1971

Studies on the etiology, hematology, and pathology of swine dysentery

Robert Dean Glock
Iowa State University

Studies on the etiology, hematology, and pathology of swine dysentery

by

Robert Dean Glock

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Veterinary Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1971

TABLE OF CONTENTS

INTRODUCTION

Swine dysentery (bloody scours, black scours, bloody diarrhea, vibrionic dysentery) is an acute to chronic disease of swine characterized by mucohemorrhagic diarrhea.

The original report of the disease (Whiting et al. 1921) included a complete description of clinical signs which included diarrhea (often with blood and mucus in the feces), dehydration, and frequently death. Mucohemorrhagic enteritis was consistently present in all or a portion of the large intestine. An adherent pseudomembrane often covered the swollen mucosa, and intestinal contents were soft to watery.

The disease has been reported in swine raising areas of many countries (Sorensen, 1970a). Economic loss is substantial but is difficult to determine exactly because of a lack of uniform methods of reporting incidence and losses. The number of herds quarantined in Iowa, where swine dysentery is reportable, was 271 in 1968 and 159 in 1969 (Harris and Glock, 1971). These figures probably represent only a small percentage of actual outbreaks due to reluctance of producers and veterinarians to report infected herds because of the inconvenience and stigma of quarantine. In 1960, 16 percent of the swine in Canada were reported to be affected with swine dysentery (Lussier, 1962).

Swine dysentery remains a major problem for the swine industry because of the lack of reliable methods for preventing the disease. Present control measures are rather empirical and reflect the fact that the etiology has never been definitely established. James (1947) and

Warner (1965) have suggested that Vibrio coli is the sole causative agent; however, other studies have refuted this assumption (Andress et al. 1968; Terpstra et al. 1968). Spirochetes have been associated with the lesions (Vallejo, 1969; Taylor and Blakemore, 1971) although their significance has not been adequately evaluated.

It is axiomatic that the determination of the etiology of swine dysentery would lead to a more definitive method of diagnosis of active and carrier cases. A practical approach to studies of biological and pharmacological control measures might also follow.

Little has been published on the subject of hematologic changes in swine dysentery although some parameters have been explored (Ruth, 1967; Sorensen, 1970a).

This study was undertaken to gain information on the pathogenesis of swine dysentery. Pigs were inoculated orally with colon contents and mucosal scrapings from infected pigs. Two major objectives were to characterize the sequential development of lesions in the large intestine with emphasis on certain associated microorganisms, and to determine specific hematologic changes.

In order to accomplish the first objective three trials were conducted in which gross, microscopic, and ultrastructural changes were examined at various stages in the disease. Phase, light, and electron microscopy were utilized to determine relative numbers of certain organisms in the large intestine with emphasis on spirochetes. The second objective (determination of hematologic changes) was accomplished by daily analysis of blood samples from three groups of inoculated pigs.

REVIEW OF LITERATURE

Swine dysentery was first reported in Indiana in 1921 (Whiting et al.) and was described as an acute to chronic diarrheal disease characterized by mucohemorrhagic enteritis involving the large intestine. Although occurring at various ages, it most commonly affected feeder pigs. The disease had been present in Indiana and probably in other swine producing areas for several years prior to this report.

Clinical Signs

Swine dysentery can occur at almost any age although it has not been reported in pigs under 2 weeks of age. It most frequently occurs in pigs 7-16 weeks old (Lussier, 1962; Alexander and Taylor, 1969) and occasionally in breeding stock (Whiting, 1928). Although some seasonal variation may occur, it has been diagnosed in all months of the year.

The incubation period for swine dysentery is extremely variable; reports range from 2 days (Hofferd, 1936) to 60 days (Doyle, 1939) with an average of about 14 days (Roberts, 1956). Prolonged treatment with preventive levels of arsenicals may extend the incubation period with clinical signs appearing following removal of the drug 3 months after exposure (Olson, 1971).

Lussier (1962) considered the disease to be divided into 4 distinct clinical syndromes: peracute, acute, subacute, and chronic. In the peracute type pigs die with no apparent history of sickness but may show

evidence of diarrhea. The acute form is most common and characteristic of the disease, while the subacute and chronic syndromes may be considered to be merely prolonged manifestations of the same general disease process.

The early clinical signs of the acute syndrome are usually moderate fever, 104 to 105 F., and partial anorexia (Whiting, 1928). Stools are initially yellow and may later become gray (Lussier, 1962; Loveday, 1964; Alexander and Taylor, 1969). After a few hours to several days there are large amounts of mucus and often flecks of blood in the stools. As the diarrhea progresses, watery stools containing blood, mucus, and shreds of white mucoid material are seen with concurrent staining of the rear quarters (Whiting et al. 1921; Lussier, 1962). Relaxation of the anal sphincter may be a factor in the apparent decline in body temperature in the late stages of the disease. Increased thirst is usually noted, and affected animals become gaunt, emaciated, depressed, and weak. The cause of death is assumed to be dehydration and electrolyte loss (Ruth, 1967).

Morbidity is usually about 75 to 80 percent (Lussier, 1962; Loveday, 1964). Mortality is about 25 percent but may be quite variable because of differences in management practices and treatment (Whiting, 1924; Lussier, 1962).

Gross Lesions

Whiting, Doyle, and Spray (1921) observed that the mucosa of the stomach, cecum, colon, and rectum was congested and hemorrhagic in

early stages of swine dysentery and became necrotic in later stages. Carcass emaciation was marked in advanced cases. Whiting (1928) found that diphtheritic colitis usually preceded the development of necrotic lesions. Doyle (1943) noted that the only consistent lesions were found in the large intestine which had a congested, reddened appearance in the early stages of the disease. There was a notable lack of lesions in the small intestine (Doyle, 1945).

Roberts (1956) described a loosely adhered necrotic exudate on the mucosas of the cecums and colons of field cases in Australia. He noted that the cases of swine dysentery that he examined were remarkably similar to those previously reported in the United States.

Deas (1960) reported on swine dysentery in Scotland and noted that the stomach of affected pigs often contained straw and extraneous matter. The small intestine was normal except for fluid, bile-tinged contents. In acute cases the large intestine was flaccid and hyperemic but rarely contained blood although contents were fluid. In less acute cases the mucosa was covered with a yellow diphtheritic exudate which could be easily removed leaving a moist, red, granular surface. The walls of the colon and rectum were thickened, and the mucosa was formed into folds with a necrotic layer at the luminal surface in chronic lesions. Mesenteric lymph nodes were swollen.

Lussier (1962) reported in detail his necropsy findings on 275 pigs from 249 different outbreaks of swine dysentery in Canada. Stomachs of affected pigs usually lacked lesions and were filled with feed or extraneous material while the small intestines were usually

empty and dilated by gas. Livers were sometimes congested. The mesentery and wall of the large intestine were hyperemic and edematous. Enteric lesions progressed from catarrhal to hemorrhagic to necrotic enteritis. In advanced cases pieces of necrotic mucosa and exudate sloughed and mixed with watery intestinal contents giving a typical "rice water" appearance. Erosions were always superficial.

Loveday (1964) reported on an outbreak of swine dysentery in South Africa. Changes in addition to those previously reported included serosal adhesions on the colon and dark-gray colonic contents.

Warner (1965) produced swine dysentery experimentally in 11 pigs either by pen contact with infected animals or by oral inoculation with Vibrio coli cultivated in embryonating eggs. These pigs were killed at various stages in the development of the disease. Stomachs were normal except for one in which the fundic portion was thickened and brilliant red. A few cases had slightly swollen livers with elevated, pale mottled areas. There was catarrhal to hemorrhagic enteritis in the large intestine. Areas of inflammation were often covered with a diphtheritic pseudomembrane. In cases having primarily a catarrhal colitis the colon wall and mesocolon were edematous, and the mucous membrane was hyperemic and had scattered hemorrhages. The lumen contained little ingesta but had large amounts of clear or slightly blood-tinged mucus. The colonic mucosa in some cases was covered with grayish yellow or white diphtheritic exudate.

Microscopic Lesions

The early reports on swine dysentery by Whiting et al. (1921) and Whiting (1924, 1928) contained descriptions of microscopic lesions. Little was done to expand these descriptions until almost 30 years later when Roberts (1956), Lussier (1962), and Warner (1965) offered complete descriptions of field and experimental cases.

The gastric lesions described by Whiting et al. (1921) included hyperemia, hemorrhage, desquamation of epithelium, and, finally, necrosis of the mucosa. Early lesions of the colon included engorged vessels and extravasation of blood into the lamina propria near the lumen. There were edema and, in some areas, hemorrhage which tended to cause separation of the epithelium from the mucosa. The crypts contained numerous goblet cells. Exudate on the mucosal surface consisted of mucus, fibrin, bacteria, lymphoid cells, desquamated epithelium, erythrocytes, and neutrophils. Edema and hemorrhage in the submucosa were also noted. Submucosal glands were often dilated and filled with mucus and cellular debris. Lymphoid nodules surrounding these glands were hyperemic or hemorrhagic. Marked leucocytic infiltration of the colon wall was seldom noted. In later stages of the disease the surface of the colonic mucosa was practically devoid of epithelium and was covered with a layer of mucus, cellular debris, and bacteria. The crypts were distended with mucus. Necrosis of the mucosa to a variable depth was evident in chronic cases, and thrombosis of blood vessels was seen in the mucosa and submucosa.

Roberts (1956) described acute enteritis of the large intestine in outbreaks of swine dysentery in Australia. He observed numerous leukocytes and increased numbers of goblet cells in the mucosa. There were numerous mitotic figures in the epithelium of the crypts. In peracute cases necrosis and hemorrhage occurred with little leukocytic infiltration and fewer mitotic figures in the crypt epithelium. Cloudy swelling was present in hepatocytes and renal cortical epithelium.

Lussier (1962) reported his findings concerning 275 pigs involved in field cases of swine dysentery. Although some cases had severe congestion and thrombosis of vessels in the fundic region of the stomach, the majority of the cases did not have microscopic lesions in the stomach. Since similar lesions are encountered in other diseases of swine, Lussier felt that stomach lesions were not specific for swine dysentery. Cloudy swelling was noted in the liver and in renal tubules.

Warner (1965) described the microscopic lesions of experimental swine dysentery. In early or mild cases the epithelium of the colon was intact, but there were increased numbers of leukocytes, many of which were neutrophils, in the mucosa. The lesions in more advanced cases were classified as acute catarrhal inflammation characterized by epithelial desquamation, excessive mucus production, hyperemia, and edema. Involvement was often patchy; altered cells were shrunken and intensely acidophilic with pyknotic nuclei. Epithelial cells of the crypts were packed and in disarray. They had large vesicular nuclei, and the basilar epithelium had increased numbers of mitotic figures. In some cases there was extravasation of large quantities of blood,

often into the lumen.

Changes seen in tissues other than the colon were hyperplasia of lymphoid nodules in the terminal ileum and of the cecal and colonic lymph nodes. Cloudy swelling was noted in hepatocytes and in the convoluted tubules of the kidney.

Etiology

The etiology of swine dysentery must be considered as undetermined at the present time although several possibilities have been suggested.

In the original publication by Whiting et al. (1921) many organisms were mentioned as being associated with the disease. Balantidium coli was frequently observed in lesions but was not considered to be a significant pathogen. Bacillus suipestifer (Salmonella choleraesuis) was isolated from 16 of 23 cases, but when experimental pigs were inoculated with this organism the resulting syndrome was very different from swine dysentery. Attempts at reproduction of the disease with cultures of Bacillus coli (Escherichia coli) and Bacillus necrophorus (Spherophorus necrophorus) also failed as did cultures of protozoa isolated from pigs affected with swine dysentery. Spirochetes were observed in lesions but failed to reproduce the disease when mixed cultures containing these organisms were fed. Comma shaped organisms were seen in sections but were not isolated.

Most authors (Warner, 1965; Powell, 1970) in agreement with Whiting et al. (1921) assumed that Balantidium coli was not a primary pathogen.

Trichomonads and other protozoa have also been eliminated as possible etiologic agents (Gorrie, 1946; Terpstra et al. 1968; Powell, 1970).

Swine dysentery has not been reproduced by bacteria-free filtrates of infectious colon contents (Whiting et al. 1921; Warner, 1965; Terpstra et al. 1968). This suggests that viruses are not etiologic agents although their possible role in a complex etiology has not been adequately investigated.

The role of Salmonella spp. is not clearly defined. Whiting (1924, 1928) was unable to produce swine dysentery with pure cultures of Bacillus suipestifer (Salmonella choleraesuis). He demonstrated that the organism was present only in certain groups of sick hogs and that the pattern of occurrence varied much the same as in hog cholera. Doyle (1945) and Gorrie (1946) believed that salmonella were secondary invaders. Powell (1970) was unable to isolate Salmonella spp. from field cases of swine dysentery. Sweeney (1966) concluded, however, that swine dysentery was merely part of the complex symptomatology of paratyphoid (Salmonellosis) on the basis of S.choleraesuis isolations from rectal swabs from 8 of 48 pigs with dysentery.

The first evidence which suggested Vibrio coli as the sole cause of swine dysentery was provided by Doyle (1944). He observed 2 morphological types of vibrio in smears and stained sections from the colons of affected swine. One type was described as a large, coarse form; the other was a finer form. A vibrio was isolated in "apparently pure culture" from the colonic mucosa of infected hogs by streaking blood agar plates which were incubated in 10 to 15 percent carbon dioxide. These

cultures were fed to 8 pigs, and 6 developed slight to marked diarrhea which was less severe than that of typical cases of swine dysentery. A sequel to this work was reported by James and Doyle (1947) using vibrio cultures as previously described. In their first trial, cultures were fed to 10 pigs with negative results, but in subsequent trials the cultures were mixed with gastric mucin and fed with ground feed. Fifty of 60 pigs infected in this manner developed typical swine dysentery as determined by clinical signs and lesions. The average incubation period was 27 days. The name Vibrio coli was suggested for the agent used in these trials (Doyle, 1948).

Roberts (1956) reported from Australia on transmission experiments using techniques similar to those described by James and Doyle (1947). Of 5 pigs fed vibrio cultures with gastric mucin, 2 developed a severe diarrhea with no blood in the feces, 2 had a mild diarrhea (one with bloody stools), and one remained clinically normal.

Truszczynski (1957) and Lussier (1961) also reported production of diarrhea in limited numbers of pigs exposed to pure cultures of V. coli although the response seemed milder than in typical cases of swine dysentery.

One of the most thorough recent investigations was that of Warner (1965). He inoculated 23 conventional and 14 caesarean-derived pigs with cultures of V. coli grown either on blood agar or in Albimi broth and then mixed with gastric mucin. In no case was typical swine dysentery produced although V. coli did become established in the caesarean-derived pigs. In a subsequent trial, embryonating eggs were inoculated

with a filtrate of infective feces which passed a 0.45 um. filter. These eggs were examined by phase microscopy and subcultured. Only V. coli was observed. When this material was fed to conventionally reared pigs, 8 of 12 developed swine dysentery.

All of the above reports of successful transmission by V. coli provide evidence for its role in the etiology. These results must, however, be considered in the light of numerous other experiments in which cultures of V. coli failed to produce swine dysentery (Boley et al. 1951; Manninger et al. 1960; Deas, 1960). Impressive evidence against the production of swine dysentery with V. coli was provided by Andress et al. (1968). In this study 15 gnotobiotic and 6 conventional pigs were infected orally with 23 strains of V. coli grown on milk media. No clinical signs of swine dysentery were produced in any of the animals although V. coli was readily established in the intestinal tracts of most pigs. Six conventional pigs were also inoculated with fresh gut material from gnotobiotic pigs in which V. coli had been established. Results of this study were also negative.

One of the major problems in transmission studies with V. coli has been the inadequacy of methods for identifying various strains of the organism. Deas (1960) described two biotypes on the basis of colony size, growth rate, and H_2S production. Both biotypes have been utilized in trials with variable results. Attempts at classification of these organisms on the basis of antigenic analysis (Lussier, 1962) revealed a heterogeneity between various isolates by agglutination

tests. DiLiello et al. (1959) assigned 5 V. coli isolates from swine to 3 subgroups on the basis of agglutination tests. Soderlind (1965) showed that some isolates of V. coli cross-reacted with V. fetus antisera in complement fixation tests. No method for definitive antigenic typing of V. coli strains has been proposed.

Warner (1965) suggested that pathogenic strains of V. coli were probably all catalase and H_2S negative. These findings were supported by Powell (1970) who found that the biochemical type of V. coli which was catalase and H_2S negative as well as glycine and nitrate positive was isolated more frequently in pigs with swine dysentery than in other pigs. He suggested that it was unfortunate that Andress et al. (1968) had used strains which were catalase and H_2S positive in their studies with gnotobiotic pigs.

Spirochetes have been observed in large numbers in feces, mucosal scrapings, and sections from pigs with swine dysentery. Whiting et al. (1921) found enormous numbers in feces by dark-field examination. Spirochetes grown in mixed cultures by the Noguchi method in meat-piece serum-water and fed to 2 pigs failed to produce clinical signs during a 12 day observation period.

Warner (1965) noted that 2 major forms of spirochetes were frequently present in colons of infected pigs. One, which he identified as Borrelia hyos, was 1.0 by 5 to 7 μm. with regular, fixed, and spiraled protoplasm. The other, which he identified as Spirochaeta sp., was 0.75 by 25 to 50 μm. He noted the presence of Spirochaeta sp. in filtrates used as inocula but felt that they did not multiply in embryonating eggs which

were fed to produce swine dysentery. He concluded that their increase in numbers following the onset of dysentery merely represented opportunistic growth.

Interest in spirochetes was renewed in 1968 when Terpstra et al. conducted a series of studies in which they were unable to reproduce swine dysentery with cultures containing E. coli, Bacteriodes spp., fusiforms, trichomonads, and V. coli. They began a serologic approach to the problem in which no agglutinating antibodies to V. coli were found in the sera of convalescing pigs. Antisera to V. coli when added to infectious intestinal material failed to reduce infectivity. Gamma globulins from pigs which had recovered from acute dysentery 1.5 months previously were conjugated with fluorescein isothiocyanate. This conjugate regularly labeled only one type of microorganism when applied to fecal smears from infected animals. These organisms could be readily differentiated from Vibrio spp.; their shape was described as longer with wider turns and more pointed ends than Vibrio spp. This morphology suggests that they were spirochetes.

Vallejo (1969) described the presence of numerous spirochetes in intestinal contents of pigs with swine dysentery. These organisms were 0.25 to 0.30 by 7 to 12 μm. in size and very motile. They were Gram-negative and easily stained with aniline dyes, especially carbolfuchsin. Electron microscopy confirmed that they lacked flagella. He suggested that they might be classified as borrelia but felt that they were probably commensals rather than primary pathogens. His observations were subsequently confirmed by others (Roberts and Simmons, 1970; Espinasse and

Redon, 1970). Todd et al. (1970) succeeded in culturing these organisms under anaerobic conditions by placing intestinal contents on a Millipore pad (pore size 0.22 μm.) resting on the surface of agar. They suggested possible classification as Borrelia hyos.

Taylor (1970) observed that more than one type of spirochete was present in feces from both normal and infected pigs. He noted that the type of spirochete described by Vallejo (1969) was present only in infected pigs. Several other types of spirochetes were also described as being present only in infected pigs. One of these which was frequently found was smaller and had more coils.

The presence of spirochetes in the intestines of pigs killed 6 days after experimental infection was noted by Blakemore and Taylor (1970). They used electron microscopy to locate organisms near the epithelium and in some areas between and within cells. A central core 0.25 to 0.3 μm. in diameter was surrounded by 13 fibrils approximately 150 Å in diameter. There was a double membrane around both giving a total diameter of 0.35 to 0.37 μm. They expanded their work in a subsequent paper (Taylor and Blakemore, 1971). Sites for examination by electron microscopy were chosen from the colon where the epithelium was intact but had been damaged. They demonstrated spirochetes within and between epithelial cells which had nuclear swelling, clumping of chromatin, reduction in number of microvilli, swelling of mitochondria, and dilation of the endoplasmic reticulum. Regardless of these findings they felt that absence of spirochetes from normal cells suggested that they were of little significance in the initial development of the disease.

Hematology

There are few reports dealing with the hematologic changes which occur in swine dysentery. Sorensen (1970a) summarized findings in field cases diagnosed at the University of Minnesota Veterinary Diagnostic Laboratory by stating that total leukocyte and differential cell counts were usually within normal ranges although occasional severe cases had leukocytosis and neutrophilia with a left shift. Varying degrees of hemoconcentration and anemia were noted. Decreased blood pH and bicarbonate values indicated an acidosis associated with diarrhea.

A comprehensive study of hematological changes was conducted by Ruth (1967) in a group of 16 pigs inoculated with infectious colon contents. Normal blood values were compared with those obtained from animals just prior to death. There was no noticeable difference in total leukocyte numbers, but large numbers of immature neutrophils were noted in samples from moribund animals. Various hematologic and serum electrolyte data are summarized in Table 1 which compares values from control animals at the beginning and end of the trial and of infected animals at the beginning of the trial and when prostrate. Hemoglobin and hematocrit values were decreased in prostrate animals as were blood pH and bicarbonate. Serum sodium and chloride decreased while potassium and phosphorus increased. Ruth stated that acidosis probably resulted from loss of extracellular sodium to the intestinal contents and from respiratory depression. He suggested the possibility that emigration of potassium and phosphorus from the cells was caused by acidosis and partial anoxia resulting from decreased extracellular fluid volume.

Table 1. Hematologic and serum electrolyte data (experimental swine dysentery)[a]

	Experimental (Beginning)	Control (Beginning)	Experimental (Prostrate)	Control (Final)
Hemoglobin (Gm./100ml.)	11.29	12.07	9.50	11.15
Hematocrit (Vol. %)	38.1	40.9	33.8	37.2
Blood pH	7.33	7.24	6.91	7.06
Bicarbonate (mEq./L.)	29.1	30.6	14.6	35.0
Sodium (mEq./L.)	146.5	153.4	117.5	152.5
Potassium (mEq./L.)	5.10	5.48	10.41	7.13
Calcium (mEq./L.)	5.51	5.97	6.22	6.30
Magnesium (mEq./L.)	3.11	2.36	4.23	3.56
Chloride (mEq./L.)	104.2	102.3	81.9	100.0
Phosphorus (mEq./L.)	3.56	3.91	10.76	6.47

[a]Modified from Ruth (1967).

MATERIALS AND METHODS

Experimental Animals

All pigs were of mixed breeding. Those in Trial 1 were from a closed herd originally stocked with caesarean-derived pigs and weighed 25 to 30 pounds at the beginning of the trial. The pigs used in the rest of the trials were obtained from a herd of conventionally reared swine with no history of swine dysentery; they weighed from 25 to 35 pounds. Diagram 1 is an outline of pigs used and the general plan of study.

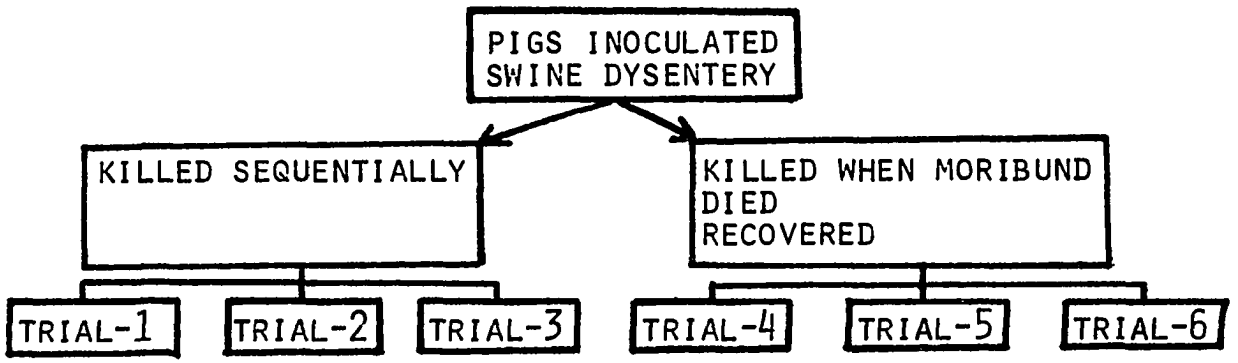
All animals were housed in isolation units at the Veterinary Medical Research Institute at Iowa State University with principals in one unit and controls in another. Feed consisted of a 16 percent protein grower ration which was free of arsenicals, antibiotics, and other drug additives. Water was provided ad lib. by automatic waterers.

Inoculum Preparation and Administration

The inoculum was produced by infecting experimental pigs with colon contents and mucosal scrapings from field cases of swine dysentery. Criteria used for selection of animals from which inoculum was obtained included clinical signs, gross and microscopic lesions, and large numbers of Vibrio coli and spirochetes as identified by phase microscopy of mucosal scrapings from the colon. Using aseptic technique, the colons were removed and separated from the mesentery. Then contents were

Diagram 1. Outline of the general plan of study

DIAGRAM 1



	TRIAL-1	TRIAL-2	TRIAL-3	TRIAL-4	TRIAL-5	TRIAL-6
NO. PRINCIPALS	11	10	5	7	6	6
NO. CONTROLS	3	5	3	0	3	2
CLINICAL OBSERVATION GROSS OBSERVATION LIGHT MICROSCOPY	+	+	+	+	+	+
PHASE MICROSCOPY	0	+	+	+	+	+
ELECTRON MICROSCOPY	+	+	+	+	0	0
BLOOD CELL CHANGES	0	0	0	+	+	+
BLOOD CHEMISTRY	0	0	0	+	+	+
BLOOD GAS ANALYSES	0	0	0	0	0	+

stripped into a beaker, and the colon was opened longitudinally. The mucosa was loosened by scraping with a glass slide, placed in a mortar, and ground with a pestle. Phosphate-buffered saline, pH 7.4, (PBS) was added to make a thick suspension. The colon contents and mucosal scrapings were mixed together. This suspension (crude gut inoculum) was examined by phase microscopy to assure that large numbers of motile vibrio and large spirochetes were present. Cultures from all inocula were positive for Vibrio coli and negative for Salmonella spp.

Material from field cases was passaged at least 2 times in experimental pigs before use in these experiments. Infected pigs were killed when they were in the early stages of acute swine dysentery and their colons were used to prepare inoculum for each of the 6 trials.

Feed was withheld from principals and controls for 24 hours prior to inoculation. From 20 to 30 ml. of the crude gut inoculum was administered orally to the principals within 4 hours of its preparation.

Clinical Observations

In all trials pigs were held in isolation units for at least 3 days prior to inoculation to allow them to become accustomed to new feed and surroundings. Rectal temperatures were taken at the same time each day. The general appearance of the pigs, consistency of the feces, and composition of the feces were recorded.

Necropsy Procedures

Pigs which did not die naturally during experiments, or which were moribund, were stunned by electrocution and exsanguinated. All major organs were examined for gross lesions, and specimens were placed in 10 percent buffered formalin for histologic study. One cm. sections of cecum and colon were placed in 1.4 percent glutaraldehyde and 10 cm. long sections in sterile plastic bags for electron microscopy and phase microscopy respectively.

Light Microscopy

Tissues were fixed in 10 percent buffered formalin for at least 48 hours, dehydrated in graded ethanols, cleared in chloroform, and embedded in paraffin. Tissues listed in Table 2 were sectioned and stained with hematoxylin-eosin. Some tissues were also stained with the McCallum-Goodpasture and Warthin-Starry methods (Armed Forces Institute of Pathology, 1968). These stains were especially useful for identification of spirochetes. The following scale was used to tabulate numbers of large spirochetes per oil immersion field with the realization that the techniques used provided only a subjective estimate of average numbers in the section: + = 1-10; ++ = 11-25; +++ = 26 or more.

