited by Leman, A. D., ed.: *Swine Health: Common Diseases Affecting Baby Pigs-A Research Review*. College of Veterinary Medicine, University of Illinois, Urbana-Champaign, Illinois, (1970): 25.

Namioka, S., and Murata, M.: Studies on the pathogenicity of *Escherichia coli*. II. The effect of a substance obtained from pseudoeosinophils of rabbits on the organism. Cornell Vet., 52, (1962): 289-296.

Nielsen, N. O., and Sautter, J. H.: Infection of ligated intestinal loops with hemolytic *Escherichia coli* in the pig. Can. Vet. J., 9, (1968): 90-97.

Ørskov, F., Ørskov, I., Jann, B., and Jann, K.: added at proof stage. Acta Path. Microbiol. Scand., 79B, (1971): 142-152. Cited by Sojka, W. J.: Enteric diseases in the newborn piglets, calves and lambs due to *Escherichia coli* infection. Vet. Bulletin, 41, (1971): 509-522.

Pierce, N. F., Greenough, W. B., and Carpenter, C. C. J.: *Vibrio cholerae* enterotoxin and its mode of action. Bact. Rev., 35, (1971): 1-13.

Porter, P.: Porcine colostral IgA and IgM antibodies to *Escherichia coli* and their intestinal absorption by the neonatal pig. Immunology, 17, (1969a): 617-626.

Porter, P.: Transfer of immunoglobulins IgG, IgA and IgM to lacteal secretions in the parturient sow and their absorption by the neonatal piglet. Biochim. et Biophys. Acta, 181, (1969b): 381-392.

Saunders, C. N., Stevens, A. J., Spence, J. B., and Betts, A. O.: *Escherichia coli* infection: Reproduction of the disease in 'pathogen free' piglets. Res. Vet. Sci., 4, (1963): 347-357.

Saunders, C. N., Stevens, A. J., Spence, J. B., and Sojka, W. J.: *Escherichia coli* infections in piglets. Res. Vet. Sci., 1, (1960): 28-35.

Shafer, D. E., Lust, W. D., Sercor, B., and Goldberg, N. O.: Elevation of adenosine 3'5' cyclic monophosphate concentration in intestinal mucosa after treatment with cholera toxin. Proc. Nat. Acad. Sci., 67, (1970): 850-855.

Sharp, G. W. G., and Hynie, S.: Stimulation of intestinal adenyl cyclase by cholera toxin. Nature, 229, (1971): 266-269.

Smith, H. W., and Gyles, C. L.: *Escherichia coli* enterotoxin. Vet. Rec., 85, (1969): 694.

Smith, H. W., and Gyles, C. L.: The relationship between two apparently different enterotoxins produced by enteropathogenic strains of *Escherichia coli* of porcine origin. J. Med. Microbiol., 3, (1970): 387-401.

Smith, H. W., and Halls, S.: Observations by the ligated intestinal segment and oral inoculation methods on *Escherichia coli* infections in pigs, calves, lambs and rabbits. J. Path. Bact., 93, (1967a): 499-529.

Smith, H. W., and Halls, S.: Studies on *Escherichia coli* enterotoxin. J. Path. Bact., 93, (1967b): 531-543.

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

Smith, H. W., and Jones, J. E. T.: Observations on the alimentary tract and its bacterial flora in healthy and diseased pigs. J. Path. Bact., 86, (1963): 387-412.

Sojka, W. J.: *Escherichia coli* in domestic animals and poultry. Review Series No. 7, Commonwealth Bureau of Animal Health, London, (1965): 104-156.

Sojka, W. J.: Enteric diseases in newborn piglets, calves and lambs due to *Escherichia coli* infection. Vet. Bull., 41, (1971): 509-522.

Staley, T. E., Jones, E. W., and Corley, L. D.: Attachment and penetration of *Escherichia coli* into epithelium of the ileum in newborn pigs. Am. J. Path., 56, (1969): 371-392.

Staley, T. E., Jones, E. W., and Marshall, A. E.: The jejunal absorptive cell of the newborn pig. An electron microscopic study. Anat. Rec., 161, (1968): 497-515.

Stevens, A. J.: Coliform infections in the young pig and a practical approach to the control of enteritis. Vet. Res., 75, (1963a): 1241-1245.

Stevens, A. J.: Enteritis in pigs-A working hypothesis. Brit. Vet. J., 119, (1963b): 520-525.

Taylor, J., Maltby, M. P., and Payne, J. M.: Factors influencing the response of ligated rabbit-gut segments to injected *Escherichia coli*. J. Path. Bact., 76, (1958): 491-499.

Taylor, J., Walkins, M. P., and Payne, J. M.: Relation of rabbit-gut reaction to enteropathogenic *Escherichia coli*. Brit. J. Exptl. Path., 42, (1961): 43-52.

Waxler, G. L., Christie, B. R., and Drees, D. T.: Use of the gnotobiotic pig in the study of *Escherichia coli* infection. Ann. N. Y. Acad. Sci., 176, (1971): 141-149.

Waxler, G. L., Schmidt, D. A., and Whitehair, C. K.: Technique for rearing gnotobiotic pigs. Am. J. Vet. Res., 27, (1966): 300-307.

White, F., Wenham, G., Shorman, G. A. M., Jones, A. S., Rattray, E. A., and McDonald, J.: Stomach function in relation to a scour syndrome in the piglet. Brit. J. Nutr., 23, (1969): 847-862.

Wilson, M. R.: Role of immunity in the control of neonatal colibacillosis in pigs. J.A.V.M.A., 160, (1972): 585-590.

Wilson, M. R., and Svendsen, J.: Immunity to *Escherichia coli* in pigs. The role of milk in protective immunity to *E. coli* enteritis. Can. J. Comp. Med., 35, (1971): 239-243.

# VITA

The author was born on September 2, 1942, in Ashland, Ohio. His primary and secondary education was completed in Michigan, graduating from Farmington High School in 1960.

The author entered Michigan State University in September of 1960 and was graduated in 1966 with the degree of Doctor of Veterinary Medicine. Shortly thereafter he was married to Anna Lottie Sollenberger, and entered the U.S. Army, being stationed in the Military District of Washington. In August of 1968 he entered private practice in Southfield, Michigan, in association with Dr. D. L. Young.

In August of 1969 the author returned to Michigan State University and entered the Master's degree program of the Department of Pathology, accepting a teaching assistantship. In September 1970 he entered the employment of Dr. Barnett Rosenberg, Department of Biophysics, Michigan State University, to begin research into the potential antiviral properties of various platinum coordination complexes.

The author is a member of the A.V.M.A., M.V.M.A., Phi Zeta, and Sigma Xi.