Table 2. Summary of tissues collected for light microscopy

Tissue	Trial Number 1	2	3	4	5	6
liver	+	+	+	+	+	+
kidney	-	+	+	-	+	+
spleen	+	+	+	-	+	+
adrenal gland	-	+	-	-	+	-
colonic lymph node	+	+	+	+	+	-
stomach	+	+	+	+	+	+
jejunum	+	+	-	-	+	+
ileum	+	+	+	+	+	+
cecum	+	+	+	+	+	+
colon 1[a]	+	+	+	+	+	+
colon 2[b]	+	+	+	+	+	+
colon 3[c]	+	+	+	+	+	-
rectum	+	+	+	-	-	+
spinal cord (lumbar)	-	-	+	-	-	-
sciatic nerve	-	-	+	-	-	-

[a]Colon 1 - proximal one-third of colon.

[b]Colon 2 - apex of colon.

[c]Colon 3 - distal one-third of colon.

Phase Microscopy

Sections of intestine were opened longitudinally and gently rinsed under running tap water to remove ingesta. The corner of a microscope slide was used to make a deep mucosal scraping which was suspended in a drop of PBS on a sterile microscope slide. A cover slip was applied, and the suspension was examined under oil immersion at a magnification of 970x with a phase microscope.

Certain organisms were recognized on the basis of morphology and motility. Vibrio were identified as short, rapidly motile,

comma-shaped rods. Short, thick, motile spiral shaped organisms were identified as Spirilla. Fusiform rods were large and straight with tapered ends. Spirochetes were classified as small and large. Small spirochetes were usually 3 to 5 μm. long and had a tightly coiled spiral shape while the large spirochetes were 5 to 10 μm. long and more loosely coiled. Motility was much more pronounced in the large spirochetes and appeared to result from flexion of the organism. The following scale was used to tabulate numbers of organisms per oil immersion field with the realization that the the techniques used provided only a subjective approximation of actual numbers: + = 1-5; ++ = 6-20; +++ = 21 or more.

Electron Microscopy

On Trials 1 through 4 sections of colon and cecum approximately 1 cm. square were removed as rapidly as possible after death. They were placed in 1.4 percent glutaraldehyde in 0.1 molar sodium cacodylate buffer at pH 7.4 for approximately 6 hours. They were then rinsed in 0.1 M. sodium cacodylate buffer at pH 7.4 containing 9.2 percent sucrose. The serosa and tunica muscularis were trimmed away, and approximately 1 mm. square sections of the tunica mucosa were placed in a second rinse where they were held at 4 C. until further processing could be accomplished. Before dehydration, all tissues were post-fixed in sodium cacodylate-buffered osmium tetroxide at pH 7.4. After a quick rinse in distilled water the tissues were dehydrated by passage through graded

ethanol solutions of 50, 70, 95, and 100 percent. Ethanol was then replaced with propylene oxide, and the tissues were embedded in epon. The epon mixture was prepared as follows:

Mixture A

25.0 ml. Dodecenylsuccinic Anhydride
15.5 ml. Epon Resin 812[a]

Mixture B

19.6 ml. Nadic Methyl Anhydride
22.0 ml. Epon Resin 812

Final Mixture

Combine Mixture A and Mixture B
Add 1.6 ml. DMP-30

Sections 1 to 2 μm. thick were cut using glass knives and an LKB 4801A Ultrotome[b]. After mounting on glass slides at 60 C. the sections were stained with Paragon Multiple Stain[c] for 90 seconds and washed with tap water. Cover slips were mounted with Permount. After selection of specific areas to be examined, blocks were trimmed to give a face approximately 0.5 mm. square. Ultrathin sections were cut at approximately 500 Å with an LKB 4801A Ultrotome using diamond knives and were mounted on copper grids.

Bacteria were negatively stained by placing a drop of a bacteria-PBS

[a]Fisher Scientific Co., 711 Forbes Avenue, Pittsburgh, Pennsylvania.

[b]LKB Instruments, Inc., 12221 Parklawn Drive, Rockville, Maryland.

[c]Paragon C. & C. Co. Inc., 190 Willow Avenue, Bronx, New York.

suspension on a copper grid coated with Parlodion[a] and carbon. After 30 to 60 seconds the excess was absorbed with filter paper and the grids were rinsed with a drop of sterile distilled water. A drop of 2.5 percent phosphotungstic acid stain, pH 6.8, (PTA) was placed on the grid for 30 to 60 seconds, the excess absorbed with filter paper, and the grid allowed to dry.

Ultrathin sections and negatively stained preparations were examined with a Hitachi HU-11A electron microscope at 50 Kv.

Bacteriology

One Gm. of inoculum for each trial and 1 Gm. of colon contents from each pig on which necropsy was performed were placed in tetrathionate broth media[b] and incubated for 24 hours at 37 C. Then 0.1 ml. was streaked on brilliant green agar[b] and incubated at 37 C. Swabs from each specimen were also streaked on Turgitol-7 agar[b]. Colonies resembling Salmonella spp. were selected for biochemical tests and serologic typing.

Vibrio coli were isolated from all inocula according to the method of Kronlund and Kendrick (1969).

Commensal cultures of Vibrio coli and large spirochetes were grown by placing a drop of mucosal-scraping suspension on a cellulose-acetate membrane (average pore diameter of 0.22 μm.) which was resting

[a]Mallinckrodt Chem. Works, St. Louis, Missouri.

[b]Difco Laboratories Inc., Detroit, Michigan.

on the surface of bovine-blood agar (Taylor, 1970). Incubation was at 37 C. under anaerobic conditions in a GasPak Anaerobe Jar[a].

Isolation of large and small spirochetes from filtrates of mucosal scraping suspensions was accomplished by the method described by Harris et al. (1972).

Hematologic Procedures

Blood sample collection

Blood samples were collected at approximately the same time each morning by the vena caval method (Carle and Dewhirst, 1942). The potassium salt of ethylenediamine-tetracetic acid (EDTA) was used as an anticoagulant. Serum samples were obtained by allowing blood to clot at room temperature for 2 to 3 hours. Serum was removed after centrifugation and recentrifuged to remove any remaining cells.

Whole blood for blood gas analysis was drawn into plastic syringes which had been flushed with a heparin solution (3,000 units per ml.). The needles were sealed with rubber stoppers and the syringes were immersed in ice water until determinations could be made within 1 to 2 hours.

Blood cell counts

Erythrocyte (RBC) and leukocyte (WBC) counts were determined with the aid of a Coulter electronic cell counter[b]. Differential leukocyte

[a]BBL, Division of BioQuest, Cockeysville, Maryland.

[b]Coulter Electronics, Inc., Hialeah, Florida.

counts were made on smears stained with Wright's blood stain, and absolute numbers were calculated.

Packed cell volume

Packed Cell Volume (PCV) was determined by the micro-hematocrit method using capillary tubes. The tubes were heat-sealed and centrifuged at 15,000 r.p.m. for 3 minutes. The percent of packed red blood cells was determined.

Erythrocyte sedimentation rate

The erythrocyte sedimentation rate (ESR) was determined by the Wintrobe method (Wintrobe and Landsberg, 1935) within 2 hours after blood samples were obtained. Blood was thoroughly mixed and drawn into a pipette which was inserted to the bottom of a disposable glass Wintrobe tube[a]. Blood was gradually expelled keeping the tip of the pipette near the surface of the blood. The tubes were placed vertically, and the rate of sedimentation in mm. was recorded at the end of 1 and 2 hours.

Plasma protein studies

Total plasma protein (TP) was determined with a direct reading refractometer[b]. Fibrinogen levels were found by centrifuging whole blood in capillary tubes to obtain plasma which was then drawn into a second capillary tube. After heating the plasma at 58 C. for 5 minutes

[a]Clay Adams Division of Becton Dickinson Co., Parsippany, New Jersey.

[b]TS-meter, AO Instrument Co., Buffalo, New York.

the sample was centrifuged, and the amount of remaining protein was again determined with a refractometer. The difference between readings of unheated and heated plasma was calculated as fibrinogen (Schalm et al. 1970).

Serum protein electrophoresis

Serum protein electrophoresis was performed with a Model R-100 Microzone Electrophoresis System[a] according to the method described by Spinco Division of Beckman Instruments, Inc. (1967). Membranes were dried and then scanned on a Gelman densitometer and recorder[b] to determine relative percentages of each protein fraction.

Serum glutamic oxaloacetic transaminase

Serum glutamic oxaloacetic transaminase (SGOT) levels were determined by the colorimetric method of Reitman and Frankel (1957) as described in Sigma Technical Bulletin 505[c].

Serum electrolytes

Serum sodium (Na) and potassium (K) levels were determined with a Perkin-Elmer 303 atomic absorption spectrophotometer with a Perkin-Elmer DCR-1 direct concentration readout[d]. Procedures were as described in the Perkin-Elmer Analytical Methods Manual.

---

[a]Spinco Division, Beckman Instruments, Inc., Palo Alto, California.

[b]Gelman Instrument Co., Ann Arbor, Michigan.

[c]Sigma Chemical Co., St. Louis, Missouri.

[d]Perkin-Elmer Corp., Norwalk, Connecticut.

Serum chloride was determined by a colorimetric method[a] which utilized a color reagent with ferric and mercuric ions in equilibrium with nitrate and thiocyanate ions. Addition of chloride ions resulted in the formation of mercuric chloride. This shifted the equilibrium with formation of excess ferric thiocyanate which was golden-brown in color. The results in mEq./L. were determined colorimetrically at 480 mm.

Blood gases and pH

Heparinized samples of blood as described under the section on blood sample collection were analyzed for pH, partial pressure of O_2 (pO_2), and partial pressure of CO_2 (pCO_2). The analyses were accomplished with an IL model 113-S2 pH-blood gas analyzer[b]. Blood bicarbonate (HCO_3) concentration was determined by using a conversion scale based on the Henderson-Hasselbalch equation. It was provided with the instrument to derive blood HCO_3 levels from pH and pCO_2 values.

[a]American Monitor Corp., Indianapolis, Indiana.

[b]Instrumentation Laboratory Inc., 113 Hartwell Ave., Lexington, Kentucky.

EXPERIMENTATION

Trial 1

Experimental design

Trial 1 was a pathogenesis study with 14 specific-pathogen-free (SPF) pigs, 11 principals and 3 controls. Principals were killed and necropsies performed at 24, 48, 60, 72, 84, 96, 132, 144, 168, 228, and 264 hours after inoculation. Necropsies of controls were performed at the termination of the trial. Specific times and procedures are summarized in Table 3.

Clinical signs

The first clinical signs noted in the principals were soft to loose stools 6 days post-inoculation (DPI). At 7 DPI the pigs were less active than normal and the loose stools contained blood and mucus. By day 8 the principals had a watery mucohemorrhagic diarrhea. Pigs were dehydrated, gaunt, and inactive. They overextended their rear legs, and mild posterior incoordination was evident. When standing, they lifted their rear legs repeatedly as if in pain. A 1 to 3 F. increase of rectal temperature occurred in most pigs with the onset of clinical signs.

Control animals remained normal.

Gross lesions

Distention of the cecum and colon with soft ingesta, the first

Table 3. Correlation of clinical signs, lesions, and presence of large spirochetes in the colons of pigs. Trial 1

Pig No.	DPI	Clinical Signs	Gross	Lesions: Light Microscopy	Lesions: Electron Microscopy	Large Spirochetes: Light Microscopy	Large Spirochetes: Electron Microscopy
5571 B	11C[a]	-	-	-	-	-	-
5572 B	11C	-	-	-	-	-	-
5575 B	11C	-	-	-	-	-	-
5582 G	1	-	-	-	-	-	-
5581 B	2	-	-	-	-	-	-
5574 B	2	-	-	-	-	-	-
5528 B	3	-	-	-	-	+[b]	+
5570 B	3	-	-	-	-	+	-
5571 G	4	-	-	-	-	++	-
5573 B	5	-	-	-	-	+	+
5581 G	6	+	+	++	++	+++	+++
5580 B	7	++	-	+	+	-	-
5580 G	9	+++	+++	++	+++	+++	+++
5583 B	11	+++	+++	+++	+++	+++	+++

[a]C = control animal in this and subsequent tables.

[b]The following symbols are used in this and subsequent tables: -, no lesions or organisms; +, mild lesions or few organisms; ++, moderate lesions or moderate numbers of organisms; +++, severe lesions or large numbers of organisms.

change in the principals, was observed 5 DPI (pig 5573B). The serosal surface of the colon was pink and edematous 6 DPI (pig 5581 G) and mesenteric lymph nodes were slightly swollen (Figure 1). Fluid colon contents contained increased amounts of mucus and a small quantity of blood. The mucosa of the entire colon was markedly hyperemic and swollen. Mild distention of the colon was noted at 7 DPI (pig 5580 B). On days 9 (pig 5580 G) and 11 (pig 5583 B) the cecum and colon were hyperemic, edematous, and dilated. Contents of the colon were watery and contained blood and mucus. The mucosa of the cecum and colon was hyperemic and often covered with a thin yellow pseudomembrane (Figure 2). There was a small amount of clear ascitic fluid in the abdominal cavities of these pigs.

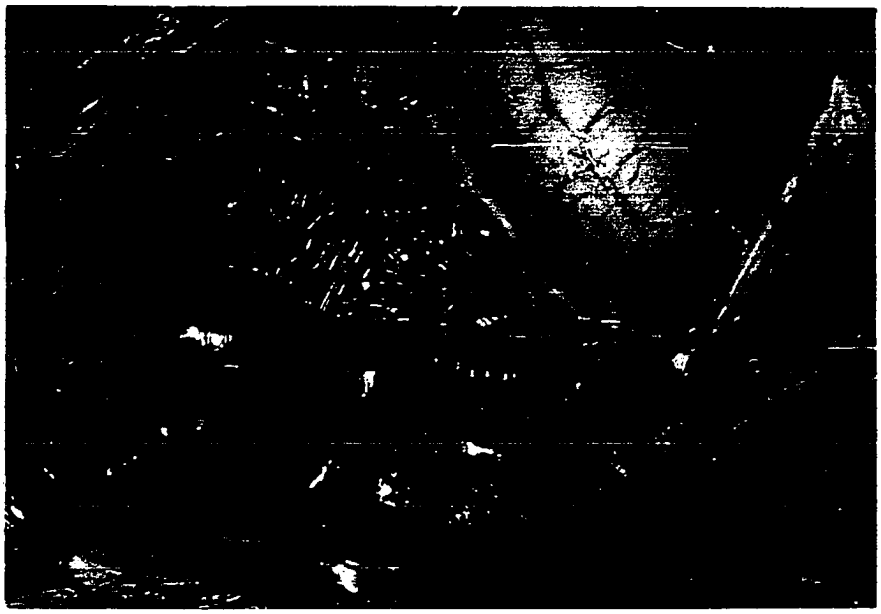
Stomachs of 6 principals and 2 controls were quite hyperemic in the fundic area. Scattered ascarid scars were present in the liver capsules of most pigs. No enteric lesions were noted in controls.

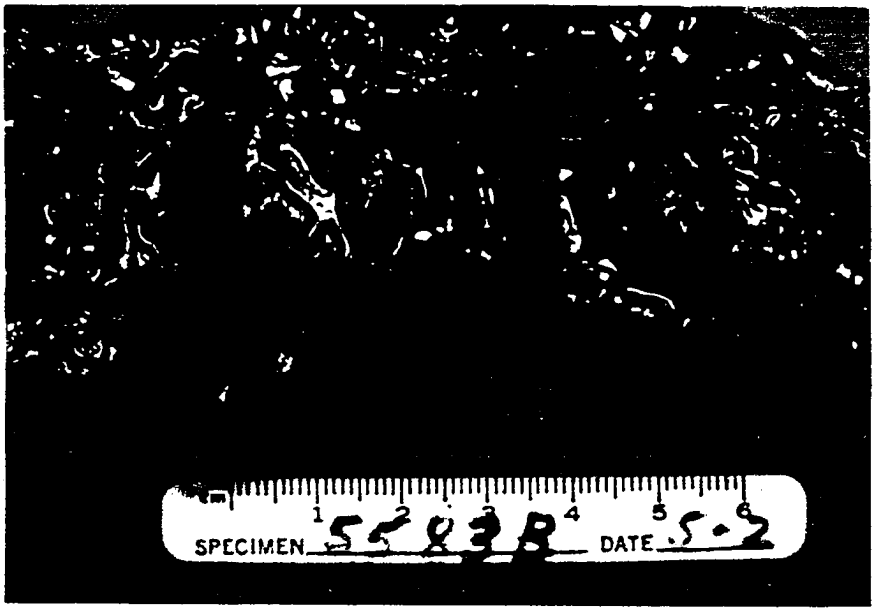
Light microscopy

Small numbers of large spirochetes were first noted in the lumens of colon sections stained by the Warthin-Starry method 3 to 5 DPI. At 6 DPI the mucosa of the entire colon was edematous and approximately twice as thick as normal. The intensity of staining in the luminal portion of the mucosa was greatly reduced. Vessels in the lamina propria were congested, especially near the lumen. Epithelial cells at the base of the crypts were deeply basophilic and had numerous mitotic figures. Crypts in some areas were distended with mucus. Small foci of

Figure 1. Trial 1, 6 DPI. Abdominal viscera of a pig with early lesions of swine dysentery. Note edema and hyperemia of spiral colon at bottom center

Figure 2. Trial 1, principal, 11 DPI. Colon. Mucosal surface of spiral colon. The mucosa has a thick layer of mucus and focal hemorrhages. A mucofibrinous pseudomembrane is seen at far left




SPECIMEN
DATE

epithelial cells were separated from the lamina propria at the luminal surface (Figure 3), and a few Balantidium coli were present in the lumen.

Increased numbers of large spirochetes were seen in the lumens and crypts of colon sections on day 6. In some crypts spirochetes were the only organisms present, but in others they were mixed with rod-shaped bacteria (Figure 4). In some instances spirochetes could be seen in goblet cells and between epithelial cells (Figure 5).

No spirochetes were observed on day 7, and the only lesion was in the lamina propria which was edematous.

Lesions on day 9 (Figure 6) were most pronounced in the proximal colon and consisted of mucofibrinous to fibrinonecrotic colitis. The submucosa was edematous, and lymphatics were dilated. The mucosa was thickened and pale-staining except for the epithelial cells at the base of some of the crypts which were strongly basophilic and elongated and which had numerous mitotic figures. The lamina propria was edematous, and numerous neutrophils were present in and around dilated vessels. Crypts were dilated, and many large goblet cells were noted in the walls along with swollen epithelial cells. Broad areas of mucosal epithelium were necrotic at the luminal surface which was covered with a mucofibrinous exudate containing bacteria, leukocytes, necrotic epithelial cells, and occasional erythrocytes. Masses of large spirochetes were seen in the lumen, in crypts, in goblet cells, and between epithelial cells. Rod-shaped bacteria were also present in the crypts but not in the epithelium.

At 11 DPI the lesions in the colon resembled those on day 9, but

Figure 3. Trial 1, principal, 6 DPI. Colon. Early lesions of swine dysentery consisting of congestion in the lamina propria and separation of groups of epithelial cells from the lamina propria at the luminal surface. H & E stain; X350

Figure 4. Trial 1, principal, 6 DPI. Colonic crypt. The long spiral organisms were identified as large spirochetes (arrow). The short curved rods resemble vibrio. MacCallum-Goodpasture stain; X1400

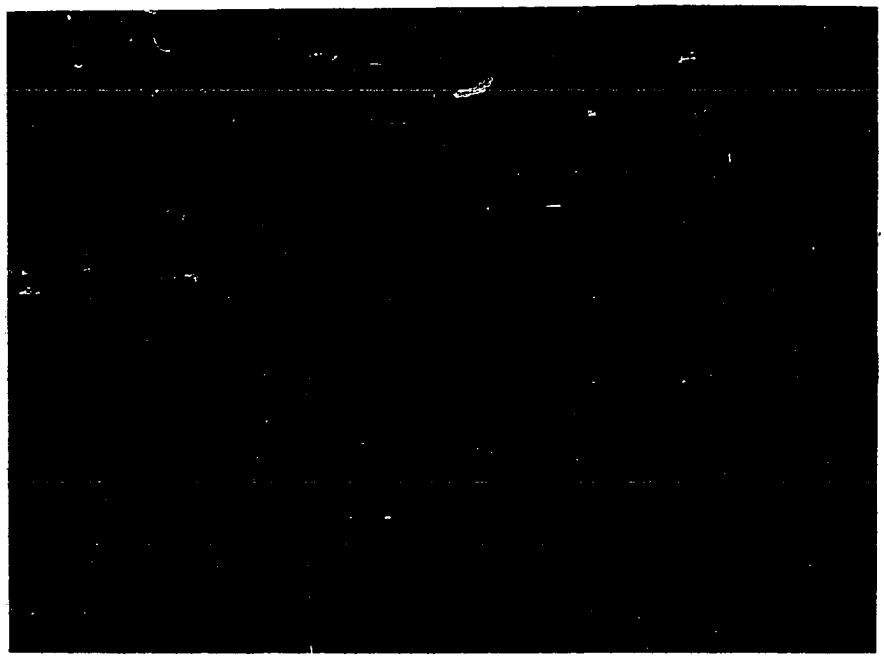
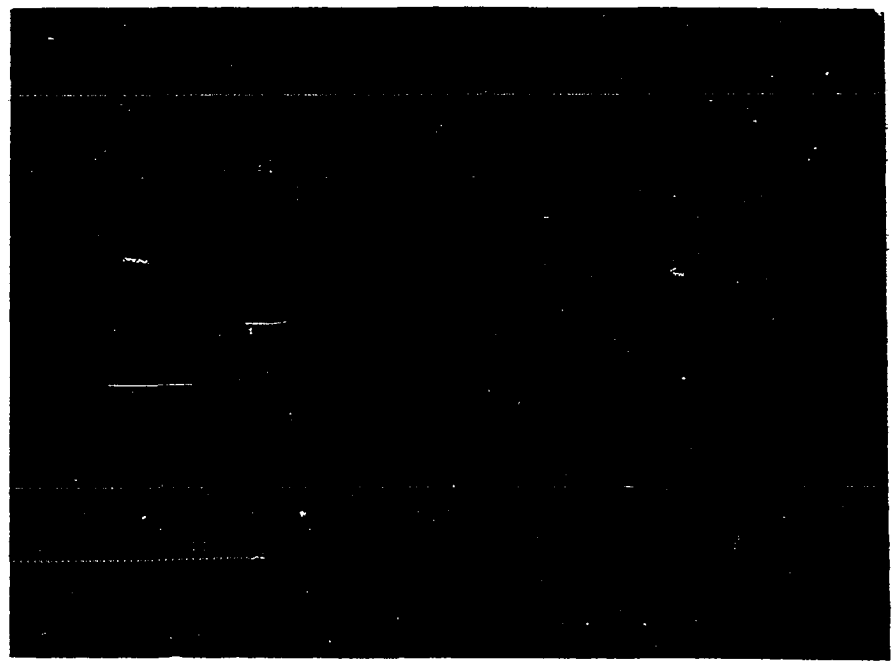
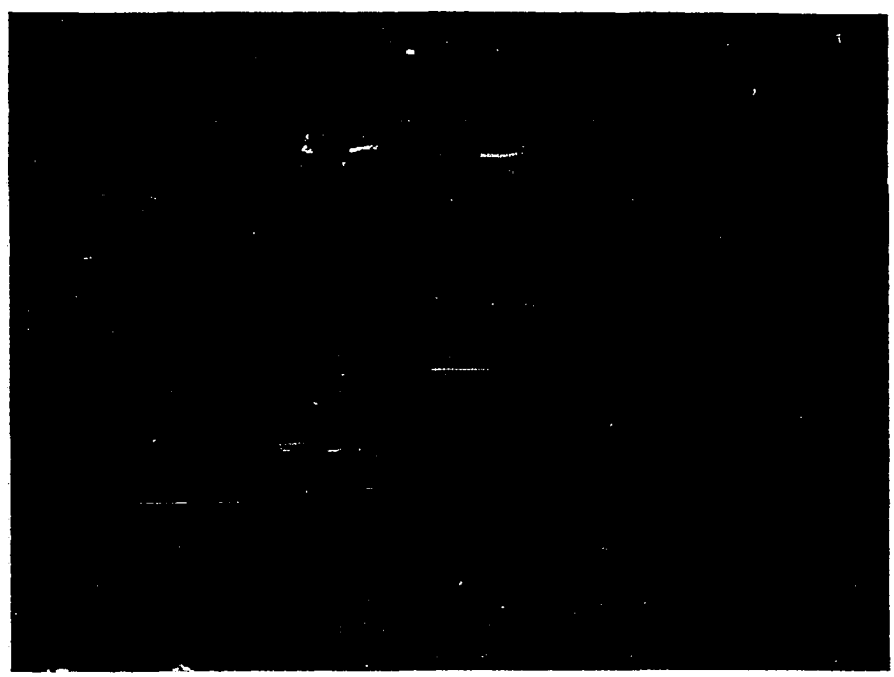


Figure 5. Trial 1, principal, 6 DPI. Colonic crypt. Large spirochetes (stained dark-brown) are seen in the lumen of the crypt, in goblet cells, and in and between epithelial cells. Warthin-Starry stain; X1400

Figure 6. Trial 1, principal, 9 DPI. Colon. Note edema and leukocytes in the submucosa and mucosa. The luminal surface of the mucosa is eroded and covered by mucofibrinous exudate. H & E stain; X60





there was less edema in the submucosa which was infiltrated with lymphocytes. The mucosa was thickened but mitotic figures were not numerous. Large numbers of both mononuclear and polymorphonuclear leukocytes filled the lamina propria near the lumen, and large segments of the luminal surface were devoid of epithelium. There were large spirochetes as on day 9, and a few B. coli were present in the lumen.

There were no lesions in the colons of controls.

Lesions were not found in sections of kidney, spleen, lung, or stomach from principals or controls in this trial. The only alterations observed in liver were occasional accumulations of leukocytes including numerous eosinophils in the interlobular septums of some pigs. Sections of duodenum and ileum from all pigs were normal except for a focal area of necrosis in the terminal ileum of the principal killed on day 9.

Electron microscopy

A few spirochetes were present at the luminal surface of the colon at 3 and 5 DPI. The only inflammatory change noted during the first 5 days of the experiment was an apparent increase in numbers of leukocytes in the lamina propria 3 DPI (pig 5528 B).

At 6 DPI numerous spirochetes were either scattered near the surface of colonic epithelial cells or massed in the crypts (Figure 7). The most consistent change in adjacent epithelial cells was alteration of microvilli which were shortened, irregular, and sparse in contrast to those from control animals (Figure 8). Mitochondria in some cells

Figure 7. Trial 1, principal, 6 DPI. Colonic crypt. Numerous large spirochetes are present in the lumen. Their structure consists of a protoplasmic cylinder with axial fibrils (arrow) along one side. The organisms are surrounded by an envelope (E). X23,600

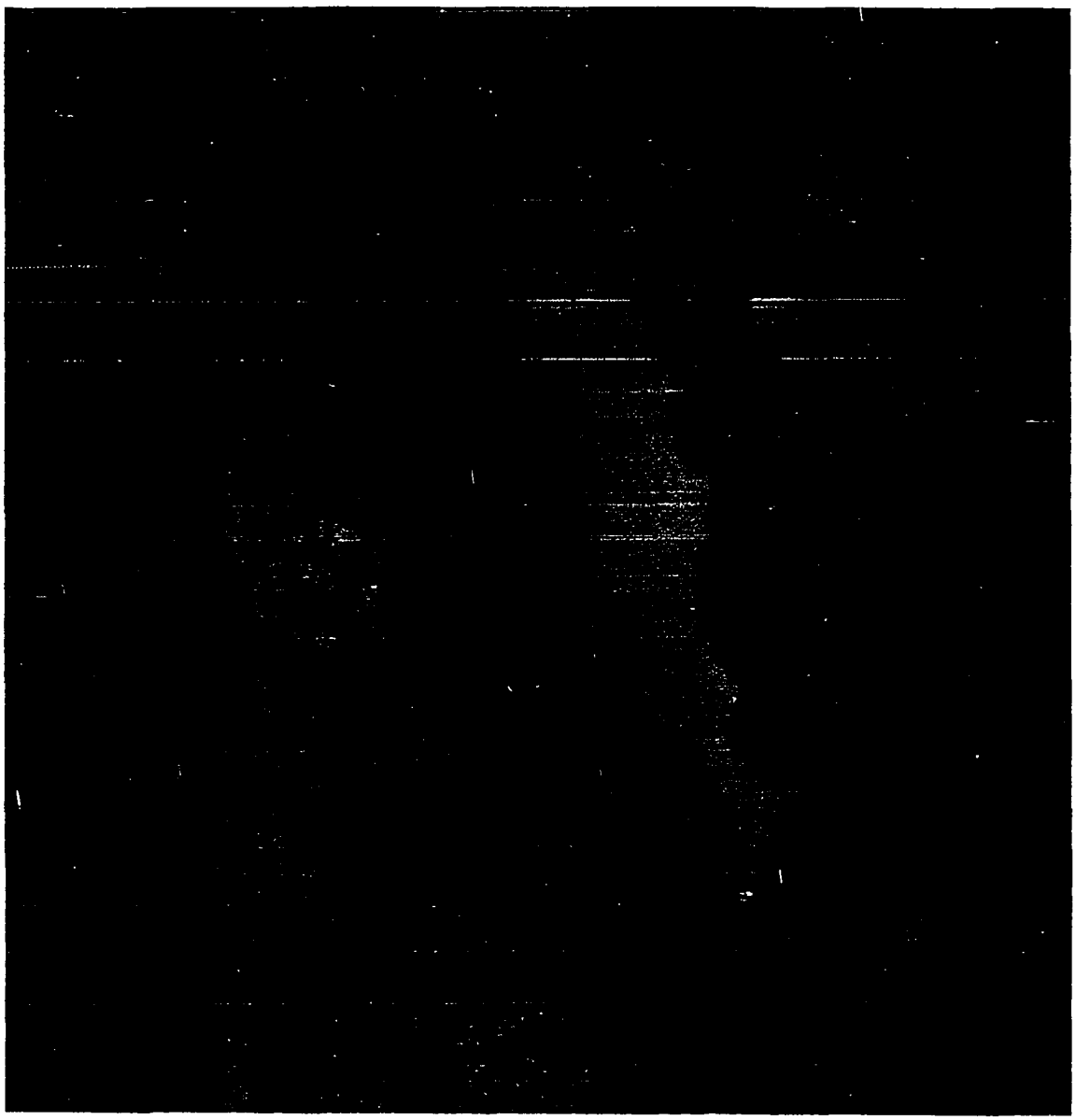
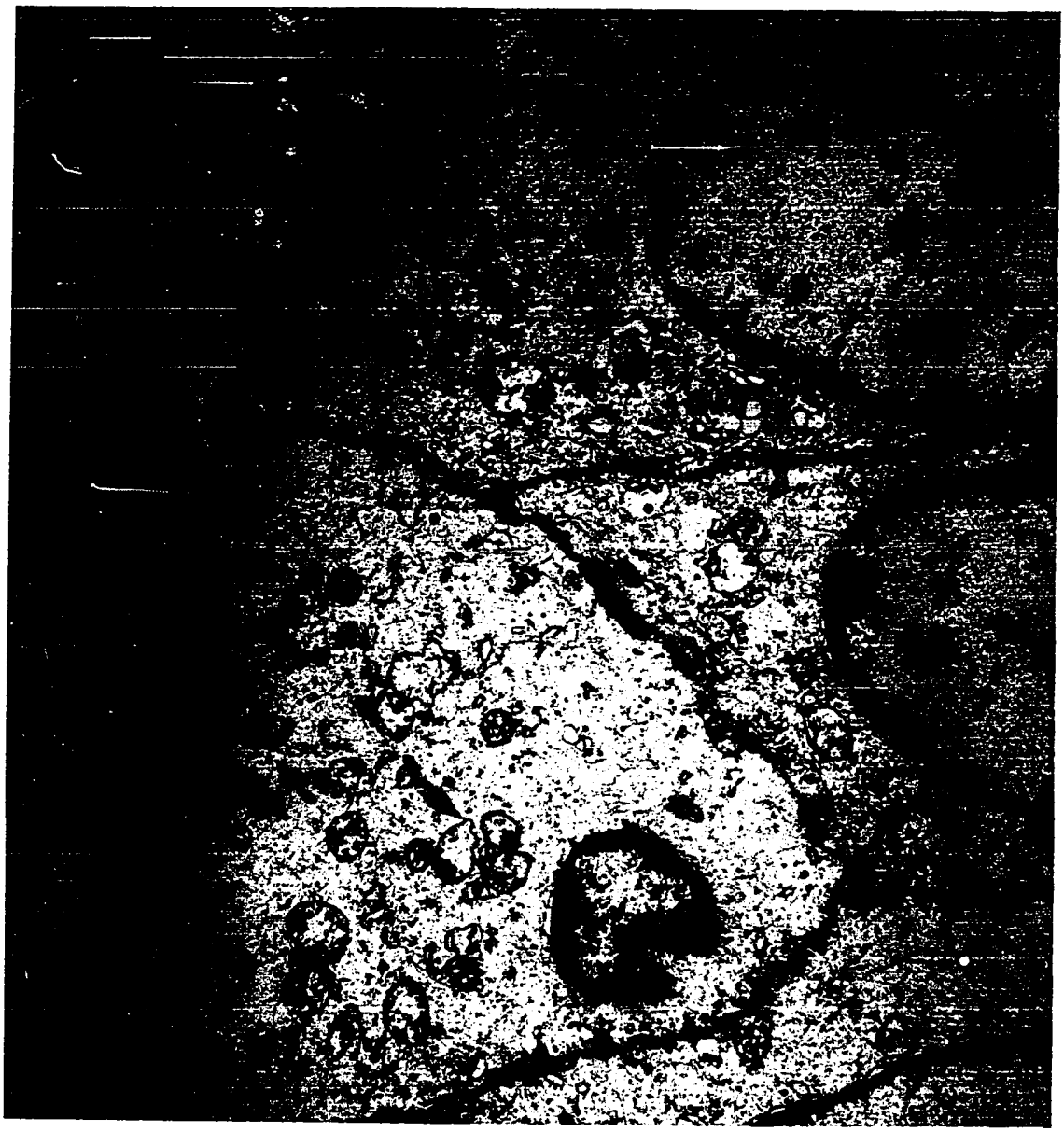


Figure 8. Trial 1, control. Normal colonic epithelium. The microvilli are regularly spaced and uniform in size. X8000



were swollen (Figure 9). There were spirochetes in the mucigen droplets and cytoplasm of goblet cells. The cytoplasm surrounding the mucigen in those cells appeared to be condensed and contained granular material in the endoplasmic reticulum and cytoplasmic matrix. Adjacent epithelial cells were often lightly stained and had decreased numbers of organelles and sparse, irregular microvilli (Figure 10).

The spirochetes observed in these sections had a protoplasmic cylinder approximately 0.30 μm. in diameter with an amorphous granular appearance and no organelles (Figure 11). The protoplasmic cylinder was surrounded by an envelope made up of 2 thin membranes. Maximum diameter including the envelope was approximately 0.38 μm. The organisms had a loose spiral appearance, and a bundle of 12 to 14 axial fibrils was seen spiraling around the organisms between the protoplasmic cylinder and the envelope. The fibrils were approximately 10 to 12 nm. in diameter.

At 7 DPI (pig 5580 B) some colonic epithelial cells were swollen and a few were shrunken with dense granular material in the endoplasmic reticulum and hyaloplasm. The most notable changes were in the lamina propria which had numerous neutrophils in and around vessels with swollen endothelial cells. Edema in the area was evidenced by intercellular and intracellular swelling (Figure 12).

Colonic lesions were more advanced at 9 DPI (pig 5580 G). Epithelial cells were lightly stained and swollen. Microvilli were sparse, and some cells near the lumen had signs of advanced degeneration including swollen endoplasmic reticulum and myelin figures. Scattered dark

Figure 9. Trial 1, principal, 6 DPI. Epithelial cells in colonic crypt. Mitochondria (M) are swollen and microvilli are short and irregular. Note large spirochetes in lumen. X 23,600

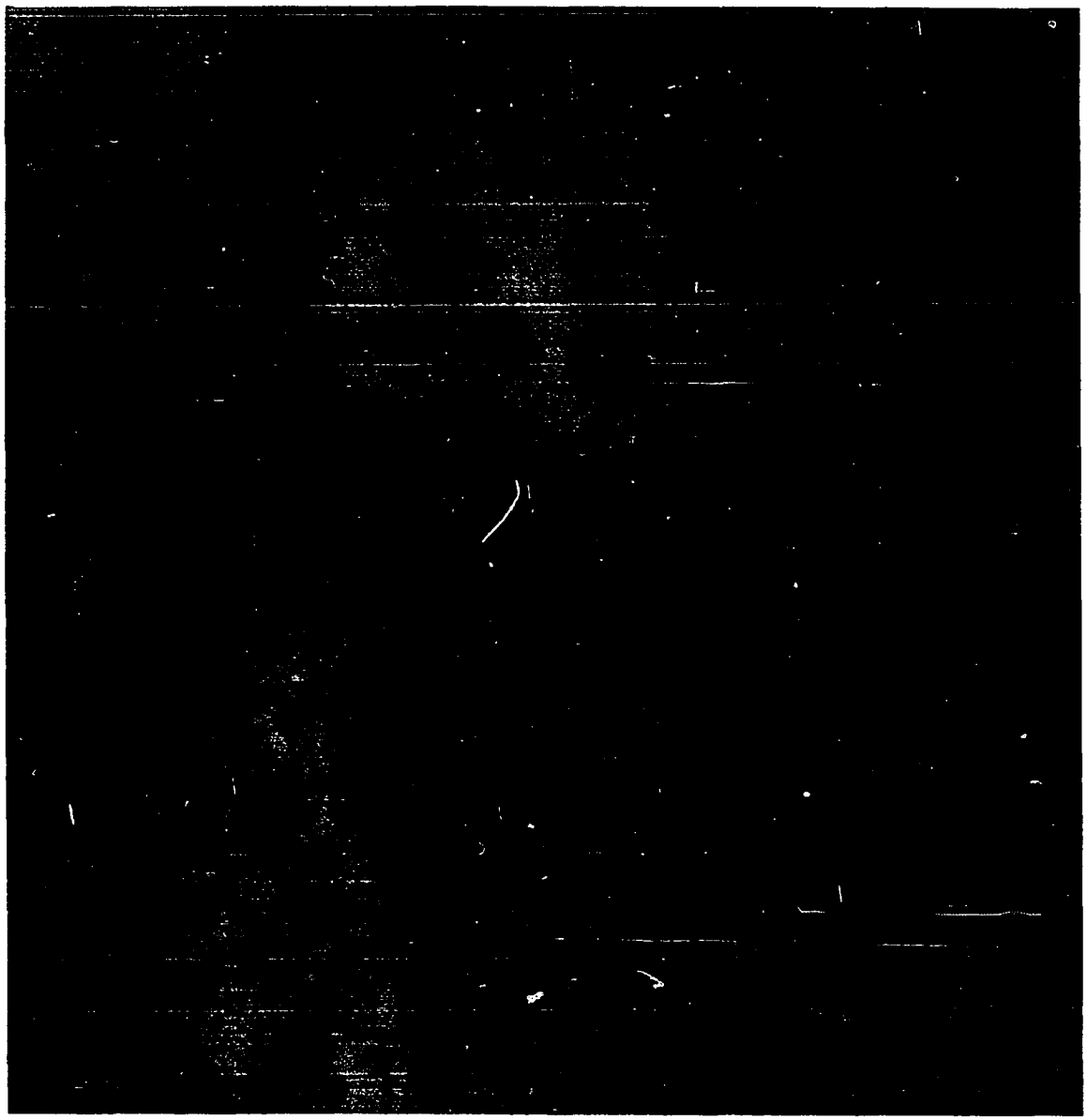
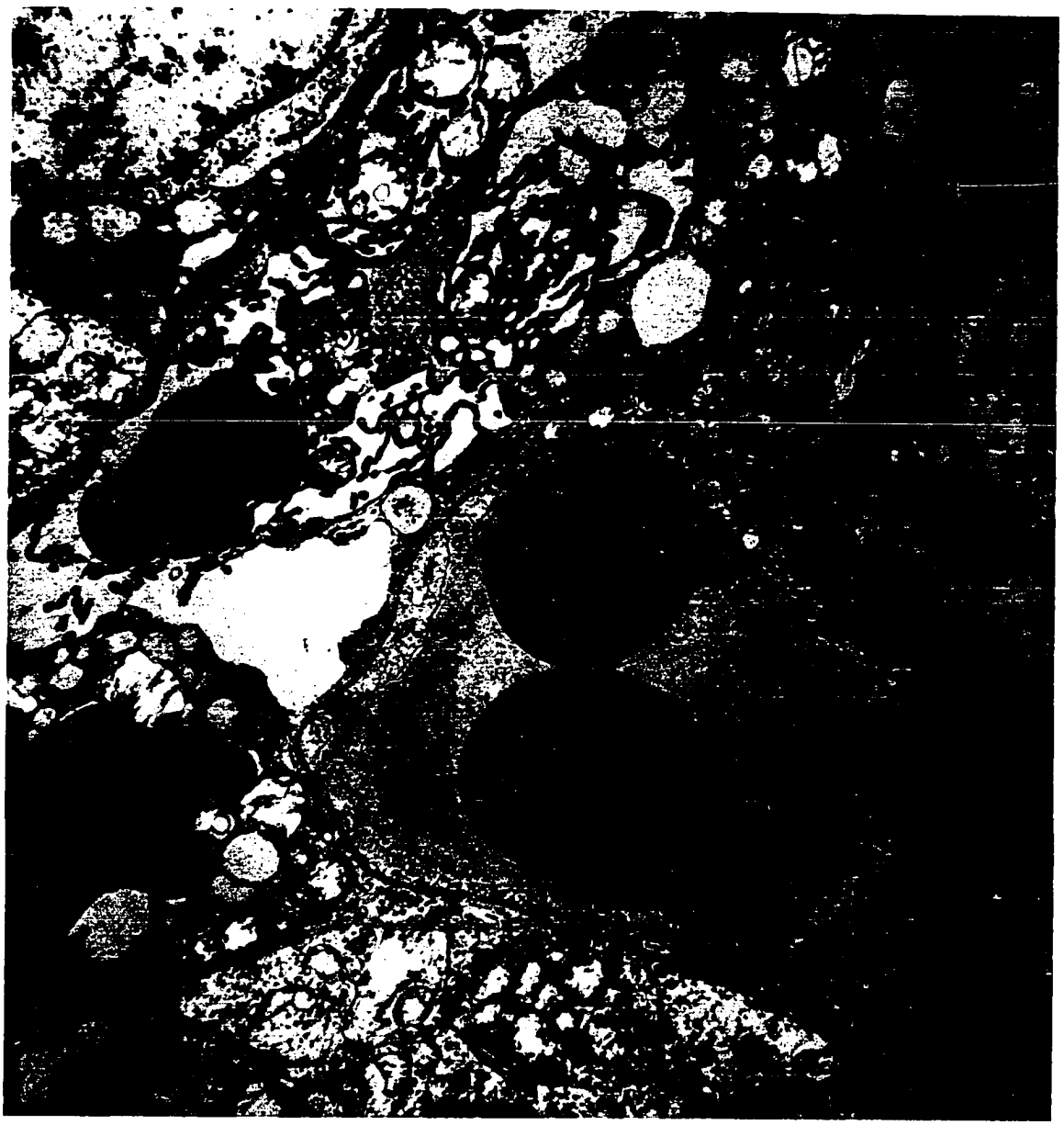


Figure 10. Trial 1, principal, 6 DPI. Epithelium of colonic crypt. There are large spirochetes in the lumen and within the mucigen and cytoplasm of a goblet cell (far left). An adjacent epithelial cell (center) has sparse microvilli and light staining cytoplasm with few organelles. The cell at the far right is less severely damaged. X 17,200

Figure 11. Trial 1, principal, 6 DPI. Large spirochete in colonic crypt. Note spiral appearance, protoplasmic cylinder (P), envelope (E), and axial fibrils (A). X30,400


A

Figure 12. Trial 1, principal, 7 DPI. Lamina propria, colonic mucosa. Note lightly stained areas of edema in and between cells. X6000



epithelial cells contained clusters of spirochetes similar to those seen on day 6 (Figure 13). Edema and neutrophils were evident in the lamina propria.

At 11 DPI (pig 5583 B) distribution of spirochetes and lesions were similar to day 9, but cellular degeneration was more advanced (Figure 14). High magnification of spirochetes confirmed the morphology previously described and suggested that the envelope was composed of 2 trilaminar membranes (Figure 15). Axial fibrils were approximately 11 nm. in diameter. Some of the necrotic cells contained bacteria other than spirochetes.

Commensal cultures of vibrio and large spirochetes from the colons of principals were negatively stained to further characterize the morphology of the spirochetes.

Large spirochetes had the same structure as those seen in ultrathin sections of colonic mucosa and most varied in length from approximately 5.6 to 7.0 μm. Half of the axial fibrils originated from each end of the organism and overlapped in the middle. The fibrils were contained within a thin envelope which surrounded the protoplasmic cylinder (Figure 16). Vibrio were short curved rods with polar flagella (Figure 17).

Bacteriology

Vibrio and large spirochetes from the colons of principals were grown in commensal cultures. No Salmonella spp. were isolated.

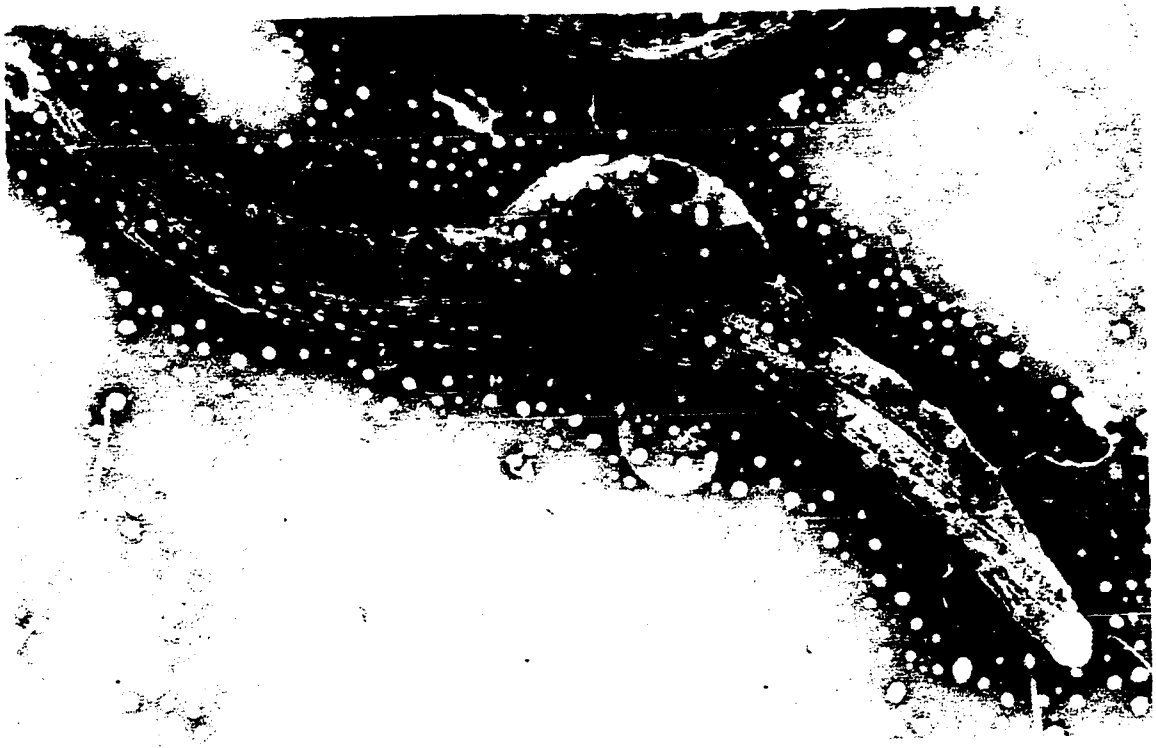
Figure 13. Trial 1, principal, 9 DPI. Epithelium of colonic crypt. Large spirochetes (S) are present in a cell with swollen endoplasmic reticulum (ER). X34,000

Figure 14. Trial 1, principal, 11 DPI. Epithelium of colonic crypt. Groups of large spirochetes (S) are present in degenerate cells. X7,400

Figure 15. Trial 1, principal, 11 DPI. Large spirochetes in epithelial cell. Note the presence of axial fibrils (A) and an envelope (E) composed of 2 trilaminar membranes. X112,000

Figure 16. Large spirochete. Axial fibrils are seen originating from both ends of the organism. PTA stain; X26,800

Figure 17. Vibrio. Note curved shape and polar flagellum. PTA stain; X26,800



Trial 2

Experimental design

Trial 2 was designed as a repeat of Trial 1 with the addition of phase microscopy of colon contents and mucosal scrapings. Fifteen conventional pigs (10 Yorkshire and 5 Hampshire) were divided into 2 groups, 10 principals and 5 controls. The principals were killed for necropsy at 2 through 7 DPI except on day 5 when 2 pigs died spontaneously. Controls were electrocuted and necropsies were performed at 2, 3, 5, 6, and 8 DPI. Table 4 is a summary of animals and observations.

Clinical signs

All principals except H-1 and Y-3 had diarrhea and were inactive 2 DPI. Blood and mucus were present in the feces of pig Y-4. On day 3 all had diarrhea, and feces of 5 pigs (H-3, H-4, Y-4, Y-5, and Y-6) contained blood and mucus. All except one (Y-2) were dehydrated and gaunt with posterior weakness exhibited as a wobbling irregular gait. They repeatedly lifted and replaced their rear feet as if in pain. By day 5 all pigs had watery, mucohemorrhagic diarrhea and were very gaunt and weak. A 2 to 3 F. increase in rectal temperature occurred in all pigs on day 2 to day 3.

The only clinical signs noted in control pigs were soft stools and an increase in rectal temperature of approximately 2 F. on days 3 and 4 in pig H-6.

Table 4. Correlation of clinical signs, lesions, and presence of large spirochetes in pigs. Trial 2

Pig No.	DPI	Clinical Signs	Gross	Lesions: Light Microscopy	Lesions: Electron Microscopy	Large Spirochetes: Phase Microscopy	Large Spirochetes: Electron Microscopy
Y-8	2-C	-	-	-	-	-	-
H-7	3-C	-	-	-	-	-	-
Y-9	5-C	-	-	-	-	-	-
H-6	6-C	-	-	-	-	-	-
Y-10	8-C	-	-	-	-	-	-
Y-3	2	-	-	-	-	+	-
H-1	2	-	+	++	+	+++	++
Y-2	3	+	++	++	++	+++	+++
Y-5	3	++	+++	+++	+++	+++	+++
Y-6	4	++	+++	+++	++	++	++
H-3	5	+++	+++	+++	+++	++	+++
H-4	5	+++	+++	+++	+++	++	++
Y-1	6	+++	+++	+++	+++	+++	+++
Y-4	6	+++	++	++	+++	++	+++
Y-7	7	+++	+++	+++	+++	+++	+++

Gross lesions

At 2 DPI the contents of the cecum in pig Y-3 were softer than normal. Colon contents were soft in pig H-1 and there was a mild fibrinous colitis in the proximal end of the colon. The colonic mesentery glistened as if mildly edematous.

At 3 DPI the mucosa of the proximal one-third of the colon of pig Y-2 was hyperemic and edematous with focal hemorrhages. Lesions were more advanced in pig Y-5. The serosa of the large intestine was mildly edematous and pink. The mucosa of the cecum and colon was thickened and contained focal hemorrhages. A thin mucofibrinous pseudomembrane lined the cecum and anterior colon. Colon contents were watery and contained blood and mucus. There were dark red areas 1 to 2 cm. in diameter in the fundic portion of the stomach of pig Y-5, and the serosa of the small intestine was mildly congested.

Gross lesions at 4 DPI (pig Y-6) were very similar to those in pig Y-5 on day 3.

The mesentery and wall of the large intestine were congested and very edematous in the two pigs (H-3 and H-4) which died at 5 DPI. The swollen mucosa of the cecum and colon was covered by a thick, tightly adherent yellow to gray pseudomembrane. Rectal lesions were less severe. The fundic portion of the stomach and the serosa of the jejunum in pig H-4 were congested.

Principals killed at 6 and 7 DPI had a mucofibrinous enteritis similar to that noted on day 5. Ecchymoses were present in the fundus of the stomach in one pig (Y-1).

Principals with marked enteritis had swollen colonic lymph nodes and variable amounts (10 to 50 ml.) of clear fluid in their abdominal cavities.

Both principals and controls had ingesta in their stomachs, a few intestinal ascarids, and ascarid scars on their livers. No enteritis was observed in controls.

Light microscopy

Of the 2 principals killed 2 DPI pig Y-3 had no apparent microscopic lesions. The mucosa of the large intestine of the other pig (H-1) was mildly edematous. Focal areas of the epithelium were eroded exposing vessels in the lamina propria. Numerous neutrophils were present in and around dilated vessels near the lumen.

Lesions in pigs Y-2 and Y-5 at 3 DPI were similar to pig H-1 at 2 DPI. However, in pig Y-5 necrosis of the luminal epithelium was more extensive and the mucosa was covered with a thick layer of mucofibrinous exudate containing cellular debris and bacteria. B. coli were observed in both pigs.

Pig Y-6 at 4 DPI had a mucofibrinous enteritis similar to pig Y-5 on the previous day. There was also infiltration of the submucosa by mononuclear and polymorphonuclear leukocytes.

On days 5 through 7, lesions in the large intestine progressed from mucofibrinous to fibrinonecrotic enteritis. Necrosis of the mucosa was superficial at first but later extended nearly to the muscularis mucosa in some pigs. Numbers of neutrophils in the lamina propria increased

and formed a line adjacent to the necrotic portion of the mucosa. Goblet cell hyperplasia was extensive until 7 DPI (pig Y-7) when goblet cells in the mucosa were less numerous. B. coli were observed in the lumen of the large intestines of the pigs killed on days 6 and 7.

Numerous mitotic figures were present in epithelial cells at the base of colonic crypts in principals and controls.

Colonic lymph nodes of principals and controls appeared similar with the exception of 2 principals (H-3 and Y-1) in which lymphoid depletion was noted. Mild serous lymphadenitis was also present in the colonic lymph node from pig H-3.

The only gastric lesions in principals or controls were large hemorrhagic infarcts in the mucosa of one principal (Y-1) at 6 DPI.

B. coli were present in one control pig. No enteric lesions were observed in the controls.

Phase microscopy

Variable numbers of vibrio and spirilla were in preparations from the colons of all pigs--principals and controls. Small spirochetes were present in all but one pig and were often observed in colon contents but not in mucosal scrapings. Fusiform bacteria were present in a majority of principal and control pigs. Large spirochetes were found only in principals and occurred in high numbers except at 2 DPI in pig Y-3 which had only a few. The large spirochetes were usually most numerous around and between clumps of epithelial cells in mucosal scrapings.

Relative numbers of the various organisms are summarized in Table 5.

Electron microscopy

Sections from the proximal third and apex of the colons of all pigs in the trial were examined. Lesions and large spirochetes were first noted in one of the 2 principals (pig H-1) at 2 DPI. Epithelial cells and cells of the lamina propria were swollen and had numerous pinocytotic vesicles adjacent to the plasma membrane.

The 2 principals killed 3 DPI had numerous large spirochetes in the lumen and crypts of the colon. Epithelial cells were often separated by intercellular edema (Figure 18) and microvilli of most were irregular, short, and in some cases swollen. The apical portion of some epithelial cells contained clear spaces and few organelles (Figure 19). The endoplasmic reticulum was often dilated, and swollen mitochondria had disorganized christae (Figure 20). Cells contained large vacuoles which in some cases were surrounded by dense, granular hyaloplasm (Figure 18). Spirochetes were often present in this type of cell (Figure 21). Edema in the lamina propria resulted in separation of connective tissue cells, and there were numerous pinocytotic vesicles in capillary endothelial cells.

Lesions later in the course of the disease were similar to those at 3 DPI but more cells were damaged. Myelin figures were seen occasionally in necrotic cells. In one case a spirochete was found partially embedded in the apical cytoplasm of an epithelial cell (Figure 22).

Table 5. Results of phase microscopic observation of mucosal scrapings from the colons of pigs. Trial 2

Pig No.	DPI	Gross Lesions	Large Spirochetes	Small Spirochetes	Vibrio	Spirilla	Fusi-forms
Y-8	2-C	-	-	+	+	++	-
H-7	3-C	-	-	+	+	+	+++
Y-9	5-C	-	-	+	+	+	++
H-6	6-C	-	-	+	++	+	++
Y-10	8-C	-	-	+	+	+	-
Y-3	2	-	+	+	++	++	-
H-1	2	+	+++	+	+	+	-
Y-2	3	++	+++	+	+	+	+
Y-5	3	+++	+++	+	+++	+	++
Y-6	4	+++	+++	-	+	+	+++
H-3	5	+++	++	+	+	+	++
H-4	5	+++	++	+	+	+	-
Y-1	6	+++	+++	+	+++	+++	+++
Y-4	6	++	+++	+	++	++	+
Y-7	7	+++	+++	+	+	+	+

Figure 18. Trial 2, principal, 3 DPI. Colonic epithelium. There is edema in and between degenerating cells some of which have dark granular hyaloplasm. Also note irregular microvilli. X13,400

Figure 19. Trial 2, principal, 3 DPI. Epithelium of colonic crypt. Numerous large spirochetes are seen in the lumen. The adjacent epithelial cell has irregular microvilli and few organelles. X16,000

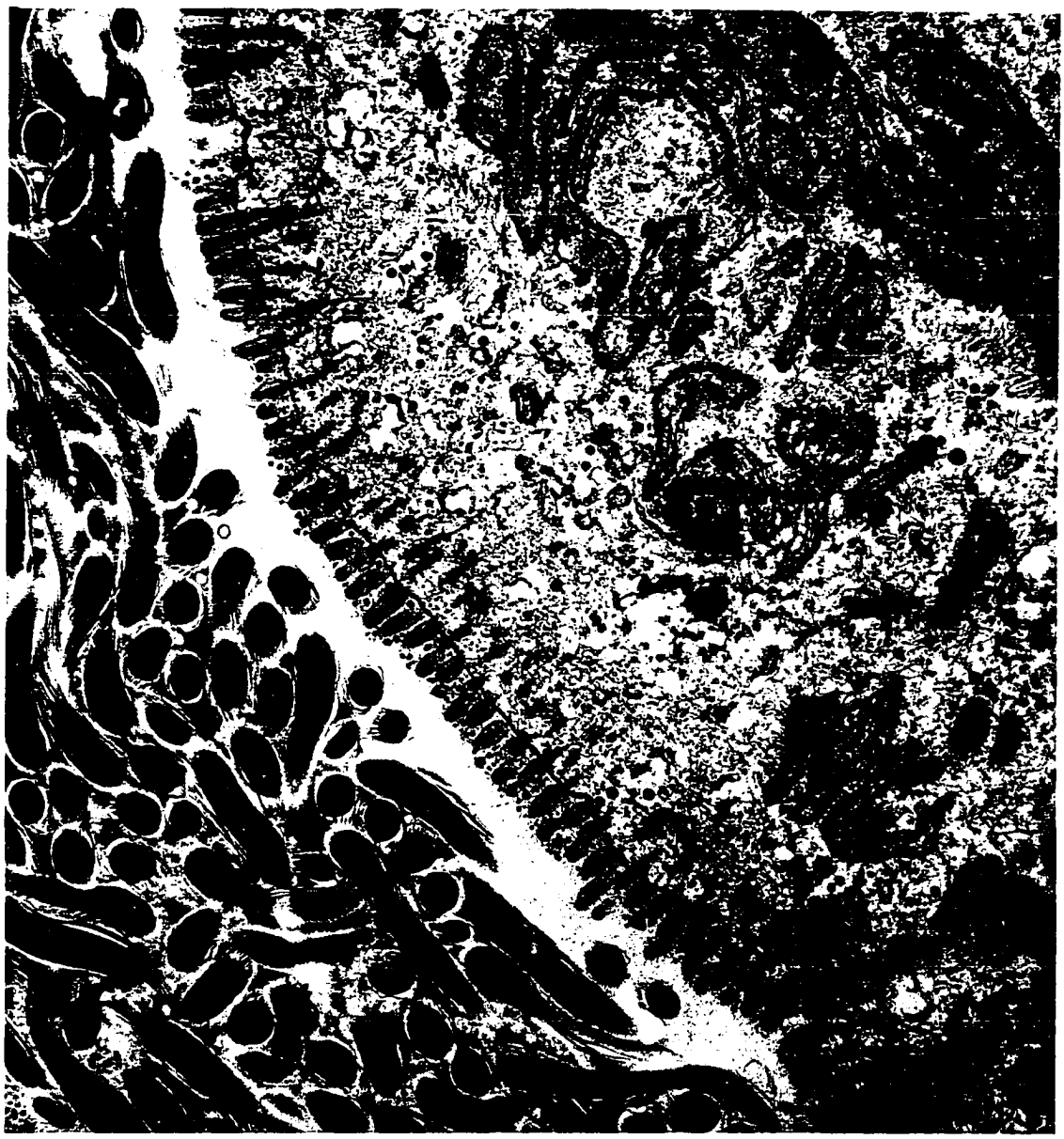


Figure 20. Trial 2, principal, 3 DPI. Epithelium of colonic crypt. Mitochondria and microvilli are swollen. X18,800

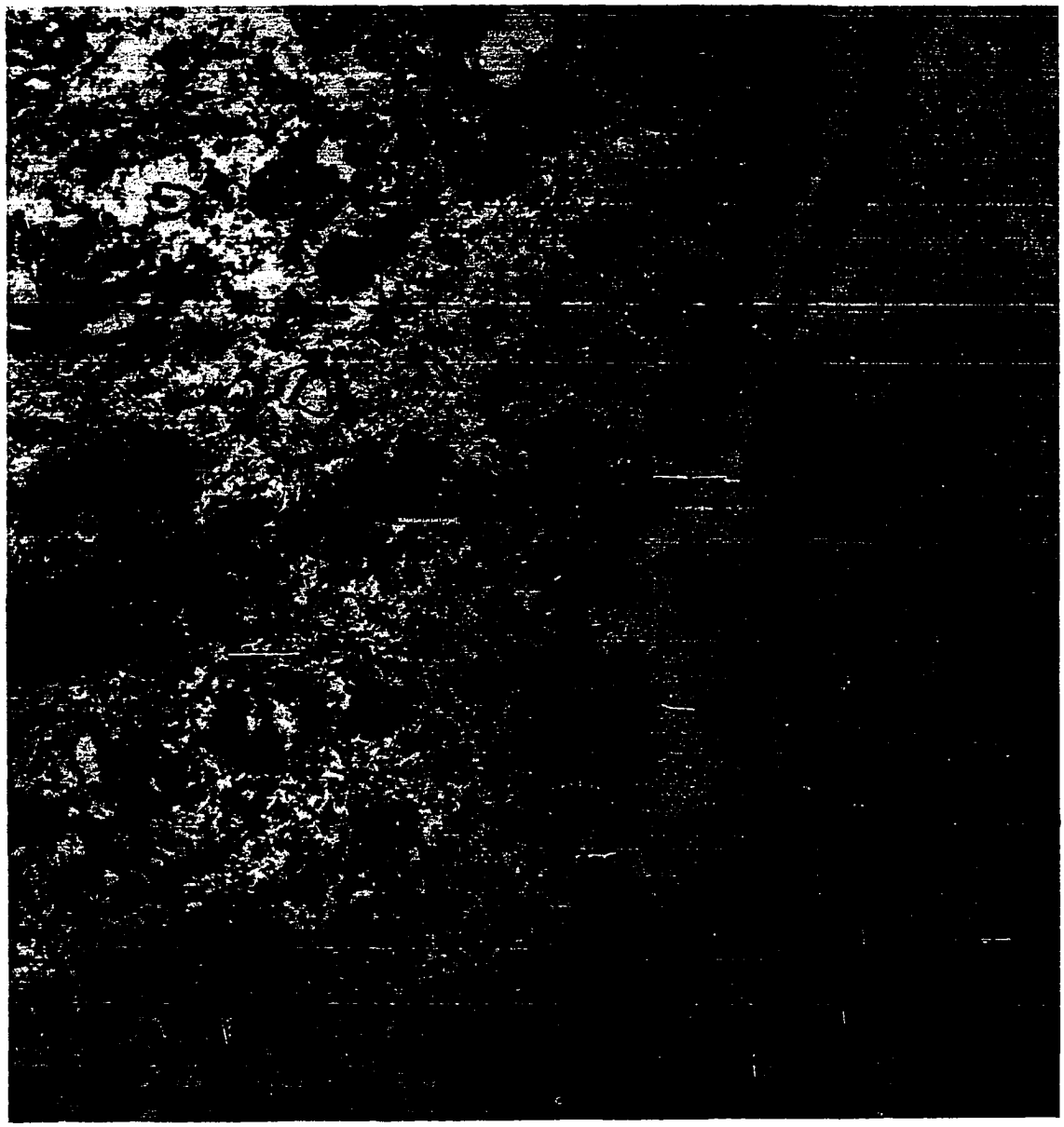


Figure 21. Trial 2, principal, 3 DPI. Epithelium at the base of a colonic crypt. Note the large spirochetes within vacuoles (V) in a degenerating cell. The cytoplasm of the cell is electron dense and contains swollen endoplasmic reticulum (ER). X11,000

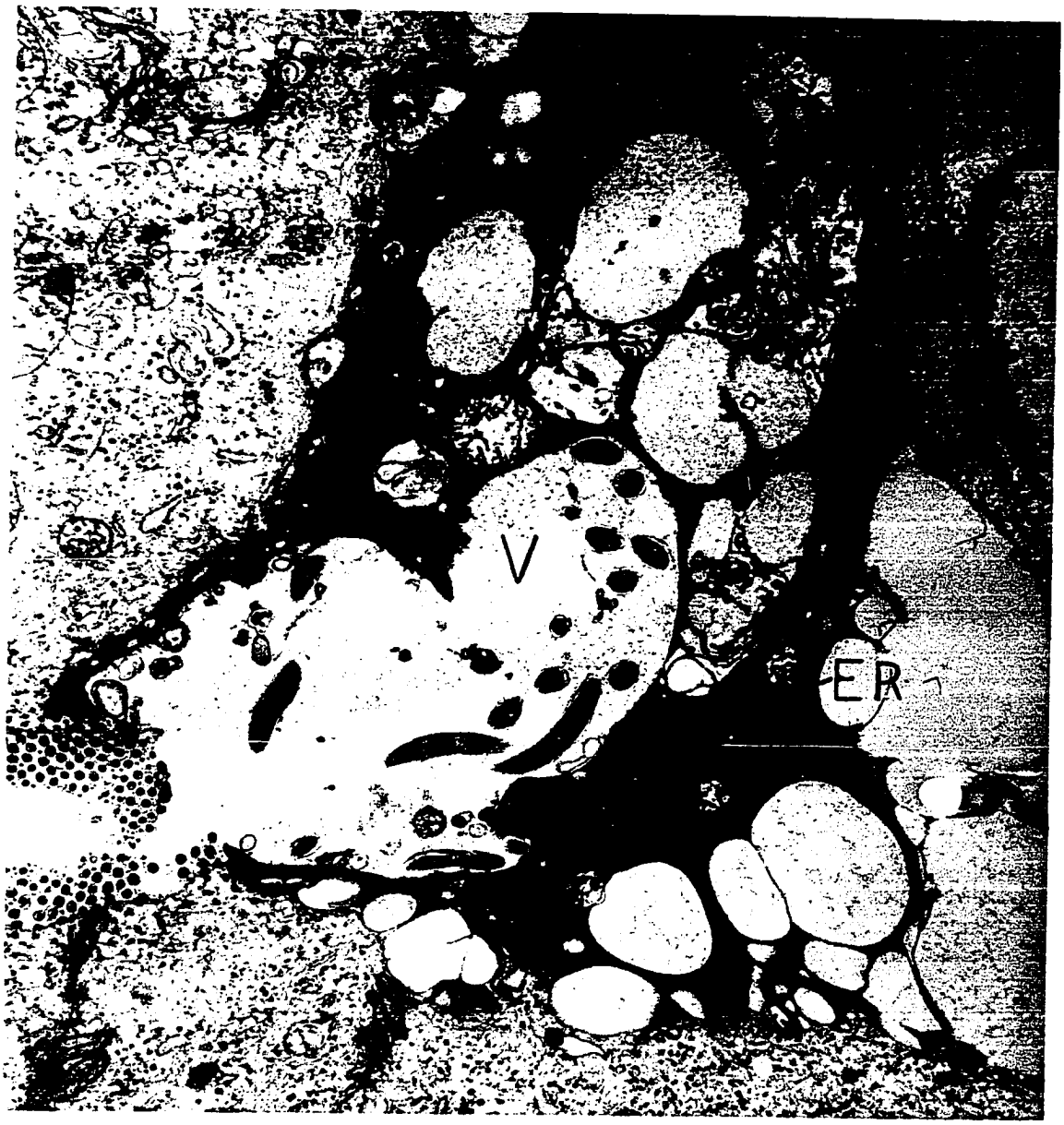

V
ER

Figure 22. Trial 2, principal, 6 DPI. Epithelium of colon. A large spirochete is partially embedded in a degenerate epithelial cell. X18,800

These organisms were often observed adjacent to the basement membrane of the epithelium and in a few cases in the lamina propria (Figure 23). Bacteria other than spirochetes were sometimes found in epithelial cells, rarely before 6 DPI and more frequently at 6 and 7 DPI. Endothelial cells in the lamina propria were swollen, and numerous neutrophils and lymphocytes could be seen in and around vessels.

The spirochetes observed in this trial were similar to those in Trial 1 (Figure 11). They were approximately 0.30 to 0.36 μm. in diameter with a granular protoplasmic cylinder and surrounding envelope. In some cases the envelope had a subunit structure in which the two membranes could not be seen (Figure 24). The number of axial fibrils varied from 12 to 16.

Bacteriology

No Salmonella spp. were isolated from the colons of any pigs in this trial.

Figure 23. Trial 2, principal, 7 DPI. Colonic mucosa. There are large spirochetes at the base of a degenerate epithelial cell (lower right) adjacent to the lamina propria (upper left). X9,800

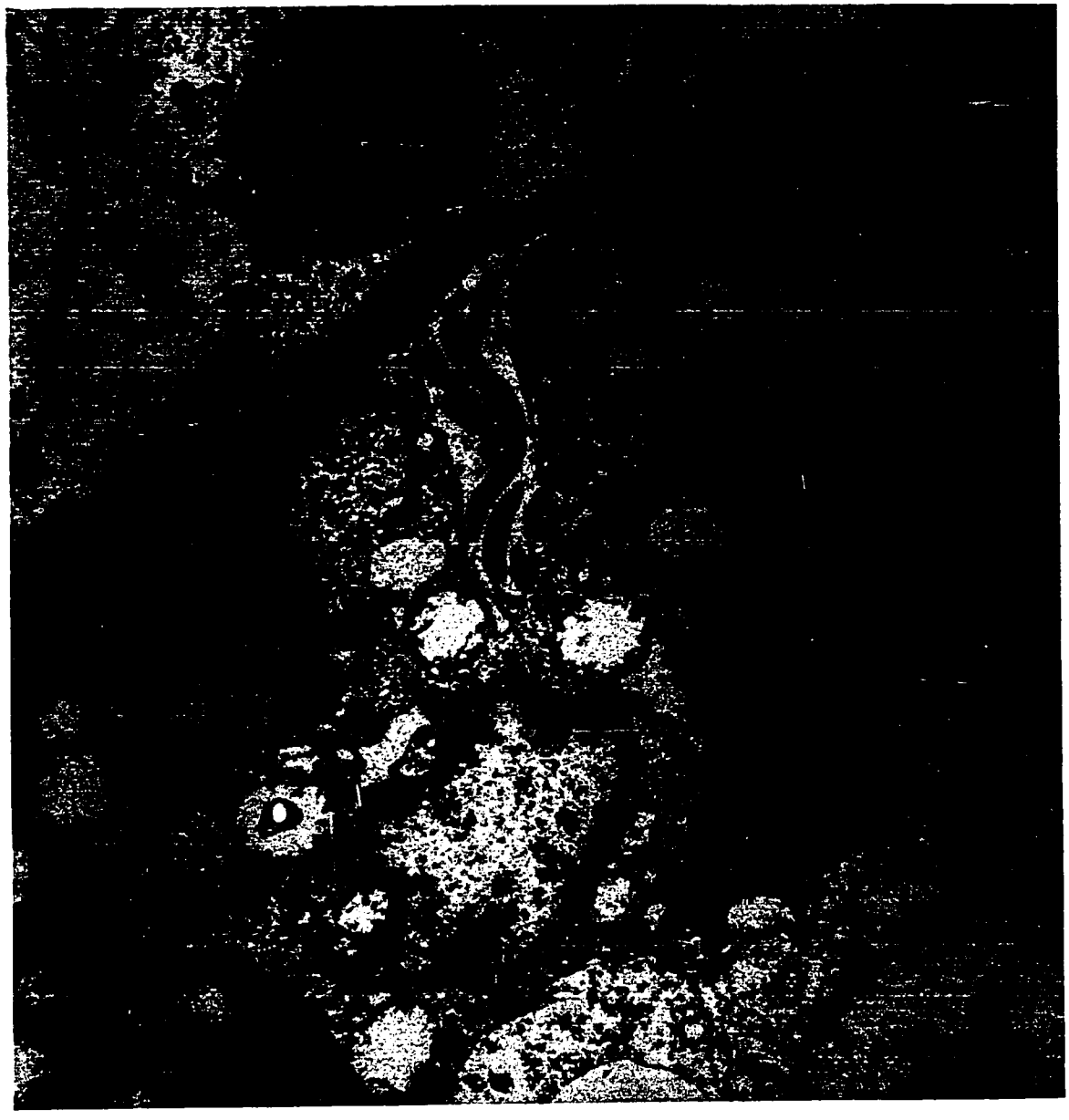


Figure 24. Trial 2, principal, 3 DPI. Large spirochetes in colonic crypt. The envelope appears to have a subunit structure (arrow). Only one membrane is discernible. X35,000

Trial 3

Experimental design

In Trial 3 the lesions in the cecum and three levels of colon were correlated with relative numbers and the location of large spirochetes. Eight conventional Yorkshire-Duroc crossbred pigs were divided into 2 groups, 5 principals and 3 controls. Principals were killed at 1 through 4 DPI. Controls were observed for clinical signs of enteritis for 10 days after which they were challenged and developed swine dysentery. Results of Trial 3 are summarized in Table 6.

Clinical signs

The principals were normal until 3 DPI when the 2 remaining pigs had loose yellow stools. The one pig remaining at 4 DPI had a watery diarrhea and was gaunt and weak as evidenced by posterior incoordination. No clinical signs were noted in the 3 controls.

Gross lesions

The cecal mucosa of the pig killed one DPI was mildly edematous, hyperemic, and covered by a thin fibrinous exudate.

One pig at 2 DPI had no gross lesions. The serosal surface of the cecum and of the proximal third of the colon of the other pig was mildly edematous and hyperemic. The mucosa was inflamed, edematous, and covered by mucofibrinous exudate. Contents of the proximal large intestine were loose and contained blood while in the middle third of the colon the ingesta was normal in consistency but was tightly adhered to

Table 6. Correlation of clinical signs, lesions, and presence of large spirochetes in various portions of the large intestines of pigs. Trial 3

Pig No.	DPI	Clinical Signs	Section of Intestine	Lesions			Large Spirochetes		
				Gross	Light Microscopy	Electron Microscopy	Light Microscopy	Phase Microscopy	Electron Miscroscopy
40	1	-	Cecum	+	+	++	+++	+++	+++
			Colon 1[a]	-	-	-	+	-	-
			Colon 2	-	-	-	+	-	-
			Colon 3	-	-	-	-	-	-
10	2	-	Cecum	-	-	-	-	-	-
			Colon 1	-	-	-	-	-	-
			Colon 2	-	-	-	-	-	-
			Colon 3	-	-	+	+	+	+
20	2	-	Cecum	++	++	+++	+++	+++	+++
			Colon 1	+	++	+++	+++	+++	+++
			Colon 2	-	+	+	++	+	-
			Colon 3	-	-	-	-	-	-
30	3	+	Cecum	+	++	++	++	ND[b]	-
			Colon 1	+	++	+++	+++	+++	+++
			Colon 2	+	++	ND	+++	+	ND
			Colon 3	+	++	+	+++	+++	++
2013	4	++	Cecum	+++	+	ND	-	+	ND
			Colon 1	+++	+++	++	++	++	+
			Colon 2	+++	+++	++	+	++	+
			Colon 3	+++	+++	++	++	++	+

[a]Colon 1= proximal one-third of colon; colon 2= apex of colon; colon 3= distal one-third of colon.

[b]ND = Not done.

the mucosa.

At 3 DPI the serosa of the entire large intestine was hyperemic and edematous. The mucosa of the cecum and colon was hyperemic, edematous, and covered with mucus. Similar but less severe lesions were seen in the rectum.

Lesions at 4 DPI consisted of fibrinonecrotic colitis and cecitis. Contents were watery but contained little mucus and no blood.

The fundic portion of the stomach was hyperemic in all pigs except one which was killed at 2 DPI.

Light microscopy

Lesions were present in the cecum at one DPI and included edema, congestion, and infiltration by polymorphonuclear leukocytes. The luminal epithelium was detached or eroded in some areas. Thick fibrinous exudate on the mucosal surface contained numerous neutrophils. There were masses of large spirochetes in the crypts, within goblet and epithelial cells, and in the lamina propria near the lumen. Lesions were not detected in the colon, but short curved bacteria and a few large spirochetes were present in colonic crypts.

Enteric lesions were not found in the first pig (10) killed at 2 DPI, but small rods were seen in the crypts of the distal colon and there were a few large spirochetes in the lumen. Lesions in the large intestine of the second pig (20) were similar to those on day 1 but extended into the proximal colon. Lesions at the apex of the colon were minimal although some large spirochetes were found in the distal

colon.

Lesions at 3 DPI were similar to those in pig 20 at 2 DPI and were found throughout the cecum and colon.

At 4 DPI lesions were not apparent in the cecum, but spiral organisms with greater dimensions than large spirochetes were in the crypts. Crypts throughout the colon were dilated with mucus, but goblet cells were not numerous. Epithelial cells at the base of the crypts were strongly basophilic and elongated. The lamina propria was edematous and contained numerous neutrophils and lymphocytes. The luminal surface of the mucosa was necrotic and covered by mucofibrinous exudate which contained a few B. coli. Crypts contained large spirochetes and some rod-shaped bacteria.

Scattered foci of mononuclear leukocytes were noted in the renal medullas of pigs killed on days 1, 2, and 4.

Phase microscopy

Large spirochetes were observed in mucosal scrapings of cecum, proximal colon, colon apex, or distal colon in all cases where gross lesions were present (Table 7). They were often most numerous around clumps of epithelial cells. Small spirochetes were rarely seen. Vibrio and spirilla were frequently present both in normal tissues and in those with gross lesions. Fusiform rods were present only at 3 and 4 DPI.

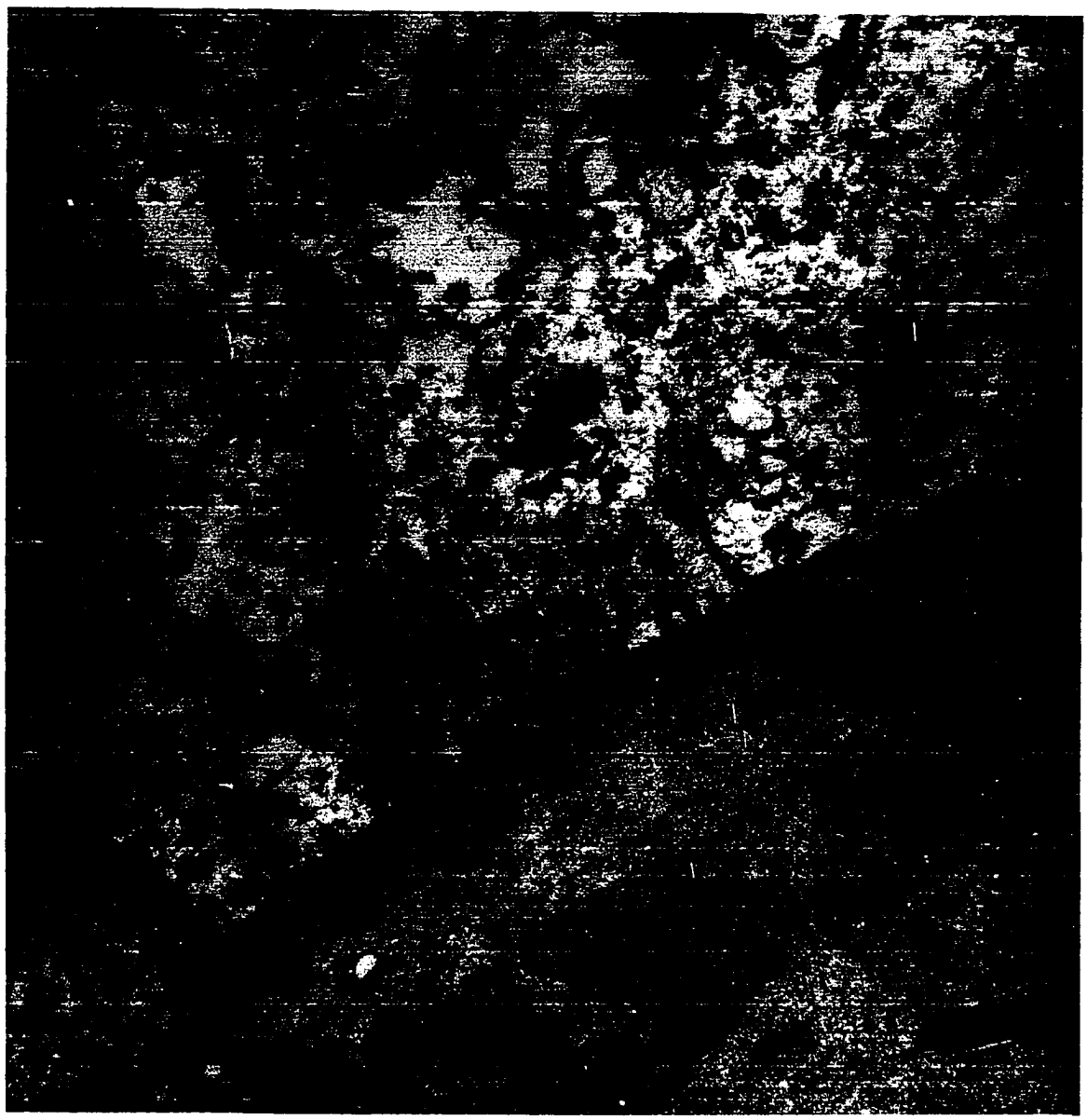
Electron microscopy

At one DPI (pig 40) lesions in the cecum included mitochondrial swelling and a reduction in number and length of microvilli (Figure 25).

Table 7. Results of phase microscopic observation of mucosal scrapings from the large intestines of pigs. Trial 3

Pig No.	DPI	Tissue	Gross Lesions	Large Spirochetes	Small Spirochetes	Vibrio	Sprilla	Fusiforms
40	1	Cecum	+	+++	-	+	+	-
		Col 1	-	-	-	-	+	-
		Col 2	-	-	-	-	+	-
		Col 3	-	-	-	-	+	-
10	2	Cecum	-	-	-	+	+	-
		Col 1	-	-	+	+	+	-
		Col 2	-	-	+	+	+	-
		Col 3	-	+	-	+	-	-
20	2	Cecum	++	+++	-	+++	+++	-
		Col 1	+	+++	-	+	+	-
		Col 2	-	+	+	+	-	-
		Col 3	-	-	-	+	+	-
30	3	Cecum not done						
		Col 1	+	+++	-	+++	+	-
		Col 2	+	+	-	+	+	-
		Col 3	+	+++	-	+	+	+
2013	4	Cecum	+++	+	-	+	-	-
		Col 1	+++	+	-	+	+	+
		Col 2	+++	+	-	+	+	+
		Col 3	+++	+	-	+	+	+

Figure 25. Trial 3, principal, one DPI. Cecal epithelium. Mitochondria are swollen and microvilli are distorted. X20,000



Occasional epithelial cells were in advanced stages of degeneration with vacuolation, degenerate organelles, and absence of microvilli (Figure 26). Numerous neutrophils and some lymphocytes were seen in the lamina propria, between epithelial cells, and in the lumen. A number of the neutrophils in the lamina propria were within vessels with swollen endothelial cells (Figure 27). Numerous large spirochetes were present in crypts and in goblet cells. The colon was normal with the exception of numerous goblet cells which in some areas outnumbered the epithelial cells.

At 2 DPI lesions in one pig (10) were confined to the distal colon and consisted of edema and infiltration of neutrophils between cells in the lamina propria and epithelium. A few large spirochetes were seen in crypts. The other pig (20) had lesions in the cecum and proximal colon similar to those seen in the cecum at one DPI (pig 40). Numerous large spirochetes were seen in crypts and within epithelial cells. In some cases clusters of these organisms could be found within dilated rough endoplasmic reticulum (Figure 28). Lesions at the colon apex were less severe and the distal colon was normal.

Lesions at 3 DPI (pig 30) and 4 DPI (pig 2013) were pronounced and very similar to those described for pig 20 at 2 DPI. Large spirochetes were observed in all sections.

Bacteriology

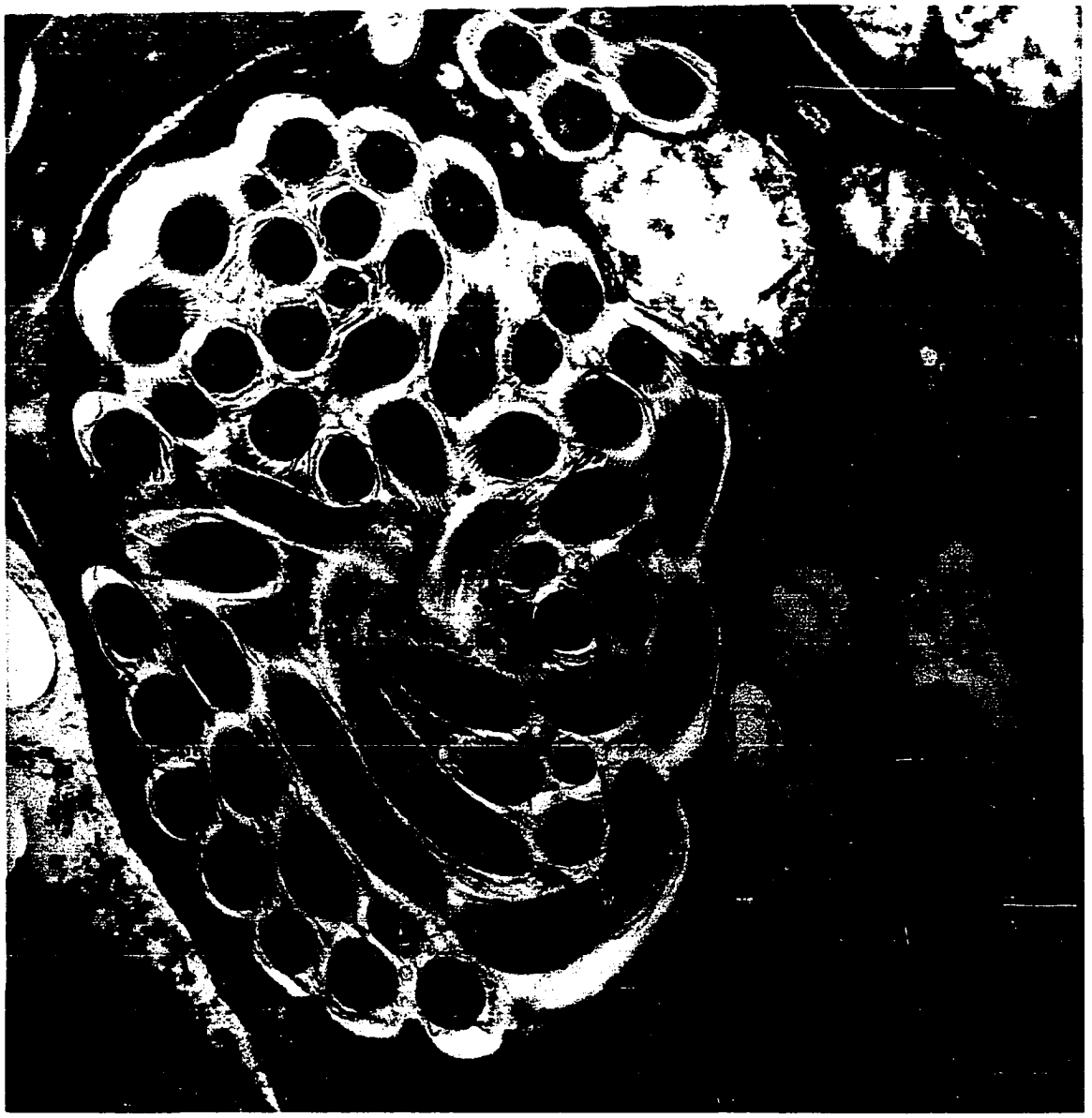
No Salmonella spp. were isolated from the colons of any pigs.

Figure 26. Trial 3, principal, one DPI. Cecal epithelium. There are large spirochetes and other bacteria adjacent to a necrotic epithelial cell. X12,800

Figure 27. Trial 3, principal, one DPI. Lamina propria of cecum. There are neutrophils (N) within and adjacent to a vessel with swollen endothelial cells (E). X7,000


E
N
N

Figure 28. Trial 3, principal, 2 DPI. Colonic epithelial cell. There is a group of large spirochetes within a greatly dilated portion of the endoplasmic reticulum. X29,700



Trial 4

Experimental design

Trial 4 was designed primarily to study hematologic changes, but lesions were also characterized. Experimental animals consisted of 3 Hampshire and 4 Yorkshire pigs all of which received crude gut inoculum. Blood samples were collected daily for 8 DPI and every 2 days thereafter. Pigs were killed either when moribund or at 22 DPI and specimens collected for light, phase, and electron microscopy as well as for bacteriology. Table 8 is a summary of results.

Clinical signs

One pig (6270) remained afebrile but developed diarrhea at 5 DPI and was moribund at 7 DPI; however, the feces never contained blood or mucus. The other 6 pigs had diarrhea beginning at 4 to 6 DPI and had temperature increases of from 2 to 3 F. above pre-inoculation values within 2 days after the onset of diarrhea. The course of the disease varied from 3 to 17 days. All pigs were dehydrated, gaunt, and had watery stools with blood and mucus. Four of these were killed when moribund, but two (6281 and 6282) were beginning to recover when killed at 22 DPI.

Gross lesions

Gross lesions in all except the 2 pigs killed at 22 DPI were similar and consisted of abdominal ascites as well as hyperemia of the mesentery and serosa of the large intestine. The mucosa of the large

Table 8. Correlation of clinical signs, lesions, and presence of organisms in the colons of pigs. Trial 4

Pig No.	DPI	Clinical Signs	Lesions			Large spirochetes		Salmonella
			Gross Lesions	Light Microscopy	Electron Microscopy	Phase Microscopy	Electron Microscopy	Culture
6271	6	+++	+++	+++	+++	+	++	-
6270	7	++	+++	++	++	+	++	+
6272	8	+++	+++	++	++	+++	++	-
0000	20	+++	+++	+++	+++	++	+	+
6280	20	+++	+++	++	++	++	++	-
6281	22	+++	++	++	+++	+	++	-
6282	22	+++	+	++	+	+	-	-

intestine was edematous, hyperemic, and covered with a mucofibrinous to fibrinonecrotic pseudomembrane. Contents of the large intestine were very soft to watery and contained blood and mucus.

Lesions were noted only in the colon of the 2 pigs killed at 22 DPI and consisted of catarrhal enteritis.

Light microscopy

Enteric lesions in all pigs were limited to the large intestine and consisted of edema, congestion, and variable numbers of mononuclear leukocytes and neutrophils in the submucosa and mucosa. Goblet cell hyperplasia was seen in the crypts which were distended with mucus. All but 2 pigs had superficial erosion of the epithelium at the luminal surface which was covered by a layer of mucofibrinous exudate. In most cases only focal areas were eroded, but occasionally the entire surface of the mucosa lacked epithelium. The 2 exceptions were pig 6271 killed at 6 DPI which had focal necrotic areas which extended to the submucosa and pig 0000 killed at 20 DPI in which the superficial one-half of the mucosa was necrotic. A thick fibrinonecrotic pseudomembrane covered the mucosa in both of these pigs.

Lesions other than enteritis consisted of focal accumulations of neutrophils and mononuclear leukocytes in hepatic portal and lobular areas in pigs 6270 and 0000.

Phase microscopy

Large and small spirochetes, vibrio, and spirilla were observed in colon contents or mucosal scrapings from all but pig 6270 at 7 DPI which

had no spirilla.

Electron microscopy

The lesions in the colon of pig 6282 at 22 DPI were mild and consisted of edema, numerous leukocytes, and swollen endothelial cells in the lamina propria. No large spirochetes were seen.

Lesions in the remainder of the pigs were similar and reflected the late stage in the disease at which the pigs were killed. Neutrophils, concentrated in and around swollen vessels, and edema were commonly seen in the lamina propria. Changes in the epithelium resembled those described in Trials 1 through 3 but were more severe as indicated by large numbers of necrotic cells near the lumen. Numerous large spirochetes were present in crypts and in necrotic cells which also contained bacilli in some cases. In some pigs bacilli with spores were observed in the lumen.

Large spirochetes isolated from pig 6272 were negatively stained for electron microscopy. Morphology was similar to large spirochetes observed in ultrathin sections from large intestines in this and previous trials. Size varied from 0.28 to 0.36 μm. in diameter and from 5 to 8 μm. in length. The large spirochetes had a granular protoplasmic cylinder with 12 to 16 axial fibrils spiraling around it (Figure 29). Half of the axial fibrils originated at implantation discs (Figure 30) at either end of the organism and overlapped in the middle. The organisms were surrounded by an envelope.

Small spirochetes were also isolated. They had 2 to 4 axial fibrils

Figure 29. Trial 4. Large spirochete isolated from the colon of a principal (6272). Note axial fibrils spiraling around the protoplasmic cylinder. The envelope was disrupted by exposure to distilled water. PTA stain; X20,300

Figure 30. Trial 4. Large spirochete isolated from the colon of a principal (6272). Half of the axial fibrils originate from implantation discs (arrow) near each end of the organism. PTA stain; X40,500

Figure 31. Trial 4. Small spirochete isolated from the colon of a principal (6272). There are only 4 axial fibrils and the organism is more acutely curved than large spirochetes. PTA stain; X25,200

and were approximately 0.20 to 0.26 μm. in diameter and 4.5 to 5.5 μm. in length (Figure 31). They were more acutely curved than the large spirochetes.

Bacteriology

Salmonella manhattan[a] was isolated from colon contents of pigs 6270 and 0000. Large and small spirochetes were isolated from pig 6272.

Hematology

Packed cell volumes Changes in PCV were not consistent as shown in Table 9. There was no detectable alteration in 3 pigs but increases of from 5 to 10 percent occurred in the other four. Maximum values coincided with severe clinical signs from 0 to 3 days prior to the days on which the pigs were killed.

Red blood cell numbers Total RBC numbers varied throughout the experiment but increases of from 200,000 to 800,000 erythrocytes/cmm. were noted in the same pigs and at about the same times as increases in PCV (Table 9). Marked increases on the day of inoculation were disregarded because they followed a 24 hour period during which feed was withheld.

Erythrocyte sedimentation rates There was no change in the ESR of pig 6270. The rest of the group had increases which began at about the time marked clinical signs were noted (Table 9). Maximum increases varied from 3 to 13 times pre-infection values and usually occurred 2 or more days prior to death. Erythrocyte sedimentation rates never

[a]Serological typing by Dr. B. O. Blackburn, National Animal Disease Laboratory, Ames, Iowa.

Table 9. Packed cell volumes (PCV), red blood cell counts (RBC), and erythrocyte sedimentation rates (ESR) of pigs. Trial 4

DPI		-6	-5	-4	0	1	2	3	4
6271	PCV	32[a]	31	34	33	35	32	34	32
	RBC	6.94[c]	5.81	6.22	8.12	7.39	6.15	6.42	5.81
	ESR	3[d]	3	2	2	3	4	2	15
6270	PCV	30	28	28	30	30	29	31	29
	RBC	6.54	5.49	5.14	7.04	5.59	6.31	5.70	5.80
	ESR	2	3	4	3	4	3	4	2
6272	PCV	35	35	34	33	37	34	35	33
	RBC	6.83	6.46	6.19	7.47	6.65	6.61	7.36	6.17
	ESR	2	2	2	2	5	4	4	3
0000	PCV	32	32	32	34	34	32	33	31
	RBC	6.91	6.45	6.85	7.26	6.32	6.66	6.26	6.17
	ESR	3	4	3	2	7	5	4	3
6280	PCV	30	30	33	28	34	29	33	29
	RBC	7.30	5.76	7.03	7.33	6.35	6.04	4.99	5.64
	ESR	2	2	1	1	5	3	3	2
6281	PCV	30	30	34	ND	33	32	34	31
	RBC	6.19	6.21	6.32	ND	6.85	6.44	ND	6.25
	ESR	3	4	1	ND	3	2	2	2
6282	PCV	29	27	31	29	31	29	32	28
	RBC	6.10	5.08	6.78	7.43	5.88	6.04	7.49	6.33
	ESR	4	5	2	1	5	6	6	4

[a]Percent.

[b]Pig died.

[c]Millions/cmm.

[d]mm./hour.

5	6	7	8	10	11	13	16	18	20
33	34	D[b]	D	D	D	D	D	D	D
5.89	6.07	D	D	D	D	D	D	D	D
38	3	D	D	D	D	D	D	D	D
28	30	28	D	D	D	D	D	D	D
5.29	5.66	5.20	D	D	D	D	D	D	D
2	2	4	D	D	D	D	D	D	D
33	35	37	47	D	D	D	D	D	D
5.87	5.97	6.45	7.65	D	D	D	D	D	D
2	1	13	6	D	D	D	D	D	D
30	30	30	29	28	28	28	35	32	30
5.65	5.48	5.44	5.47	ND	4.94	4.92	5.52	5.90	4.84
6	14	34	36	38	20	3	1	6	9
32	35	29	33	30	33	38	37	39	36
6.06	6.63	5.66	6.07	ND	5.81	6.67	6.73	7.02	7.30
11	2	3	2	10	8	4	10	6	27
33	35	33	32	31	35	36	ND	39	35
6.28	6.41	6.38	6.34	ND	6.25	6.73	ND	7.19	7.04
1	1	3	5	6	5	2	ND	7	14
30	32	31	30	27	30	33	37	36	34
5.48	5.82	5.60	5.52	ND	5.42	5.76	6.84	6.95	6.74
4	2	4	3	7	6	3	6	22	7

exceeded 5 mm./hour before infection but were as high as 38 mm./hour in infected animals.

White blood cell counts Pre-inoculation WBC counts ranged from 13,600 to 21,900 with a mean of 17,000 cells/cmm. Moderate increases occurred during the course of the disease but sharp increases were noted in all but one pig just prior to death (Table 10). White blood cell counts on the last sample before death varied from 22,600 to 68,000 cells/cmm. with a mean of 37,600 cells/cmm.

Differential leukocyte counts Lymphocytes were the predominant cell type in pre-inoculation blood smears. Their numbers ranged from only slightly higher to more than twice the number of mature neutrophils. General trends were obvious during the trial although daily fluctuations in specific numbers were marked in some instances. After clinical signs appeared, 3 of the 7 pigs had a sharp drop in numbers of segmented neutrophils with a concurrent increase in band neutrophils which in some cases greatly exceeded the mature forms. In the rest of the pigs, usually less acutely affected, there was a gradual increase in both segmented and band neutrophils to several times higher than pre-infection numbers. Increases in neutrophilic metamyelocytes to as high as 2,700/cmm. coincided with the increases in band neutrophils. Lymphocytes in most pigs increased in the later stages of the disease to approximately twice pre-infection levels. No change was noted in monocytes, eosinophils, or basophils.

Total plasma protein and fibrinogen levels Simultaneous increases in TP and fibrinogen were noted during clinical disease in 6 of the 7

Table 10. White blood cell counts in pigs. Trial 4

DPI	-6	-5	-4	0	1	2	3	4	5	6	7	8	10	11	13	16	18	20	22
Pig No.																			
6271	20.1[a]	19.9	21.9	30.1	17.9	20.8	21.2	23.5	26.2	44.0	D	D	D	D	D	D	D	D	D
6270	14.2	15.2	16.8	24.8	17.1	22.8	21.8	21.9	21.1	20.6	22.6	D	D	D	D	D	D	D	D
6272	18.1	17.8	19.7	15.2	16.1	21.6	23.2	19.4	21.2	18.3	16.1	26.6	D	D	D	D	D	D	D
0000	14.8	18.4	15.8	16.9	14.4	18.4	16.0	15.8	17.7	15.7	15.4	15.5	24.6	18.4	14.9	29.8	23.6	38.8	D
6280	13.6	14.3	17.0	18.7	13.7	17.9	15.8	20.1	17.2	21.4	19.2	18.3	25.3	19.3	27.5	31.5	41.8	68.0	D
6281	15.6	14.6	16.0	ND	13.8	14.1	18.2	17.6	17.2	15.6	17.3	15.6	20.0	22.2	20.7	ND	25.4	28.3	28.1
6282	16.5	16.9	21.1	19.5	16.6	15.7	21.8	18.2	19.8	18.3	16.8	17.3	23.7	19.0	22.0	29.8	23.0	28.7	35.3

[a]Thousands/cmm.

pigs as shown in Table 11. Peak levels were reached in the last blood sample from 4 moribund animals killed during the trial and 2 to 4 days prior to the day pigs 6281 and 6282 were killed at the termination of the experiment. Pre-inoculation TP levels ranged from 4.4 to 5.5 Gm./100ml. and maximum post-inoculation values ranged from 6.0 to 8.8 Gm./100ml. Pre-inoculation fibrinogen levels ranged from 0.1 to 0.3 Gm./100ml. except for pigs 6281 and 6282 which had higher values in the first 2 blood samples. Maximum post-inoculation fibrinogen levels were from 0.6 to 1.0 Gm./100ml. Reductions in TP/fibrinogen ratios coincided with increased fibrinogen.

No significant alterations occurred in either TP or fibrinogen levels in pig 6270.

Table 11. Total plasma protein (TP), plasma fibrinogen (Fib), and total plasma protein/fibrinogen ratios in pigs. Trial 4

	-6	-5	-4	0	1	2	3	4	5	6	7	8	10	11	13	16	18	20	22
Pig No. 6271																			
TP	4.7[a]	4.5	4.5	5.4	5.6	5.2	5.1	5.2	5.8	6.4	D	D	D	D	D	D	D	D	D
Fib	.1[a]	.2	.2	.3	.2	.2	.2	ND	.8	.9	D	D	D	D	D	D	D	D	D
TP/Fib	47.0	22.5	22.5	18.0	28.0	26.0	25.5	--	7.3	7.1	D	D	D	D	D	D	D	D	D
Pig No. 6270																			
TP	4.8	4.6	4.4	4.7	4.5	4.6	4.4	4.7	4.3	4.5	4.7	D	D	D	D	D	D	D	D
Fib	.2	.2	.1	.1	.1	.1	.1	ND	.1	.2	.1	D	D	D	D	D	D	D	D
TP/Fib	24.0	23.0	44.0	47.0	45.0	46.0	44.0	--	43.0	22.5	47.0	D	D	D	D	D	D	D	D
Pig No. 6272																			
TP	5.2	5.2	5.0	5.3	5.8	5.2	5.2	5.5	5.2	5.4	6.2	8.4	D	D	D	D	D	D	D
Fib	.2	.3	.3	.2	.3	.3	.2	ND	.2	.3	.4	1.0	D	D	D	D	D	D	D
TP/Fib	26.0	17.3	16.7	26.5	19.3	17.3	26.0	--	26.0	18.0	15.5	8.4	D	D	D	D	D	D	D
Pig No. 0000																			
TP	5.3	5.2	5.1	5.4	5.3	5.3	5.0	5.3	5.1	5.0	5.4	5.0	5.5	5.6	5.9	5.7	5.6	6.0	D
Fib	.2	.2	.1	.1	.2	.1	.2	ND	.3	.5	.7	.8	ND	ND	.3	.3	.5	.5	D
TP/Fib	26.5	26.0	51.0	54.0	26.5	53.0	25.0	--	17.0	10.0	7.7	6.3	--	--	19.7	19.0	11.2	12.0	D
Pig No. 6280																			
TP	4.7	4.7	4.7	4.9	5.4	4.6	4.6	4.8	5.1	5.3	4.6	5.3	5.4	5.6	7.1	7.1	8.1	8.8	D
Fib	.1	.2	.1	.1	.1	.1	.1	ND	.1	.2	.1	.1	ND	ND	.3	.6	.8	.8	D
TP/Fib	47.0	23.5	47.0	49.0	54.0	46.0	46.0	--	51.0	26.5	46.0	53.0	--	--	23.7	11.8	10.1	11.0	D
Pig No. 6281																			
TP	5.2	5.1	5.5	ND	5.7	5.2	5.2	5.3	5.5	5.4	6.1	5.6	5.4	5.5	6.2	ND	7.5	8.1	6.4
Fib	.5	.4	.3	ND	.3	.2	.1	ND	.3	.2	.2	.2	ND	ND	.2	ND	.6	.5	.4
TP/Fib	10.4	12.8	18.3	--	19.0	26.0	52.0	--	18.3	27.0	30.5	23.0	--	--	31.0	--	12.5	16.2	16.0
Pig No. 6282																			
TP	5.1	4.6	4.9	5.0	5.1	5.1	5.1	5.0	5.2	5.3	5.4	5.1	4.9	5.1	5.6	7.0	7.3	7.1	6.5
Fib	.6	.3	.3	.1	.1	.3	.3	ND	.2	.2	.1	.1	ND	ND	.1	.7	.8	.6	.5
TP/Fib	8.5	15.3	16.3	50.0	51.0	17.0	17.0	--	26.0	26.5	54.0	51.0	--	--	56.0	10.0	9.1	11.8	13.0

[a]Gm./100ml.

Trial 5

Experimental design

The main purpose of Trial 5 was to study hematologic changes in swine dysentery. Nine Yorkshire-Duroc crossbred pigs, 6 principals and 3 controls, were placed in isolation units. Daily collection of blood samples began 2 days prior to inoculation. Only 3 pigs developed clinical signs typical of swine dysentery. One of these (pig 9) died at 7 DPI. Blood samples were collected from the other 2 pigs with dysentery until 15 DPI but were discontinued in the rest of pigs in the trial at 11 DPI. All pigs were held until 17 DPI to determine whether clinical signs would appear and principals were then killed. Controls were challenged with crude gut inoculum and developed swine dysentery.

Clinical signs

Response to the inoculum was erratic. Pig 9 had watery stools containing blood and mucus 4 DPI and died 7 DPI. Pig 1 began to recover after a period, 7 through 12 DPI, of mucohemorrhagic diarrhea. Pig 7 had loose stools with blood and mucus at 10 DPI but began to recover at 11 DPI. Loose stools were noted in pig 6 at 5 through 10 DPI. There was no detectable change in rectal temperature in any of the pigs. Only transient soft stools were observed in the other 2 principals. The 3 controls remained normal.

Gross lesions

Gross lesions in pig 9 which died at 7 DPI included fibrinous

cecitis and fibrinonecrotic colitis. The stomach was empty. The serosa of the small intestine was hyperemic but the mucosa was normal. The only enteric lesions in the 5 principals killed at the termination of the experiment were mild hyperemia in the colon of pig 3 and hyperemia and edema in the colon of pig 7.

Light microscopy

Lesions in the cecum and colon of pig 9 included congestion, edema, and necrosis of the mucosa. The zone of necrosis in some areas of the colon extended to the muscularis mucosa and was demarcated by a band of neutrophils. The only enteric lesions noted in the rest of the principals were congestion, edema, and focal erosions of the superficial epithelium of the large intestine in pigs 3 and 7. Large spirochetes were seen in the colons of pigs 3, 6, 7, and 9.

Phase microscropy

There were large spirochetes in mucosal scrapings from the colons of principals 3, 6, 7, and 9. Small spirochetes were observed in principals 1, 2, 7, and 9. All but pig 7 had vibrio. Spirilla and fusiform rods were not recorded.

Hematology

Since diarrhea was observed in only 4 principals, tables of results that follow are condensed to include only those 4 pigs listed in decreasing order of severity of clinical signs.

Packed cell volumes An increase of from 2 to 5 percent in PCV

was detected in all pigs in the trial on the day of inoculation which followed 24 hours during which feed was withheld. The 3 pigs with marked clinical signs had PCV values which varied from 4 to 7 percent above pre-inoculation values and roughly coincided with periods of diarrhea.

Erythrocyte sedimentation rates Increases in ESR occurred in only 2 pigs. Five days after diarrhea began (12 DPI), pig 1 had a transient increase to 13 mm./hour from a previous high of 2 mm./hour. The ESR of pig 9 was 22mm./hour 2 days prior to death and 4 mm./hour 1 day prior to death. The maximum previous rate for pig 9 was 2 mm./hour.

White blood cell counts Marked increases in WBC counts occurred in pigs 1 and 9 at about the time clinical signs were noted, but no significant changes in WBC counts were observed in other pigs. A decline in WBC numbers in most principals was evident at 3 through 5 DPI (Table 12).

Table 12. White blood cell counts in pigs. Trial 5.

DPI:	-2	-1	1	2	3	4	5	6	7	8	9	10	11
Pig No:													
9	18.8[a]	20.1	19.7	30.9	37.9	38.8	28.6	42.7	D	D	D	D	D
1	13.0	15.2	14.8	23.7	12.5	11.6	11.5	9.7	22.3	28.5	34.8	34.2	26.2
7	ND	17.5	ND	25.2	10.9	14.1	14.8	14.7	18.7	22.7	21.9	19.1	24.4
6	19.2	16.8	20.8	25.0	8.9	14.6	13.3	11.8	20.6	19.5	25.6	18.8	22.4
3	19.6	19.1	23.2	29.3	26.8	13.6	18.7	17.6	20.4	28.1	23.4	20.2	24.1
2	13.1	15.3	12.7	19.3	ND	9.4	9.0	9.2	18.1	18.8	23.9	16.2	17.1
5-c	ND	23.6	20.9	25.4	27.0	27.9	26.3	27.7	ND	31.1	27.8	ND	27.0
8-c	16.9	14.9	18.6	ND	18.8	15.8	16.7	15.3	ND	20.0	17.5	ND	14.1
10-c	16.2	15.5	12.7	17.3	18.5	16.1	12.7	16.8	ND	16.5	14.8	ND	13.0

[a]All values recorded as thousands/cmm.

Differential leucocyte counts The number of lymphocytes was approximately equal to or much greater than the number of neutrophils in nearly all pigs without clinical signs of swine dysentery. Significant changes in relative and absolute numbers of neutrophils and lymphocytes were noted in 3 of 4 pigs listed in Table 13. Pig 9 had a marked left shift with numbers of band neutrophils much greater than segmented neutrophils. There were also 1,700 neutrophilic metamyelocytes/cmm. on the day before death. There was an absolute but not a relative increase in lymphocytes. Similar but less marked changes were noted in pig 1 which was not as severely affected, and only increased numbers of mature neutrophils were seen in pigs 6 and 7.

Total plasma protein and fribrinogen levels Transient increases in TP were noted in principals and controls for 2 days after inoculation which followed 24 hours of feed withdrawal. The only significant changes in levels of TP occurred in 4 principals during periods of diarrhea and consisted of increases above a pre-inoculation mean of 6.1 Gm./100ml. to maximum values of from 7.0 to 8.9 Gm./100ml. (Table 14).

Plasma fibrinogen levels in all samples remained below 0.3 Gm./100ml. except in pigs 1, 7, and 9 which had maximum values during periods of diarrhea of 1.0, 0.4, and 0.7 Gm./100ml. respectively (Table 14). These changes were reflected in decreased TP/fibrinogen ratios.

Serum protein fractions A marked decrease in albumin/globulin ratios occurred during diarrhea (Table 15) as a result of decreased serum albumin and increased alpha globulin levels. The increase in alpha globulin was primarily in the alpha-2 fraction. Beta and gamma

Table 13. Absolute numbers of neutrophils and lymphocytes in pigs. Trial 5

Pig no.	Cell Type	DPI -2	-1	1	2	3	4	5
9	Lymph[a]	12.4[b]	14.1	15.0	14.2	13.6	12.4	8.6
	SN[c]	6.0	5.2	3.9	15.5	24.3	25.6	13.2
	BN[d]	0	0	0	0	0	0	4.6
	NM[e]	0	0	0	0	0	0	1.1
1	Lymph	9.9	9.4	10.1	13.3	6.0	7.4	6.4
	SN	2.9	4.9	3.2	10.4	6.0	3.3	5.1
	BN	0	0	0	0	0	0	0
	NM	0	0	0	0	0	0	0
7	Lymph	ND	12.9	ND	15.6	4.1	7.6	12.4
	SN	ND	4.2	ND	9.6	5.9	6.2	2.1
	BN	ND	0	ND	0	0	0	0
	NM	ND	0	ND	0	0	0	0
6	Lymph	13.4	11.4	14.5	12.5	4.4	6.1	6.9
	SN	5.0	4.7	4.6	12.0	4.3	7.6	6.1
	BN	0	0	0	0	0	0	0
	NM	0	0	0	0	0	0	0

[a]Lymph = lymphocytes.

[b]All values recorded as thousands/cmm.

[c]SN = segmented neutrophils.

[d]BN = band neutrophils.

[e]NM = neutrophilic metamyelocytes.

6	7	8	9	10	11	12	13	15
19.7	D	D	D	D	D	D	D	D
6.8	D	D	D	D	D	D	D	D
14.5	D	D	D	D	D	D	D	D
1.7	D	D	D	D	D	D	D	D
6.0	13.0	19.9	24.7	18.5	13.7	9.3	7.3	10.1
3.1	6.2	4.6	5.9	12.3	11.5	15.5	17.2	13.2
0	3.1	4.0	3.8	3.4	0.5	0.5	0	0.2
0	0	0	0	0	0	0	0	0
10.3	9.6	8.6	15.8	9.2	14.6	13.9	12.5	10.8
4.0	8.2	13.6	6.1	9.2	9.8	14.8	22.3	17.6
0	0	0	0	0	0	0.3	0	0
0	0	0	0	0	0	0	0	0
7.8	9.5	7.8	11.3	10.9	13.9	14.9	ND	ND
4.0	10.7	11.3	13.8	7.5	8.3	13.1	ND	ND
0	0	0	0	0	0	0	ND	ND
0	0	0	0	0	0	0	ND	ND

Table 14. Total plasma protein (TP), plasma fibrinogen (Fib), and total plasma protein/fibrinogen ratios (TP/Fib) in pigs. Trial 5

Pig no.	DPI:	-2	-1	1	2	3	4	5	6	8	9	10	11	12	13	15
9	TP	5.7	5.5	6.0	5.8	5.7	5.7	6.1	7.4	D	D	D	D	D	D	D
	Fib	0.2	0.2	0.2	0.2	0.1	0.2	0.3	0.7	D	D	D	D	D	D	D
	TP/Fib	28.5	27.5	30.0	29.0	57.0	28.5	20.3	10.6	D	D	D	D	D	D	D
1	TP	6.2	6.4	7.0	7.2	6.8	6.5	6.0	6.3	7.8	8.7	8.9	8.4	7.4	6.8	6.7
	Fib	0.2	0.2	0.2	0.2	0.2	0.3	0.1	0.3	0.8	1.0	0.8	0.6	0.5	0.3	0.3
	TP/Fib	31.0	32.0	35.0	36.0	34.0	21.6	60.0	21.0	9.8	8.7	11.1	14.0	14.8	22.6	22.3
7	TP	ND	6.1	ND	7.3	6.6	6.6	6.3	6.5	6.7	7.3	7.1	6.5	6.7	6.8	7.2
	Fib	ND	0.2	ND	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4
	TP/Fib	ND	30.5	ND	26.3	22.0	22.0	31.5	21.7	22.3	26.3	23.6	21.6	22.3	17.0	18.0
6	TP	6.5	6.2	6.9	7.1	7.2	6.6	6.3	6.4	6.7	7.0	6.7	7.0	6.6	ND	ND
	Fib	0.3	0.2	0.1	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.2	0.2	ND	ND
	TP/Fib	21.7	31.0	69.0	35.5	36.0	33.0	36.5	21.5	22.5	23.5	33.5	23.3	33.0	ND	ND

Table 15. Serum protein fractions in pigs. Trial 5

Pig no.	DPI:	-2	-1	1	2	3	4	5	6	7	8	9	10	11
9	Albumin	49[a]	55	54	58	64	43	46	32	D	D	D	D	D
	Alpha	20	23	17	23	23	24	27	38	D	D	D	D	D
	Beta	17	11	22	11	9	15	14	15	D	D	D	D	D
	Gamma	14	11	7	8	5	18	13	15	D	D	D	D	D
	A/G[b]	.96	1.22	1.17	1.38	1.75	.75	.85	.47	D	D	D	D	D
1	Albumin	47	49	54	48	49	49	54	46	43	37	31	32	29
	Alpha	25	26	23	28	22	22	20	27	29	36	38	39	38
	Beta	13	15	11	11	15	13	15	13	16	12	14	14	15
	Gamma	15	10	12	13	14	16	11	14	12	15	17	15	18
	A/G	.89	.96	1.17	.92	.96	.96	1.17	.85	.75	.58	.45	.47	.41
7	Albumin	50	50	48	46	49	49	48	46	45	42	39	37	49
	Alpha	22	26	16	24	24	25	21	25	25	29	30	31	21
	Beta	13	13	23	17	14	14	15	15	14	15	14	15	14
	Gamma	15	11	13	13	13	12	16	14	16	14	17	17	16
	A/G	1.00	1.00	.92	.85	.96	.96	.92	.85	.82	.72	.64	.58	.96
6	Albumin	46	52	42	41	43	49	48	41	45	41	45	38	42
	Alpah	24	23	23	21	24	22	21	25	24	26	24	26	25
	Beta	15	14	17	18	17	15	16	18	15	15	17	15	12
	Gamma	15	11	18	20	16	14	15	16	16	18	14	21	21
	A/G	.85	1.08	.72	.70	.75	.96	.92	.70	.82	.70	.82	.61	.72

[a]Values in percent of TP.

[b]A/G = Albumin/Globulin ratio.

globulin levels remained fairly constant. Little variation was seen in serum proteins in pig 6 which had only mild diarrhea.

Serum electrolytes Serum Na and K levels were somewhat variable in all animals. Values for clinically affected animals are shown in Table 16. Serum Na levels declined to minimums of 126.1 mEq./L. in pig 1 at 10 DPI and 130.5 mEq./L. in pig 9 at 6 DPI. These values were approximately 5 to 8 mEq./L. below values in clinically normal animals. A slight decrease also occurred in pig 6 at 11 DPI.

Serum K levels 2 to 3 days after the onset of diarrhea ranged from 6.1 to 6.9 mEq./L. as compared to a pre-inoculation mean of 4.7 mEq./L.

Table 16. Serum sodium and potassium levels in pigs. Trial 5

Pig No.	DPI:	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12
9	Na	141.4[a]	141.4	139.2	134.9	145.7	139.2	135.5	130.5	D	D	D	D	D	D
	K	3.6	4.2	4.5	4.1	3.8	4.1	4.2	6.7	D	D	D	D	D	D
1	Na	134.9	137.0	139.2	147.9	147.9	137.0	139.2	141.4	143.6	139.2	134.9	126.1	130.5	134.9
	K	4.4	4.6	5.1	5.2	5.0	4.7	4.6	4.9	6.3	6.0	6.9	5.8	5.9	5.8
7	Na	143.6	137.0	137.0	147.9	147.9	141.4	141.4	143.6	145.7	145.7	141.4	141.4	139.2	141.4
	K	5.5	4.7	ND	ND	5.1	6.0	5.6	5.6	5.5	5.3	6.0	6.1	5.5	5.8
6	Na	141.4	134.9	137.0	143.6	147.9	141.4	139.2	143.6	150.1	150.1	150.1	139.2	134.9	137.4
	K	5.4	5.1	5.4	5.1	5.5	5.4	5.0	4.9	6.7	5.5	6.4	4.7	5.6	5.4

[a]All values expressed in mEq./L.

Trial 6

Experimental design

Trial 6 was a repeat of Trial 5 because of the erratic clinical response in Trial 5. Ten Yorkshire-Duroc crossbred pigs were placed in an isolation unit, and 2 days later daily sampling of blood was begun. Two pigs were eliminated from the trial--one because of severe arthritis and the other because of abnormal pre-inoculation hematologic findings. The remaining 8 pigs were divided into two groups, 6 principals (pigs 1, 2, 3, 6, 8 and 10) and 2 controls (pigs 4 and 9). Controls were killed when the last principal died.

Clinical signs

Typical signs of swine dysentery were seen in all principals. Pig 1 had a mild diarrhea at 2 DPI and died at 3 DPI. Watery diarrhea began in the remainder of the group at 2 DPI at which time the feces of 2 pigs contained blood. By 5 DPI all had blood and mucus in their stools and varied in clinical appearance from weak and dehydrated to moribund. Watery stools containing blood and mucus were noted until the time of death which varied from 5 to 12 DPI. Four of the 6 principals had rectal temperature increases of from 1.2 to 2.6 F. above pre-inoculation peak levels. The only clinical abnormality in the controls was in pig 4 which had soft stools and a 1-2 F. temperature increase on the second through the fourth day.

Gross lesions

Pig 1 at 3 DPI and pig 6 at 11 DPI had an acute fibrinous cecitis and colitis with focal hemorrhages of the mucosa. The serosa was congested in the ileum of pig 1 and the fundic portion of the stomach was hyperemic in both pig 1 and 6. A thin, white fibrinonecrotic pseudomembrane with focal hemorrhagic areas was found on the mucosal surface throughout the large intestines of the rest of the principals. Walls of the cecum and colon were hyperemic and mildly edematous.

No enteric lesions were noted in the controls. Pigs 2, 3, 6, and 9 had red consolidated areas typical of mycoplasmal pneumonia at the apex of the apical and cardiac lobes of the lungs.

Light microscopy

In most principals lesions were found throughout the large intestine. There were edema, congestion and infiltrations of lymphocytes and a few neutrophils in the submucosa and mucosa. Edema was usually mild. Mucosal crypts were dilated and there were basophilic, elongated epithelial cells with numerous mitotic figures at the base of the crypts. There were large spirochetes in crypts, in and between epithelial cells, and in the lamina propria in some areas. Necrosis of the mucosa was extensive but superficial and there were often neutrophils at the junction between necrotic and normal tissue. A thick mucofibrinous to fibrinonecrotic pseudomembrane lined the lumen. Dilated submucosal glands contained mucus, necrotic debris, and bacteria.

Villi in the duodenum and jejunum of pig 6 were atrophied. A layer of

short rod-shaped bacteria was adjacent to the epithelium in these tissues. There was congestion near the gastric lumen in pigs 1 and 3. Small foci of mononuclear leukocytes were present in the renal medullas of pigs 2, 3, 8, and 10.

The only lesions observed in the controls were scattered small foci of mononuclear leukocytes in the liver of pig 4.

Phase microscopy

Numerous large spirochetes were found in mucosal scrapings from the colons of all principals but there were none in the controls. Small spirochetes were seen in 4 principals and 1 control, and vibrio in 4 principals and both controls. Incidence of other organisms was not recorded.

Bacteriology

No Salmonella spp. were isolated.

Hematology

Packed cell volumes Maximum increases in PCV in the principals occurred 2 to 5 DPI and varied from 1 to 11 percent (Table 17). A gradual decrease to or slightly below previous levels was noted in pigs surviving past day 5. Values in control pig 9 remained constant; however, there was a slight increase in pig 4 at 1 through 3 DPI.

Red blood cell numbers RBC numbers varied widely throughout the trial. There was a tendency for increases to parallel PCV values (Table 17).

Table 17. Packed cell volumes (PCV), red blood cell counts (RBC), and erythrocyte sedimentation rates (ESR) of pigs. Trial 6

Pig no.	DPI	-3	-2	-1	1	2	3
1	PCV	30[a]	32	32	33	43	D
	RBC	5.9[b]	6.5	7.2	6.3	8.0	D
	ESR	7[c]	8	7	9	6	D
2	PCV	42	37	35	37	40	44
	RBC	7.0	6.6	7.0	7.4	6.7	7.4
	ESR	6	9	10	19	18	7
10	PCV	38	37	37	35	40	39
	RBC	7.5	7.7	9.2	7.6	8.0	7.0
	ESR	6	8	9	15	13	20
8	PCV	36	37	36	37	38	40
	RBC	7.0	6.4	6.9	8.0	6.5	8.2
	ESR	1	6	2	22	13	8
6	PCV	38	35	36	37	38	38
	RBC	7.9	7.6	7.0	8.5	7.6	7.4
	ESR	26	10	9	6	4	1
3	PCV	42	38	38	38	45	40
	RBC	8.1	7.1	7.7	7.4	7.9	8.0
	ESR	1	4	4	3	1	3
4-C	PCV	35	35	35	38	38	37
	RBC	7.0	7.4	6.5	8.6	7.0	7.0
	ESR	8	7	8	2	10	6
9-C	PCV	34	34	32	35	33	29
	RBC	7.0	6.7	6.5	7.2	6.0	5.7
	ESR	5	13	10	23	6	3

[a]Percent.

[b]Millions/cmm.

[c]mm./hour.

4	5	6	7	8	9	10	11	12
D	D	D	D	D	D	D	D	D
D	D	D	D	D	D	D	D	D
D	D	D	D	D	D	D	D	D
44	43	D	D	D	D	D	D	D
8.2	6.7	D	D	D	D	D	D	D
3	3	D	D	D	D	D	D	D
43	45	D	D	D	D	D	D	D
8.2	7.3	D	D	D	D	D	D	D
29	2	D	D	D	D	D	D	D
44	42	38	35	D	D	D	D	D
7.4	6.3	6.5	5.8	D	D	D	D	D
19	13	22	54	D	D	D	D	D
36	38	36	35	37	38	35	D	D
7.8	7.7	7.6	7.0	7.5	8.3	7.2	D	D
4	2	15	48	50	20	4	D	D
40	41	40	39	38	37	36	37	35
7.7	6.8	7.3	8.9	7.6	8.5	7.0	7.2	6.7
15	36	11	42	45	8	4	5	15
34	34	33	32	32	ND	32	31	34
6.4	6.2	6.1	6.0	6.3	ND	7.4	5.9	6.8
5	8	7	10	8	ND	6	7	5
31	33	33	30	33	34	32	33	33
6.0	5.5	6.1	5.0	6.0	6.6	6.3	6.5	7.1
3	4	4	1	5	3	2	2	2

Erythrocyte sedimentation rates The mean pre-inoculation ESR was 7 mm./hour. Three principals and 1 control had transient increases on the first DPI (Table 17). Increases of ESR to 22 to 50 mm./hour occurred in 4 of 6 principals 4 or more DPI. A decline to normal values occurred prior to death in pigs 3, 6, and 10. Controls had no notable increases in ESR except for pig 9 which had an isolated high value of 23 mm./hour one day after inoculation of principals.

White blood cell counts Significant increases in WBC numbers occurred in only 3 principals. Maximum values were as follows: pig 2--24,500 leukocytes/cmm. at 4 DPI; pig 3--28,500 leukocytes/cmm. at 6 DPI; and pig 10--36,600 leukocytes/cmm. at 5 DPI (Table 18).

Differential leukocyte counts All principals had a marked left shift which began shortly after inoculation and usually continued to increase in severity until death occurred. Absolute neutrophil counts remained fairly constant, but segmented neutrophils declined to minimums of from 234 to 1,240 cells/cmm. with a concurrent increase in band neutrophils to maximums of from 7,110 to 10,920 cells/cmm. A marked increase in numbers of neutrophilic metamyelocytes accompanied the left shift. This increase reached 412 neutrophilic metamyelocytes/cmm. in pig 1, which was the first to die, and ranged from 1,750 to 4,900 neutrophilic metamyelocytes/cmm. in the other 5 principals. Typical changes in absolute leukocyte numbers are illustrated in Figure 32.

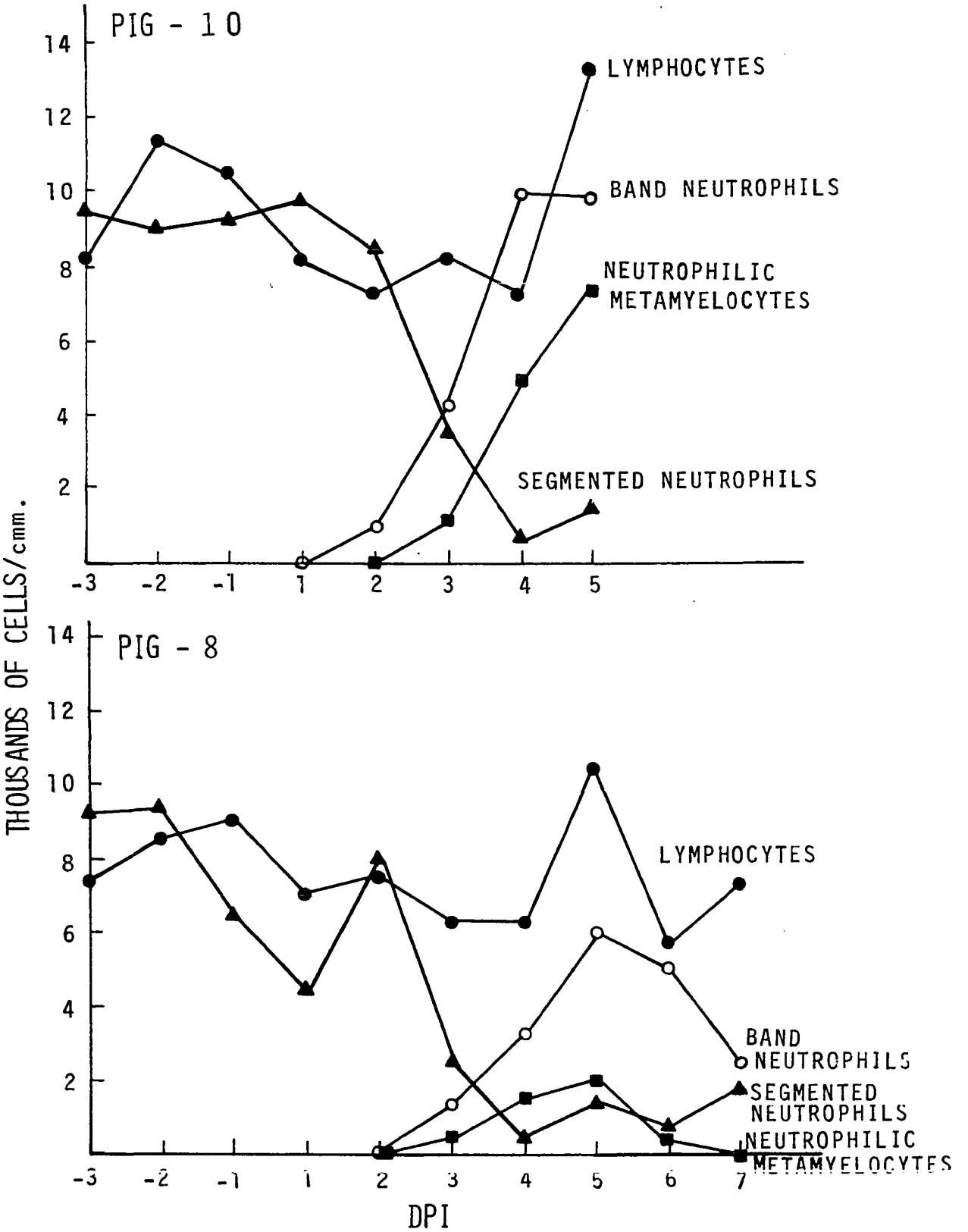
Slight increases in monocyte numbers were noted in all principals except pig 1 and reached maximum levels of 500 to 1,830 cells/cmm. Lymphocyte numbers remained fairly stable until late in the disease

Table 18. White blood cell counts in pigs. Trial 6

Pig no.	DPI: -3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12
1	18.2[a]	19.3	13.4	13.8	20.7	D	D	D	D	D	D	D	D	D	D
2	15.6	16.5	17.2	13.1	14.2	15.5	24.5	21.1	D	D	D	D	D	D	D
10	19.0	20.9	20.7	19.0	16.8	17.8	23.4	36.6	D	D	D	D	D	D	D
8	17.2	18.7	16.5	13.0	15.8	10.6	11.3	20.2	12.5	11.8	D	D	D	D	D
6	35.3	23.3	22.4	16.9	24.6	16.7	14.7	18.4	16.6	19.2	25.0	22.2	21.8	D	D
3	12.4	14.4	14.3	8.0	12.6	7.0	9.9	23.6	28.5	15.5	14.8	13.4	12.7	15.8	13.3
4-C	20.4	26.5	24.2	17.7	21.2	16.3	16.5	15.4	18.0	17.3	21.4	ND	15.2	18.1	21.1
9-C	18.3	21.8	19.6	17.7	19.9	14.6	14.1	18.4	21.2	17.5	21.6	19.0	16.6	17.5	18.4

[a]Thousands/cmm.

Figure 32. Trial 6, principals 8 and 10. Leukograms which illustrate the left shift observed in pigs with swine dysentery


PIG - 10
LYMPHOCYTES
BAND NEUTROPHILS
NEUTROPHILIC METAMYELOCYTES
SEGMENTED NEUTROPHILS
PIG - 8
LYMPHOCYTES
BAND NEUTROPHILS
SEGMENTED NEUTROPHILS
NEUTROPHILIC METAMYELOCYTES
THOUSANDS OF CELLS/cmm.
DPI

when they increased except in pig 3 which had an initial lymphocytosis followed by a decrease to below pre-inoculation numbers.

One control (pig 9) had about equal numbers of lymphocytes and segmented neutrophils throughout the trial. Segmented neutrophils were more numerous than lymphocytes in control pig 4 at the beginning of the trial, but a decline in neutrophils resulted in lymphocytes becoming the most numerous circulating leukocytes in the last few days of the trial.

Total plasma protein and fibrinogen levels Increased TP values were present in all principals. Four pigs which died prior to 7 DPI had sharp rises in TP to maximum values above 8.0 Gm./100ml. Total plasma protein levels in the pigs which lived beyond 7 DPI were elevated less but were higher than levels in the controls (Table 19).

The first pig to die (pig 1) had no fibrinogen increase, but the other 5 principals had maximum fibrinogen levels of 0.8 Gm./100ml. or more. Peak fibrinogen levels occurred at about the same times as maximum TP levels. Increased fibrinogen levels were reflected in lowered TP/fibrinogen ratios.

Serum protein fractions Albumin/globulin ratios decreased in most principals, and minimum values occurred near the time of death (Table 20). The decrease was due to a reduction in the percentage of albumin and a relative and absolute increase in the alpha-2 fraction of alpha globulin. A moderate reduction in gamma globulin was noted in most pigs; however, total globulin levels increased because of the increased alpha globulin levels. The only notable change in control pigs

Table 19. Total protein (TP), fibrinogen (Fib), and total protein/fibrinogen ratios (TP/Fib) in pigs. Trial 6

Pig no.	DPI	-3	-2	-1	1	2	3	4
1	TP[a]	5.2	5.6	5.8	6.0	8.2	D	D
	Fib[a]	.2	.2	.3	.3	.3	D	D
	TP/Fib	26.0	18.7	19.3	20.0	27.3	D	D
2	TP	6.5	6.5	6.2	6.5	7.0	8.0	8.5
	Fib	.4	.4	.3	.3	.1	.6	.7
	TP/Fib	16.3	16.3	20.6	21.6	70.0	13.3	12.1
10	TP	6.7	6.7	6.9	6.9	7.4	7.5	8.4
	Fib	.3	.3	.5	.3	.1	.5	.6
	TP/Fib	32.3	32.3	13.8	23.0	74.0	15.0	14.0
8	TP	6.1	6.2	6.4	6.9	6.6	6.9	7.7
	Fib	.4	.3	.3	.4	.1	.1	.6
	TP/Fib	15.3	20.7	21.3	17.3	66.0	69.0	12.8
6	TP	7.0	6.6	6.7	6.8	7.2	6.9	6.8
	Fib	.5	.5	.5	.3	.2	.2	.1
	TP/Fib	14.0	13.2	13.3	22.7	36.0	34.5	68.0
3	TP	6.2	6.0	6.1	6.5	7.8	6.8	6.8
	Fib	.2	.2	.2	.2	.2	.2	.3
	TP/Fib	31.0	30.0	30.5	32.5	39.0	34.0	22.6
4-C	TP	5.9	5.9	6.1	6.6	6.8	6.5	6.2
	Fib	.3	.3	.4	.3	.1	.3	.3
	TP/Fib	19.7	19.7	15.3	22.0	68.0	21.7	20.7
9-C	TP	6.6	6.6	6.3	7.0	6.9	6.1	6.4
	Fibq	.2	.3	.2	.1	.1	.4	.2
	TP/Fib	33.0	22.0	21.5	70.0	69.0	15.3	32.0

[a]TP and Fib values in Gm./100 ml.

5	6	7	8	9	10	11	12
D	D	D	D	D	D	D	D
D	D	D	D	D	D	D	D
D	D	D	D	D	D	D	D
	D						
8.5	D	D	D	D	D	D	D
.8	D	D	D	D	D	D	D
10.6	D	D	D	D	D	D	D
8.7	D	D	D	D	D	D	D
.9	D	D	D	D	D	D	D
9.7	D	D	D	D	D	D	D
8.5	8.6	8.1	D	D	D	D	D
1.0	1.0	.8	D	D	D	D	D
8.5	8.6	10.1	D	D	D	D	D
7.4	7.2	6.9	7.7	7.9	7.7	D	D
.3	.4	.4	.7	.8	.6	D	D
24.7	18.0	17.3	11.0	9.9	12.8	D	D
6.9	7.2	7.2	7.1	6.7	6.7	7.0	6.5
.7	.9	.8	.8	.6	.5	.5	.5
9.9	8.0	9.0	8.9	11.2	13.4	14.0	13.0
6.4	6.0	5.9	5.8	ND	5.7	5.6	6.0
.3	.3	.2	.3	ND	.2	.3	.3
21.3	20.0	29.5	19.3	ND	28.5	18.7	20.0
6.6	6.5	6.1	6.5	6.5	6.1	6.4	6.3
.2	.1	.1	.2	.2	.1	.2	.2
33.0	65.0	61.0	32.5	32.5	61.0	32.0	31.5

Table 20. Serum protein fractions in pigs. Trial 6

Pig no.	DPI	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12
	Data															
1	Albumin	35[a]	33	33	32	29	D	D	D	D	D	D	D	D	D	D
	Alpha	25	23	25	29	37	D	D	D	D	D	D	D	D	D	D
	Beta	17	16	16	14	17	D	D	D	D	D	D	D	D	D	D
	Gamma	23	28	26	25	17	D	D	D	D	D	D	D	D	D	D
	A/G[b]	.54	.49	.49	.47	.41	D	D	D	D	D	D	D	D	D	D
2	Albumin	36	35	35	37	43	40	ND	35	D	D	D	D	D	D	D
	Alpha	25	23	24	24	19	21	ND	30	D	D	D	D	D	D	D
	Beta	13	20	18	19	19	16	ND	15	D	D	D	D	D	D	D
	Gamma	26	22	23	20	19	23	ND	20	D	D	D	D	D	D	D
	A/G	.56	.54	.54	.59	.75	.70	ND	.54	D	D	D	D	D	D	D
10	Albumin	35	38	33	34	35	33	35	30	D	D	D	D	D	D	D
	Alpha	30	29	28	30	28	30	33	38	D	D	D	D	D	D	D
	Beta	13	13	16	13	12	14	14	15	D	D	D	D	D	D	D
	Gamma	22	20	23	23	25	23	18	17	D	D	D	D	D	D	D
	A/G	.54	.61	.49	.51	.54	.49	.54	.43	D	D	D	D	D	D	D
8	Albumin	32	33	36	34	41	34	30	26	24	38	D	D	D	D	D
	Alpha	28	28	29	27	24	26	30	38	38	40	D	D	D	D	D
	Beta	15	15	12	12	11	14	15	14	14	16	D	D	D	D	D
	Gamma	25	24	23	27	24	26	25	22	22	20	D	D	D	D	D
	A/G	.47	.49	.56	.51	.69	.51	.43	.35	.35	.31	D	D	D	D	D

6	Albumin	33	32	32	31	38	30	30	32	30	30	32	31	27	D	D
	Alpha	31	28	30	29	26	28	29	27	33	32	33	35	38	D	D
	Beta	14	15	13	14	14	13	14	13	14	14	15	15	16	D	D
	Gamma	22	25	25	26	22	29	27	28	23	24	20	19	19	D	D
	A/G	.49	.47	.47	.45	.61	.43	.43	.47	.43	.43	.47	.45	.37	D	D
3	Albumin	32	45	42	40	39	42	42	30	31	28	31	27	30	32	28
	Alpha	29	19	21	20	22	22	25	32	36	40	38	38	39	39	40
	Beta	16	11	12	19	13	12	11	14	15	16	17	17	16	14	17
	Gamma	23	25	25	21	26	24	22	24	18	16	14	18	15	15	15
	A/G	.47	.81	.72	.67	.64	.72	.72	.43	.45	.39	.45	.37	.43	.47	.39
4-C	Albumin	41	42	37	35	38	38	44	38	43	39	40	39	41	32	36
	Alpha	27	26	29	32	28	28	26	27	25	27	27	27	28	32	29
	Beta	15	14	15	13	12	14	13	13	14	14	14	15	14	14	15
	Gamma	17	18	19	20	21	20	17	22	18	20	19	19	17	22	20
	A/G	.69	.72	.59	.54	.61	.61	.75	.61	.75	.64	.67	.64	.69	.47	.56
9-C	Albumin	29	31	30	30	32	35	30	33	29	31	30	30	32	35	30
	Alpha	27	29	27	25	26	22	24	24	27	29	27	25	26	22	24
	Beta	13	12	14	17	14	13	13	13	13	12	14	17	14	13	13
	Gamma	30	28	29	28	28	30	33	30	30	29	28	28	28	30	33
	A/G	.41	.45	.43	.43	.47	.54	.43	.49	.41	.45	.43	.43	.47	.54	.43

[a]Values expressed as percent of TP.

[b]A/G = Albumin/Globulin ratio.

was a transient increase in alpha and gamma globulins in pig 4 at 11 days after the trial began.

Serum glutamic oxalaoacetic transaminase levels Levels of SGOT activity remained within the normal range in all pigs (Table 21).

Serum electrolytes Serum Na, K, and Cl levels are summarized in Table 22. Onset of diarrhea was accompanied by decreases in serum Na. Sodium levels continued to decline as diarrhea continued so that the lowest Na levels were observed in pigs which survived for the longest time. The mean pre-inoculation serum Na was 143 mEq./L.; values in the last samples from the principals ranged from 98 to 126 mEq./L. Mean pre-inoculation serum Cl content was 99 mEq./L.; final levels ranged from 69 to 93 mEq./L. Trends in serum Cl followed those in serum Na. Serum K increased 1 to 2 days prior to death with maximum values of from 6.7 to 7.8 mEq./L. The pre-inoculation mean was 5.6 mEq./L. All K values at 2 DPI were disregarded because samples were hemolyzed.

Blood gas analyses Results of blood pH analyses and blood HCO_3 calculations are included in Table 23. All values were extremely variable, but the following observations were noted in principals: (1) blood pH values of 7.06 and 7.15 in 2 pigs in the last sample before death, (2) markedly decreased blood HCO_3 levels in all principals shortly before death. The mean level of pre-inoculation blood HCO_3 was 20.9 mEq./L. and the mean level just prior to the death of principals was 16.6 mEq./L.

Table 21. Serum glutamic oxaloacetic transaminase levels in pigs. Trial 6

DPI	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12
Pig no:															
1	20[a]	22	26	33	44	D	D	D	D	D	D	D	D	D	D
2	35	26	22	26	44	37	ND	ND	D	D	D	D	D	D	D
10	31	20	18	19	ND	25	44	ND	D	D	D	D	D	D	D
8	20	18	20	20	20	18	14	24	24	15	D	D	D	D	D
6	18	20	20	19	31	18	29	31	19	20	33	37	37	D	D
3	31	35	35	20	26	33	33	42	50	33	22	18	13	11	31
4-C	15	15	16	50	28	15	15	24	18	24	18	28	22	19	24
9-C	20	19	22	31	ND	28	31	35	33	28	31	33	28	33	44

[a]Values reported as Sigma-Frankel units.

Table 22. Serum sodium, (Na), potassium (K), and chloride (Cl) levels in pigs. Trial 6

Pig no.	DPI	-3	-2	-1	1	2	3
1	Na	144[a]	139	143	137	126	D
	K	6.3	6.4	5.3	5.4	9.1	D
	Cl	99	ND	97	98	92	D
2	Na	139	141	146	141	133	131
	K	5.4	5.8	4.4	4.6	7.8	5.8
	Cl	97	ND	97	98	98	93
10	Na	146	141	139	146	137	131
	K	6.3	5.8	4.7	5.0	7.8	4.9
	Cl	102	ND	102	99	89	98
8	Na	141	146	144	141	139	137
	K	6.4	6.1	5.9	5.3	8.2	6.1
	Cl	100	ND	99	96	98	106
6	Na	141	144	141	137	135	141
	K	5.8	6.4	4.4	5.1	8.8	4.4
	Cl	100	ND	99	93	97	101
3	Na	144	137	144	137	144	146
	K	6.1	5.4	4.5	5.6	8.6	4.4
	Cl	100	ND	98	99	96	99
4-C	Na	146	148	148	139	144	150
	K	5.9	5.5	5.6	6.1	9.2	6.5
	Cl	100	ND	101	94	98	103
9-C	Na	141	141	139	139	139	141
	K	6.1	5.6	4.9	4.6	7.0	4.7
	Cl	101	ND	99	94	91	96

[a]All values expressed as mEq./L.

4	5	6	7	8	9	10	11	12
D	D	D	D	D	D	D	D	D
D	D	D	D	D	D	D	D	D
D	D	D	D	D	D	D	D	D
ND	122	D	D	D	D	D	D	D
ND	7.8	D	D	D	D	D	D	D
ND	ND	D	D	D	D	D	D	D
131	122	D	D	D	D	D	D	D
6.0	6.8	D	D	D	D	D	D	D
ND	D	D	D	D	D	D	D	D
135	126	120	118	D	D	D	D	D
4.9	6.5	6.5	6.7	D	D	D	D	D
98	94	83	69	D	D	D	D	D
137	137	139	131	125	118	111	D	D
4.6	5.0	4.2	4.9	4.5	6.7	6.3	D	D
102	102	97	95	89	86	72	D	D
141	131	128	124	115	107	111	107	98
5.8	5.4	5.5	5.5	6.0	6.1	6.0	5.6	7.4
102	97	90	83	75	81	74	72	70
135	141	144	144	139	144	144	146	144
5.3	5.9	5.0	5.5	5.0	5.6	5.6	5.1	6.0
100	105	98	97	97	98	97	97	99
144	141	141	139	141	137	139	137	141
5.4	6.7	4.6	5.1	4.5	4.7	5.0	4.9	5.5
100	100	98	104	94	96	93	93	95

Table 23. Blood pH values and bicarbonate (HCO_3) levels in pigs. Trial 6

Pig No.	DPI	-2	-1	2	3	4	5	6	7	8	9	10	11	12
1	pH	7.30	7.21	7.06	D	D	D	D	D	D	D	D	D	D
	HCO^3[a]	29.5	29.0	16.5	D	D	D	D	D	D	D	D	D	D
2	pH	7.39	7.35	7.32	7.23	7.20	7.28	D	D	D	D	D	D	D
	HCO^3	26.5	26.0	23.5	23.0	24.0	18.0	D	D	D	D	D	D	D
10	pH	7.21	7.16	7.22	7.26	7.23	7.29	D	D	D	D	D	D	D
	HCO^3	22.0	23.5	20.5	20.0	21.0	16.3	D	D	D	D	D	D	D
8	pH	7.25	7.26	7.26	7.31	7.22	7.24	7.21	7.21	D	D	D	D	D
	HCO^3	22.5	25.5	22.5	15.5	20.0	19.7	16.5	14.3	D	D	D	D	D
6	pH	7.37	7.38	7.18	7.22	7.45	7.27	7.35	7.47	7.43	7.30	7.41	D	D
	HCO^3	22.5	22.0	18.0	21.0	22.5	21.0	23.0	21.0	22.2	19.5	18.5	D	D
3	pH	7.31	7.21	7.16	7.29	7.29	7.23	7.33	7.32	7.46	7.36	7.45	7.34	7.15
	HCO^3	24.0	25.0	20.0	25.0	21.5	19.7	21.8	21.8	22.5	24.5	21.5	22.0	16.0
4-C	pH	7.23	7.26	ND	7.19	7.28	7.20	7.26	ND	7.22	7.32	7.31	7.33	7.18
	HCO^3	23.0	24.5	ND	26.0	26.0	22.6	25.9	ND	24.3	25.0	26.5	26.0	24.0
9-C	pH	7.37	7.46	ND	7.30	7.37	7.27	7.27	ND	ND	7.30	7.43	7.41	7.35
	HCO^3	27.0	22.5	ND	28.0	28.5	24.9	25.1	ND	ND	25.0	28.5	28.0	28.0

[a]Expressed in mEq./L.

DISCUSSION

Swine dysentery is an infectious disease which is readily transmitted by colon contents and/or mucosa obtained from affected pigs. Two types of bacteria, vibrio and spirochetes, have been considered to be the most likely etiologic agents associated with the disease. The lack of adequate techniques for isolation, propagation, and identification of these organisms has hampered attempts at defining their pathogenetic importance.

This study has emphasized the progressive development of the lesions of swine dysentery. Special emphasis was placed on the association of a morphologically defined organism, large spirochete, with lesion development. There was a consistent and positive correlation between the presence of large spirochetes and lesions of swine dysentery.

The incubation period in the experimentally produced disease varied from 2 to 10 days but it was quite uniform in individual trials. An exception was Trial 5 in which some pigs remained clinically normal. Early, uniform onset of dysentery was noted in Trials 2, 3, and 6 suggesting that an incubation period of 2 days may be expected following massive exposure. The short incubation period in these trials as compared with other reports (Doyle, 1939; Roberts, 1956) probably resulted from the use of large amounts of inoculum and also from withholding feed prior to exposure. There is less dilution of inoculum in an empty stomach and acid secretion is reduced. It was assumed that the causative agent or agents reached the intestine with higher infectivity as a result

of these factors.

The first clinical signs of swine dysentery were soft yellow or gray stools which became mucohemorrhagic and watery as diarrhea became more severe. A 1 to 3 F. increase in rectal temperature usually accompanied the onset of marked clinical signs. The course of the disease was quite variable with deaths occurring from 1 to 15 days after diarrhea began. Occasional pigs began to recover.

Gross lesions progressed from catarrhal to mucofibrinous to mucohemorrhagic enteritis in the large intestine and in some instances lesions in late stages of the disease included fibrinonecrotic enteritis. These findings were similar to previous descriptions (Whiting et al. 1921; Lussier, 1962; Warner, 1965).

The assignment of a specific lesion diagnosis in an individual pig was quite arbitrary except in the early stages of catarrhal enteritis or in the later fibrinonecrotic enteritis. In the intermediate stages of swine dysentery there was a mixture of mucofibrinous, hemorrhagic, and necrotic lesions which varied in intensity from one area of the large intestine to another. Lesions were consistently found in the colon, but the cecum was normal or less severely affected in some pigs. In Trial 3 (Table 6) the cecum and proximal colon were affected prior to the distal colon suggesting that lesions developed progressively from the proximal to the distal large intestine. Rectal lesions were usually less severe than colonic lesions.

Congestion was frequently noted in the fundic portion of the stomach but was also observed in some controls. Hemorrhagic gastritis occurred

only in two principals, Y-1 and Y-5, in Trial 2. The sporadic incidence of gastric lesions supports the observations of Warner (1965) and indicates that gastritis lacks importance either in the pathogenesis of swine dysentery or in its diagnosis.

No hepatic lesions were noted. This was in contrast to swollen livers with pale mottled areas described by Warner (1965). Lesions in tissues other than the large intestine and its mesentery were inconsistent and probably incidental.

The only consistent and significant histologic lesions were in the large intestine and consisted of a progression from catarrhal to mucofibrinous to fibrinonecrotic enteritis. Hyperemia and edema were invariably present.

Mucosal lesions at first consisted only of exudation and accumulation of mucus and fibrin. As early lesions progressed, groups of intact epithelial cells could be seen separated from the edematous lamina propria at the luminal surface (Figure 3). Pascal et al. (1968) showed that numerous epithelial cells were intimately attached to one underlying fibroblast and that these cells migrated as a unit from the base of colonic crypts to the luminal surface of the rabbit colon. This in addition to desmosomal attachments between cells may explain the separation of epithelial cells in groups as noted in swine dysentery. Enterorrhagia was evident at this stage and probably resulted from the exposure and rupture of small vessels.

Also noted in early lesions were increased numbers of mitotic figures in elongated hyperchromic epithelial cells at the base of colonic crypts.

The exact stimulus for increased mitotic activity in colonic crypts is not known, but there was apparently an increased rate of proliferation of epithelial cells probably as a response to inflammation as suggested by Abrams et al. (1963) and Sprinz (1969). Pigs with swine dysentery often recover rapidly after successful treatment with antibiotics. Preliminary studies have indicated that the lesions of mucohemorrhagic enteritis in the colons of pigs with swine dysentery may completely regress within 2 days after treatment (Glock and Harris, 1970). This rapid repair is most likely the result of replacement of damaged epithelium by new epithelium migrating upward from the base of the mucosal crypts. Moon (1971) demonstrated that the replacement time for mucosal epithelium in the cecum and colon of normal 3-week-old pigs was 2 to 3 days. If this rate of replacement is accelerated by inflammation, the rapid recovery after suppression of the etiologic agents is explained.

Neutrophils were numerous in the lamina propria near the lumen of the large intestine. At first they could be seen pavementing in vessels, but as lesions progressed they were distributed throughout the superficial mucosa and in the lumen. Goblet cell hyperplasia was an early and consistent finding, but in the later stages of Trials 2 and 3 goblet cells were less numerous suggesting exhaustion of secretory function.

Lesions often progressed to fibrinonecrotic cecitis and colitis, but necrosis was usually confined to the superficial mucosa. However, in Trial 4 the zone of necrotic tissue extended to or nearly to the

muscularis mucosa in 2 pigs (0000 and 6271). Salmonella manhattan was isolated from the colon of one of these, pig 0000, as well as another pig, 6270. The possible pathogenicity of S. manhattan in swine is not known. Although necrosis was slightly more extensive in pigs in this trial than in others, the role of S. manhattan was considered to be minimal especially since it was isolated from only 2 of the 7 pigs.

Large spirochetes were observed in cecal and colonic lesions in 30 of 31 pigs with swine dysentery. The one exception was pig 5580 B in Trial 1. Lesions in this pig consisted only of edema in the lamina propria and distention of the colon with ingesta. It should be emphasized that large spirochetes were never seen in controls. In Trial 1 large spirochetes were present in the lumen of the colon 3 days before clinical signs or lesions were observed. This indicated that these organisms became established in the gut as the result of inoculation and that they proliferated prior to lesion development.

Numerous large spirochetes were observed at the luminal surface and deep in mucosal crypts of the large intestine wherever early lesions were noted. These organisms were demonstrated in goblet cells and in and between epithelial cells (Figure 5). The Warthin-Starry stain was useful for identifying and locating the organisms. In slightly more advanced cases where some erosion of the epithelium could be seen, large spirochetes were noted in the lamina propria. It was apparent that necrosis of the epithelium was not necessary to facilitate invasion since crypt epithelial cells containing large spirochetes were intact as seen with light microscopy except for differences in staining intensity

in some areas. Necrotic cells were usually seen only at the luminal surface and very occasionally in mucosal crypts.

The first ultrastructural change was alteration of the microvilli of the epithelial cells at the surface and in crypts of the cecal and colonic mucosa. The microvilli were short, irregular in outline, and less numerous than in controls. Large spirochetes were usually but not invariably noted near cells with early degenerative changes (Figure 9). As lesions became more severe, the epithelial cells were often devoid of microvilli and cytoplasmic changes included edema, mitochondrial swelling, and swelling of the endoplasmic reticulum. Large spirochetes were often found in goblet cells, in and between epithelial cells (Figure 10), and occasionally near the basement membrane or in the lamina propria (Figure 23).

Epithelial cells which were electron-dense and shrunken were occasionally seen in the colonic mucosa of control pigs but were observed in increasing numbers as the lesions of swine dysentery progressed. These cells had a granular, electron-dense cytoplasmic matrix and usually had dilated endoplasmic reticulum containing granular material (Figure 21). The nuclei often appeared pyknotic. Moon et al. (1971) saw similar cells in normal rabbit and swine small intestine and, although uncertain of their significance, suggested that they may have been degenerating cells. Small numbers of degenerating epithelial cells could be expected in normal animals as a result of epithelial turnover. Principals in this study had increased numbers of these dark cells which

often contained large spirochetes. This suggests that the dark cells were in fact degenerating epithelial cells.

Additional ultrastructural lesions included intracellular and extracellular edema in the lamina propria. Endothelial cells of vessels were often swollen. Exudation of fibrin and neutrophils as a result of vascular damage was a prominent feature of the lesions observed by light and electron microscopy.

Taylor and Blakemore (1971) examined lesions of swine dysentery from the colons of 6 pigs and noted large spirochetes in and between degenerate cells of the colonic mucosa. They concluded that the absence of these organisms from normal cells indicated that they were of little significance in the initial development of the disease but may have represented opportunistic invasion of damaged cells. This conclusion is not necessarily valid for two reasons. (1) The mechanisms by which spirochetes produce lesions are unknown. Smith et al. (1968) stated only that spirochetes are not known to produce endotoxins or exotoxins and suggested that elaboration of proteolytic enzymes may produce tissue necrosis. It is premature, therefore, to assume that invasion is essential to the production of cellular degeneration. (2) The argument that large spirochetes invade only damaged cells because they were not seen in normal cells can be questioned because of the relatively small number of cells which can be examined by electron microscopy. It is possible that cellular degeneration may have begun immediately after invasion thereby requiring rather precise timing to observe the cell before damage was evident.

In evaluating the specific role of spirochetes in the pathogenesis of swine dysentery it seems appropriate to discuss some of the difficulties involved in understanding the pathogenesis of infectious disease of the large intestine. Dubos et al. (1965) noted that attention has only recently begun to focus on the incidence and significance of the anaerobic bacteria which constitute the principal flora of the intestine and especially the large intestine. They classified the enteric flora as: autochthonous microbiota, common to all members of a species; normal microbiota, those ubiquitous in a particular community; and pathogens. Savage and McAllister (1970) stressed the importance of competition between the various types of intestinal flora and the possible exclusion of pathogens by the autochthonous microbiota which are mostly anaerobes. Savage et al. (1971) also noted that the location of certain of these organisms is not random but at specific levels or layers in the mucus on the epithelium of the cecal and colonic mucosa. Spirochetes are frequently included in this group of autochthonous organisms.

A simplistic, one agent per disease, approach to the understanding of enteric pathogenesis excludes a number of currently known or suspected interrelationships. Roberts (1969) stressed the importance of synergism in mixed infections. One example is oral lesions of man in which a diphtheroid together with either a Fusobacterium sp. or a gram-negative anaerobic bacillus can enhance growth of Treponema microdentium by supplying various essential biochemical factors (Socransky et al. 1964). Vincent's angina (fusospirochetal disease) is another example of

synergism in which fusiform bacilli and spirochetes are thought to be involved in oral lesions but require the presence of vibrio and cocci (Smith et al. 1968).

Fusiform rods, and small spirochetes were demonstrated by phase or light microscopy in colons of both controls and principals as best illustrated in Trial 2 (Table 5). Small spirochetes were more frequently seen in colon contents than in mucosal scrapings which suggests that they are not found in significant numbers in close contact with the mucosa. Neither of these organisms was consistently present in infected pigs. There was no significant difference between principals and controls in the number or location of spirilla. Vibrio-like organisms were seen in mucosal crypts in both principals and controls, but this does not imply that specific strains not differentiated in this study may not be involved in the pathogenesis of swine dysentery. Lussier (1962) and Powell (1970) suggested that specific biochemical types of vibrio may be pathogenic while others may be nonpathogenic.

It is likely that 2 or more organisms function synergistically to produce swine dysentery, and the consistent presence of vibrio and large spirochetes in lesions suggests that such a relationship may exist between these 2 organisms. Difficulty in reproducing the disease with proven pure cultures serves to support this possibility. It is also conceivable that the erratic response to various drugs may result in part from lack of activity against all organisms involved. Spirochetes are especially susceptible to arsenicals which have less activity against some other organisms. The effectiveness of arsenicals in

treating outbreaks of swine dysentery adds circumstantial support to the belief that large spirochetes are involved in the pathogenesis of swine dysentery.

The role of spirochetes as pathogens is poorly understood. Spirochetes have been observed in numerous mammalian species including man (Hanson, 1970), but their role in enteric disease is not defined. Harland and Lee (1967) noted the presence of spirochetes in intestinal lesions of man but were unsure of their significance. In a later paper (Lee et al. 1971) they suggested that significance was minimal. Shera (1962) felt that spirochetes were significant but secondary factors in some types of human enteritis. Zymet (1969) found spirochetes associated with diarrhea in dogs.

This study has demonstrated the early and consistent presence of large spirochetes which have been shown to invade the epithelium and lamina propria of the colonic mucosa. Their early presence, high numbers, and invasiveness make it difficult to believe that they are inconsequential to the pathogenesis of swine dysentery. The possibility of a synergistic relationship between large spirochetes and vibrio or other agents seems likely on the basis of this and previous studies which have indicated the importance of vibrio (James and Doyle, 1947; Warner, 1965). Whether either organism can be assigned to a primary or secondary role seems relatively unimportant as long as either can be shown to be essential for the production of lesions.

The term "large spirochete" has been used throughout this discussion for lack of a better term. The name Borrelia hyos was suggested for

similar organisms by Warner (1965) and Todd et al. (1970), but although useful and descriptive it has not been accepted for two reasons: (1) Borrelia are usually considered to be arthropod borne and there is no such evidence in this case, and (2) assignment to a species on the basis of structure and source is extremely presumptuous. Listgarten and Socransky (1965) offered an excellent scheme for classification of spirochetes on the basis of morphology but recognized their work as only a temporary method for use until better classification schemes were developed.

The organisms identified as large spirochetes in this study were morphologically characterized. When seen by phase microscopy they had the appearance of a loose spiral with tapered ends. They were motile in fresh specimens and appeared to move by a series of flexions. Some affinity for epithelial cells was suggested by their increased numbers adjacent to and within clumps of these cells. Their appearance in tissue sections was similar and they stained readily with silver and Gram's stains (Figures 4 and 5).

Size was determined from electron photomicrographs of negatively stained organisms and organisms in ultrathin sections. They were usually 5 to 8 μm. in length and 0.30 to 0.38 μm. in diameter. Ultrastructure included a granular protoplasmic cylinder with a rigid wall surrounded by a thin membranous envelope which appeared to be composed of 2 membranes (Figures 11 and 15). Six to 8 axial fibrils 10 to 12 nm. in diameter originated from implantation discs on the protoplasmic cylinder near each end of the organism (Figures 29 and 30). The fibrils

overlapped in the middle of the organism to give a total of 12 to 16 axial fibrils.

Hematologic changes were studied in Trials 4, 5, and 6. While there was some variation in values between individual pigs and groups of pigs, certain trends were observed.

Packed cell volume increases of from 1 to 11 percent above pre-inoculation levels were observed in principals in Trials 4 and 6. Maximum values were reached during the period of most severe clinical signs, usually after several days of severe diarrhea. These changes indicate that hemoconcentration occurred as a result of dehydration. In pigs surviving past 5 DPI in Trial 6 the increase in PCV was less marked and a return to or below pre-inoculation values was observed although pigs still appeared dehydrated. TP often remained elevated indicating that anemia was present, possibly as the result of intestinal hemorrhage. Blood loss resulting from daily withdrawal of blood may also have been a factor in the decline of PCV (Kornegay, 1967). Changes in RBC numbers paralleled those in PCV values.

Erythrocyte sedimentation rates were determined by the Wintrobe method which, although somewhat less sensitive than the Westergren method (Cassidy, 1970), is more generally used and is less complex. Pre-inoculation mean ESR varied from 2 mm./hour in Trial 5 to 7 mm./hour in Trial 6. Readily detectable increases occurred in Trials 4, 5, and 6. Maximum values were usually observed at about the same time as maximum PCV values during severe diarrhea and ranged from 15 to

50 mm./hour. A decline in ESR was often seen 2 to 3 days prior to death (Trial 6). The ESR, therefore, reflected the early severe clinical signs observed in swine dysentery. The reason for ESR increases cannot be specifically determined, but a positive correlation was noted with increased fibrinogen levels. Lynch et al. (1969) stated that increases in alpha globulin and decreases in albumin enhance ESR, but in this study ESR often decreased while alpha globulin levels were still rising and albumin levels were falling. Rouleaux formation may have been a factor but was not observed in stained smears. Little change was noted in the ESR of mildly affected pigs in Trial 5.

There was some variation between trials in numbers of circulating leukocytes. Sharp increases to as high as 68,000 cells/cmm. were detected in the severely affected animals in Trials 4 and 5. Maximum values occurred just prior to death. Pigs with transient diarrhea in Trial 5 had little change in WBC numbers. In Trial 6 increases to from 24,500 to 36,000 cells/cmm. were observed in only 3 of the 6 severely affected pigs. The other three principals had little change in WBC numbers. Therefore, although notable increases occurred in some pigs, results were not consistent.

The most consistent leukocytic alteration in pigs with dysentery was a marked left shift. Numbers of mature neutrophils began to decline with the onset of clinical signs and in most cases continued to decrease until death. Low values of from 234 to 1240 mature neutrophils/cmm. occurred in severely affected pigs. Those with less acute clinical

signs had smaller decreases in mature neutrophils, and several in Trial 4 had a gradual increase. Increases in numbers of band neutrophils were dramatic with counts of 7,110 to 10,920 cells/cmm. in severely affected pigs. There was a corresponding rise in numbers of neutrophilic metamyelocytes. In Trial 6 maximum numbers of neutrophilic metamyelocytes ranged from 412 to 4900 cells/cmm.

Loss of mature neutrophils from the circulating pool probably resulted from exudation in the large intestine with a resultant stimulation of granulopoiesis leading to the observation of large numbers of immature neutrophils in the circulating blood.

Although lymphocyte numbers often decline during severe systemic disease, nothing more than transient reductions in lymphocyte counts was noted during this study. Increasing numbers were often noted late in the course of the disease.

The early and pronounced left shift with little increase in WBC counts until late in the disease was consistent and dramatic but should not be considered to be diagnostic for swine dysentery since other enteric diseases such as salmonellosis may produce similar changes (Sorensen, 1970b).

Mean total plasma protein in pre-inoculation blood samples ranged from 5.0 Gm./100ml. in Trial 4 to 6.3 Gm./100ml. in Trial 6. Levels of TP in pigs with dysentery were markedly increased with maximum values of from 7.0 to 8.9 Gm./100ml. observed at about the same time as increases in PCV and RBC numbers. Thus, changes in TP were probably relative and due mainly to dehydration.

The amount of plasma fibrinogen in pre-inoculation blood samples was usually less than 0.3 Gm./100ml. Levels increased to from 0.6 to 1.0 Gm./100ml. during severe dysentery. This was probably a result of increased production of fibrinogen by the liver in response to inflammation (Schalm, 1970). High levels in this study reflected the severity of the enteritis observed in swine dysentery.

A consistent trend was detected in relative proportions of serum proteins as a result of swine dysentery. Electrophoretic separation of the various fractions indicated a progressive reduction in albumin and an increase in globulins. The increase in globulins resulted from a higher level of alpha-2 globulin which overshadowed a minor reduction in gamma globulin. This type of change is common in many diseases and probably can result from any kind of stress (Vesselinovitch, 1955).

Serum glutamic oxaloacetic transaminase activity did not vary from normal levels (Wretlind et al. 1959) in any of the principals or controls.

Hematologic changes discussed to this point helped to characterize swine dysentery but were for the most part generalized reactions to inflammation and/or dehydration. Results of serum electrolyte and blood gas analyses offered more information concerning the cause of death. Application of these findings could be useful in approaches to therapy.

The effect of swine dysentery on serum Na levels was best illustrated in Trial 6. Reduction of serum Na was noted on the same day that diarrhea began, and the level steadily declined in each principal

until the time of death. Although the decline was somewhat more rapid in pigs which died early in the trial, levels in the last blood samples prior to death were much lower in pigs which survived for longer periods of time (Table 22). The lowest value recorded was 98 mEq./L. in pig 3 which survived until day 12. The decline in serum Cl paralleled that of serum Na. These changes were the result of continued loss of Na and Cl from the intestine and decreased intake because of anorexia. Depletion of these electrolytes was closely related to the duration of diarrhea but not necessarily to the time of death. Therefore, death could not be attributed solely to depletion of Na and Cl.

Serum K levels increased sharply 1 to 2 days prior to death. This terminal increase occurred at the same time as a reduction in serum HCO_3 and probably resulted primarily from displacement of intracellular K by H^+ ions. The extent of loss of intracellular K to the extracellular fluids following necrosis in the intestine could not be determined. Low serum Na may also have been a factor in displacing K from intracellular to extracellular fluids but Na levels were often depressed long before increases in K levels were noted. Maximum K levels in the last blood sample prior to the death of pigs in this study ranged from 6.7 to 7.8 mEq./L. Since the effect of high K levels is enhanced by low Na levels it is likely that the increases were a contributory factor in the death of infected pigs.

Blood pH was quite variable, and marked acidemia was detected only in 2 pigs in Trial 6. However, blood HCO_3 levels were low (14.3 to 18.5 mEq./L.) in the last blood sample prior to death of all principals.

The Kintner balance nomogram as described by Gambino (1969) was used to characterize changes in acid-base balance. Base deficit gradually became more severe in principals during the course of the diarrhea. Maximum base deficit in the last specimen obtained prior to death ranged from 6.5 to 15.5 mEq./L. thereby indicating a consistent metabolic acidosis. The fact that blood pH values in most pigs remained near normal was explained by compensatory respiratory alkalosis. Compensation was less marked or absent in the 2 pigs which had low blood pH values. Since blood samples were drawn at the same time each day rather than immediately prior to the time of death, decompensation may have occurred in some pigs after the last blood sample was obtained.

Antibiotics and arsenicals are commonly used as therapeutic agents in pigs with swine dysentery. Unfortunately some losses usually occur before the disease is arrested in affected herds. Losses may become extensive in certain herds since some experimentation may be required to find a drug which is effective. These studies indicate that electrolyte depletion or imbalance and metabolic acidosis are significant factors in debilitation and death. This indicates a need for further investigations to determine the feasibility of administering preparations containing Na, Cl, and HCO_3 to infected animals either via oral or parenteral routes.

SUMMARY

The pathogenesis of swine dysentery was studied in 6 trials with a total of 61 pigs, 45 principals and 16 controls. Principals were inoculated orally with colon contents and mucosal scrapings from pigs with swine dysentery. Special emphasis was placed on determining the extent and nature of the association of large spirochetes with lesions. Hematologic changes were also studied.

Large spirochetes were consistently found in the cecal and colonic mucosa of pigs with clinical signs or lesions of swine dysentery, but large spirochetes were never observed in controls. In 3 trials, principals were killed on consecutive days, and large spirochetes were found in the lumen of the large intestine as early as 3 days before clinical signs or enteric lesions were detected.

Enteritis was limited to the large intestine, and early lesions consisted of mucofibrinous cecitis and colitis. Ultrastructural changes in the epithelium included distortion and loss of microvilli, intracellular edema, and swelling of mitochondria and the endoplasmic reticulum. Mucosal crypts of the large intestine invariably contained numerous large spirochetes. Their invasiveness was demonstrated by their presence in goblet cells and in and between epithelial cells.

As lesions became more advanced (mucohemorrhagic enteritis) many of the epithelial cells were electron-dense with a dark granular cytoplasmic matrix and swollen endoplasmic reticulum which contained dense granular material.

Clumps of large spirochetes were present in these degenerating cells as well as in the lamina propria.

The structure of the large spirochetes associated with swine dysentery was studied by light, phase, and electron microscopy. The organisms were 5 to 8 μm. in length and 0.30 to 0.38 μm. in diameter with tapered ends. They had a spiral shape, usually with 2 to 3 spirals per organism, and were actively motile by means of flexing motions. Ultrastructure consisted of a granular protoplasmic cylinder with 6 to 8 axial fibrils originating from implantation discs at either end of the cylinder. These fibrils were 10 to 12 nm. in diameter and spiraled around the surface of the central cylinder overlapping in the middle to give a total of 12 to 16 axial fibrils. The entire organism was surrounded by an envelope which appeared to be composed of 2 trilaminar membranes.

Hematologic changes were examined in 3 trials. Hemoconcentration accompanied dysentery and was made apparent by increased packed cell volume, erythrocyte numbers, and total plasma protein. Plasma fibrinogen increased to levels of from 0.6 to 1.0 Gm./100ml., and there was a corresponding increase in erythrocyte sedimentation rate to from 15 to 50 mm./hour. Leukocytosis usually occurred shortly before death. A marked left shift began with the onset of diarrhea and intensified as the disease progressed. The number of band neutrophils was as high as 10,920 cells/cmm., and neutrophil metamyelocytes rose to as high as 4,900 cells/cmm. Albumin/globulin ratios declined as a result of decreased albumin and increased alpha globulin. Levels of serum glutamic oxaloacetic transaminase activity remained normal.

A severe disturbance in electrolyte balance was noted in pigs with dysentery. Serum Na and Cl levels declined throughout the course of the disease, and serum K levels increased shortly before death. Metabolic acidosis resulted from depletion of plasma HCO_3. In some pigs the metabolic acidosis was partially or completely compensated by respiratory alkalosis.

Further investigation is needed to find a practical approach to therapeutic replacement of depleted serum sodium, chloride, and bicarbonate.

These studies indicate that large spirochetes have an essential role in the pathogenesis of swine dysentery. The possibility that lesions result from the synergistic action of large spirochetes and vibrio is discussed.

LITERATURE CITED

Abrams, G. D., Baur, H., and Sprinz, H.: Influence of the Normal Flora on Mucosal Morphology and Cellular Renewal in the Ileum. Lab. Invest., 12, (1963): 355-364.

Alexander, T. J. L., and Taylor, D. J.: The Clinical Signs, Diagnosis and Control of Swine Dysentery. Vet. Rec., 85, (1969): 59-63.

Andress, C. E., and Barnum, D. A.: Pathogenicity of Vibrio Coli for Swine II: Experimental Infection of Conventional Pigs with Vibrio Coli. Can. Jour. Comp. Med., 32, (1968): 529-532.

Andress, C. E., Barnum, D. A., and Thomson, R. G.: Pathogenicity of Vibrio Coli for Swine I: Experimental Infection of Gnotobiotic Pigs with Vibrio Coli. Can. Jour. Comp. Med., 32, (1968): 522-528.

Armed Forces Institute of Pathology: Manual of Histologic and Special Staining Techniques. 3rd ed., McGraw-Hill Book Co., New York, N.Y., 1968.

Blakemore, W. F., and Taylor, D. J.: An Agent Possibly Associated with Swine Dysentery. Vet. Rec., 87, (1970): 59-60.

Boley, L. E., Woods, G. T., Hatch, R. D., and Graham, R.: Studies on Porcine Enteritis: II. Experimental Therapy with Sulfathalidine, Sulfamethizine, Sodium Arsanilate, and Bacitracin in a Natural Outbreak of Swine Dysentery. Cornell Vet., 41, (1951): 231.

Bunce, S. A.: Observations on the Blood Sedimentation Rate and the Packed Cell Volume of Some Domestic Farm Animals. Brit. Vet. Jour., 110, (1954): 322-328.

Carle, B. N., and Dewhirst, W. H.: A Method for Bleeding Swine. J.A.V.M.A., 101, (1942): 495-496.

Cassidy, D. R.: The Hematology of Hog Cholera. Unpublished Ph.D. Thesis, Iowa State University, Ames, Iowa, (1970).

Cornelius, C. E., Bishop, J., Switzer, J., and Rhode, E. A.: Serum and Tissue Transaminase Activities in Domestic Animals. Cornell Vet., 49, (1959): 116-126.

Deas, D. W.: Observations on Swine Dysentery and Associated Vibrios. Vet. Rec., 72, (1960): 65-69.

DiLiello, L. R., Poelma, L. J., and Faber, J. E.: Biochemical and Serological Separation of Some Members of the Genus Vibrio. Am. J. Vet. Res., 20, (1959): 532-536.

Dimopoullos, G. T.: Plasma Proteins. In Clinical Biochemistry of Domestic Animals. 2nd ed. Vol. 1. Edited by J. J. Kaneko and C. E. Cornelius. Academic Press, New York, N.Y. (1970): 97-129.

Doyle, L. P.: A Vibrio Associated with Swine Dysentery. Am. J. Vet. Res., 5, (1944): 3-5.

Doyle, L. P.: Infectious Types of Swine Enteritis. U. S. Livestock San. Assoc. Proc., 43, (1939): 224-231.

Doyle, L. P.: Swine Dysentery. J.A.V.M.A., 102, (1943): 449-451.

Doyle, L. P.: Swine Dysentery. J.A.V.M.A., 106, (1945): 26-28.

Doyle, L. P.: The Etiology of Swine Dysentery. Am. J. Vet. Res., 9, (1948): 50-51.

Dubos, R., Schoedler, R. W., Costello, R., and Hoet, P.: Indigenous, Normal, and Autochthonous Flora of the Gastrointestinal Tract. J. Exp. Med., 122, (1965): 67-76.

Elkinton, J. R., Winkler, A. W., and Danowski, T. S.: Transfers of Cell Sodium and Potassium in Experimental and Clinical Conditions. J. Clin. Invest., 27, (1948): 74-81.

Espinasse, J., and Redon, P.: The Control of Swine Dysentery. Vet. Rec., 86, (1970): 24.

Gambino, S. R.: Water, Electrolytes, Acid-base, and Oxygen. In Clinical Diagnosis by Laboratory Methods. 14th ed. Edited by I. Davidsohn and J. B. Henry. W. B. Saunders Co., Philadelphia, Pennsylvania. (1969): 645-672.

Glock, R. D., and Harris, D. L.: Pathogenesis of Swine Dysentery in Feeder Pigs. Unpublished Paper Presented at the 51st Annual Meeting of the Conference of Research Workers in Animal Diseases, Chicago, Illinois, November 30, 1970. Dept. of Veterinary Pathology, Iowa State University, Ames, Iowa (1970).

Gordon, J. H., and Dubos, R.: The Anaerobic Bacterial Flora of the Mouse Cecum. J. Exp. Med., 132, (1970): 251-260.

Gorrie, C. J. R.: Enteric Diseases of Swine: II. Swine Dysentery. Aust. Vet. J., 22, (1946): 135-138.

Govan, C. D., and Weiseth, W. M.: Potassium Intoxication. J. Ped., 28, (1946): 550-553.

Hanson, A. W.: Isolation of Spirochetes from Primates and Other Mammaliam Species. Brit. J. Ven. Dis., 46, (1970): 303-306.

Harland, W. A., and Lee, F. D.: Intestinal Spirochaetosis. Brit. Med. Jour., 3, (1967): 718-719.

Harris, D. L., and Glock, R. D.: Swine Dysentery: A Review. Iowa State Univ. Vet., 33, (1971): 4-11.

Harris, D. L., Mullin, M. T., Kinyon, J. M., and Glock, R. D.: Isolation and Propagation of Spirochetes from the Colon of Swine Dysentery Affected Pigs. Can. Jour. Comp. Med., (1972): Accepted for Publication.

Hofferd, R. M.: Swine Dysentery in Iowa from a Field Standpoint. J.A.V.M.A., 88, (1936): 299-310.

James, H. D., and Doyle, L. P.: Further Studies with a Vibrio as the Etiologic Agent of Swine Dysentery. J.A.V.M.A., 111, (1947): 47.

King, W. E., and Baeslack, F. W.: Studies on the Virus of Hog Cholera. Jour. Inf. Dis., 12, (1913): 39-41.

Kornegay, E. T.: Daily and Twice Daily Repeated Blood Sampling in Weanling Pigs. Am. J. Vet. Res., 28, (1967): 839-844.

Kronland, N., and Kendrick, J. W.: A Method of Isolating Vibrio coli from Pigs. Am. J. Vet. Res., 30, (1969): 1245.

Lee, F. D., Kraszewski, J., Gordon, J., Howie, J. G. R., McSeveney, D., and Harland, W. A.: Intestinal Spirochetosis. Gut, 12, (1971): 126-133.

Listgarten, M. A., and Socransky, S. S.: Electron Microscopy of Axial Fibrils, Outer Envelope, and Cell Division of Certain Oral Spirochetes. J. Bacteriol., 88, (1964): 1087-1103.

Listgarten, M. A., and Socransky, S. S.: Electron Microscopy as an Aid in the Taxonomic Differentiation of Oral Spirochetes. Arch. Oral Biol., 10, (1965): 127-138.

Loveday, R. K.: Swine Dysentery in South Africa--Report of an Outbreak. J. S. Afr. Vet. Med. Assoc., 35, (1964): 51-55.

Lussier, G.: Studies on Vibrionic Dysentery in Swine. Unpublished Ph.D. Thesis, University of Ontario, Guelph, Ontario, (1961).

Lussier, G.: Vibrionic Dysentery of Swine in Ontario. Can. Vet. Jour., 3, (1962): 228-237.

Lynch, M. J., Raphael, S. S., Mellor, L. D., Spare, P. D., and Inwood, M. J. H.: Medical Laboratory Technology and Clinical Pathology. 2nd ed. W. B. Saunders Co., Philadelphia, Pa., 1969.

Manninger, R., Meszaros, J., and Szentivanyi, T.: Further Investigations on the Etiology of Infectious Gastroenteritis of Pigs. Acta Vet. Hung., 10, (1960): 93-98. Abs. in Vet. Bull., 35:118.

Milicevic, M., Addleman, A. D., Mayer, D. T., and Lasley, J. F.: Breed Differences in the Number and Kinds of Leucocytes in Blood of Swine. University of Missouri Research Bulletin 731, 1960.

Miller, E. R., Ullrey, D. E., Ackerman, I., Schmidt, D. A., Hoefer, J. A., and Luecke, R. W.: Swine Hematology from Birth to Maturity. I. Serum Proteins. J. An. Sci., 20, (1961): 31-35.

Moon, H. W.: Epithelial Cell Migration in the Alimentary Mucosa of the Suckling Pig. Proc. Soc. Exper. Biol. Med., 137, (1971): 151-154.

Moon, H. W., Whipp, S. C., and Baetz, A. L.: Comparative Effects of Enterotoxins from Escherichia coli and Vibrio cholerae on Rabbit and Swine Small Intestine. Lab. Invest., 25, (1971): 133-140.

Olson, L. D.: Therapeutic Studies on Swine Dysentery. Proc. of North Central Conf. of Vet. Lab. Diagnosticians, Univ. of Missouri, Columbia, Missouri (1971).

Pascal, R. R., Kaye, G. I., and Lane, N.: Colonic Pericryptal Fibroblast Sheath: Replication, Migration, and Cytodifferentiation of a Mesenchymal Cell System in Adult Tissue. I. Autoradiographic Studies of Normal Rabbit Colon. Gastroent., 54, (1968): 835-851.

Pindak, F. F., Clapper, W. E., and Sherrod, J. H.: Inicidence and Distribution of Spirochetes in the Digestive Tract of Dogs. Am. J. Vet. Res., 26, (1965): 1391-1399.

Powell, H. S.: Studies of Swine Dysentery. Unpublished M.S. Thesis, University of Georgia, Athens, Georgia (1970).

Reitman, S., and Frankel, S.: Colorimetric Method for the Determination of Serum Transaminase Activity. Am. J. Clin. Path., 28, (1957): 56-63.

Roberts, D. S.: Editorial: Synergic Mechanisms in Certain Mixed Infections. Jour. Inf. Dis., 120, (1969): 720-724.

Roberts, D. S.: Studies on Vibrionic Dysentery of Swine. Aust. Vet. J., 32, (1956): 114-118.

Roberts, R. M., and Simmons, J. R.: An Agent Possibly Associated with Swine Dysentery. Vet. Rec., 86, (1970): 22.

Ruth, G. R.: Experimental Vibrionic Colitis in Swine. Unpublished M.S. Thesis, Michigan State University, Ann Arbor, Michigan (1967).

Savage, D. C., and McAllister, J. S.: Microbial Interactions at Body Surfaces and Resistance to Infectious Diseases. In Resistance to Infectious Disease. Edited by R. Dunlop and H. Moon. Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon (1970): 113-127.

Savage, D. C., McAllister, J. S., and Davis, C. P.: Anaerobic Bacteria on the Mucosal Epithelium of the Murine Large Bowel. Infection and Immunity, (1971) Accepted for Publication.

Schalm, O. W.: Plasma Protein: Fibrinogen Ratios in Disease in the Dog and Horse--Part II. Calif. Vet., 24, (1970): 19-22.

Schalm, O. W., Smith, R., and Kaneko, J. J.: Plasma Protein: Fibrinogen Ratios in Dogs, Cattle, and Horses. Part I. Influence of Age on Normal Values and Explanation of Use in Disease. Calif. Vet., 24, (1970): 9-11.

Shera, A. G.: Specific Granular Lesions Associated with Intestinal Spirochaetosis. British J. Surg., 50, (1962): 68-77.

Smith, D. T., Conant, N. F., and Willett, H. P.: Zinsser Microbiology. 14th ed. Meredith Corp., New York, N.Y., 1968.

Socransky, S. S., Loesche, W. J., Hubersak, D., and Macdonald, J. B.: Dependency of Treponema microdentium on Other Oral Organisms for Isobutyrate, Polyamines, and a Controlled Oxidation-reduction Potential. J. Bact., 88, (1964): 200-209.

Soderlind, O.: The Isolation of Vibrio coli from Pigs. Vet. Rec., 77, (1965): 193-196.

Sorensen, D. K.: Dysentery. In Diseases of Swine. 3rd ed. Edited by H. W. Dunne. Iowa State University Press, Ames, Iowa. (1970a): 486-498.

Sorensen, D. K.: Salmonellosis. In Diseases of Swine. 3rd ed. Edited by H. W. Dunne. Iowa State University Press, Ames, Iowa. (1970b): 499-507.

Sorensen, D. K., Martinsons, E., and Perman, V.: Clinical and Hematological Manifestations of Hog Cholera. In Symposium on Hog Cholera. Edited by G. T. Mainwaring and D. K. Sorensen. University of Minnesota, St. Paul, Minnesota (1961): 29-42.

Spinco Division of Beckman Instruments, Inc.: Methods Manual--Model R-100 Microzone Electrophoresis System. Stanford Industrial Park, Palo Alto, California, 1967.

Sprinz, H.: Pathogenesis of Intestinal Infections. Arch. Path., 87, (1969): 556-562.

Swain, R. H. A.: Electron Microscopic Studies of the Morphology of Pathogenic Spirochaetes. J. Path. Bact., 69, (1955): 117-128.

Sweeney, E. J.: The Aetiology of Dysentery of Swine. Vet. Rec., 78, (1966): 372-375.

Tasker, J. B.: Fluids, Electrolytes, and Acid-base Balance. In Clinical Biochemistry of Domestic Animals. 2nd ed. Vol. 2. Edited by J. J. Kaneko and C. E. Cornelius. Academic Press, New York, N.Y. (1971): 62-110.

Taylor, D. J.: An Agent Possibly Associated with Swine Dysentery. Vet. Rec., 86, (1970): 416.

Taylor, D. J., and Blakemore, W. F.: Spirochaetal Invasion of the Colonic Epithelium in Swine Dysentery. Res. Vet. Sci., 12, (1971): 177-179.

Tennant, B. C., and Ewing, G. O.: Gastrointestinal Function. In Clinical Biochemistry of Domestic Animals. 2nd ed. Vol. 2. Edited by J. J. Kaneko and C. E. Cornelius. Academic Press, New York, N.Y. (1971): 111-153.

Terpstra, J. I., and Akkermans, J. P. W. M.: Dysentery in Swine. Proc. 19th World Vet. Congress, 2, (1971): 422-424.

Terpstra, J. I., Akkermans, J. P. W. M., and Ouwerkerk, H.: Investigations into the Etiology of Vibrionic Dysentery (Doyle) in Pigs. Neth. J. Vet. Sci., 1, (1968): 5-13.

Todd, J. N., Hunter, D., and Clark, A.: An Agent Possibly Associated with Swine Dysentery. Vet. Rec., 86, (1970): 228.

Truszczynski, M.: Further Investigations on the Aetiology of Swine Dysentery. Roczn. Nauk. Rol., 68, (1957): 141.

Vallejo, M. T.: Spirochaetales Micro-organisms: An Agent Possibly Associated with Swine Dysentery. Vet. Rec., 85, (1969): 562-563.

Vesselinovitch, S. D.: Electrophoretic Studies of Oedema Disease in Swine. Brit. Vet. J., 111, (1955): 398-403.

Warner, S.: Studies on the Pathogenesis of Vibrio coli Infection in Swine. Unpublished Ph.D. Thesis, University of Minnesota, St. Paul, Minnesota (1965).

Weide, K. D., and Twiehaus, M. J.: Hematological Studies of Normal, Ascarid-Infected, and Hog Cholera-Vaccinated Swine. Am. J. Vet. Res., 20, (1959): 562-567.

Whiting, R. A., Doyle, L. P., and Spray, R. S.: Swine Dysentery. Purdue Univ. Agr. Exp. Station Bull. 257, (1921).

Whiting, R. A.: Swine Dysentery. J.A.V.M.A., 17, (1924): 600-610.

Whiting, R. A.: Swine Dysentery. J.A.V.M.A., 25, (1928): 721-728.

Wintrobe, M. M., and Landsberg, J. W.: A Standardized Technique for the Blood Sedimentation Test. Am. J. Med. Sci., 189, (1935): 102.

Wretlind, B., Orstadius, K., and Lindberg, P.: Transaminase and Transferase Activities in Blood Plasma and in Tissues of Normal Pigs. Zentr. Veterinarmed., 6, (1959): 963-970.

Zymet, C. L.: Canine Spirochetosis and Its Association with Diarrhea. Small Animal Clinician, 64, (1969): 883-887.

ACKNOWLEDGMENTS

My sincere appreciation goes to Dr. F. K. Ramsey for his enthusiastic support and for sharing his usually excellent and never dull philosophy. This appreciation also extends to Dr. J. P. Kluge for his encouragement and generous assistance during this project.

Thanks are extended to the members of my graduate committee and especially to Dr. C. J. Mare for his example and guidance in the art of research.

A special thanks is expressed to Dr. D. L. Harris who provided direct assistance, thoughtful criticism, and moral support throughout this endeavor.

I wish to acknowledge the helpfulness and stimulating environment provided by the faculty and staff of the Department of Veterinary Pathology. Special gratitude goes to Miss Carol Wehling, Mrs. Jean Bischof, and Mrs. Elizabeth Yoder for their technical assistance and counsel.

I thank my wife, Ruth, and my children, Karen, Jeffrey, and Douglas for their patience during the past four years.

I would also like to express my gratitude to those who have been most helpful but have not received individual mention.