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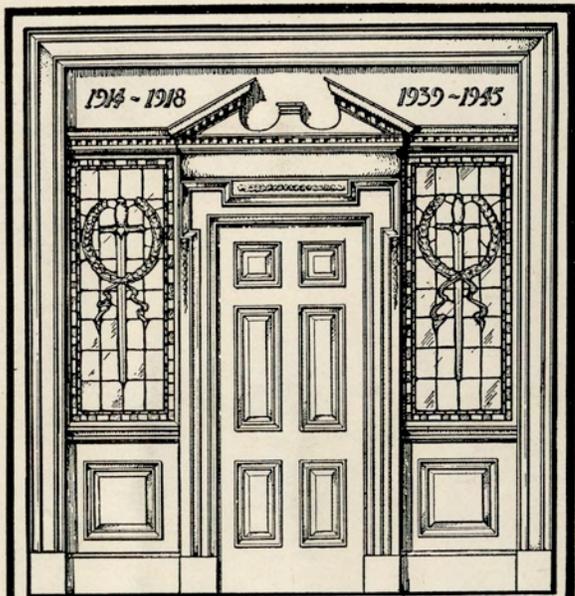
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STUDIES ON VIBRIONIC DYSENTERY
IN SWINE

A Thesis

Presented to the School of Graduate Studies
of

The University of Toronto

by

LOUIS GILLES LUSSIER

In partial fulfilment of the requirements

for the degree of

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BIOGRAPHICAL SKETCH

The author was born October 5, 1934 at St-Charles of St-Hyacinthe, Quebec, where he received his primary education. He then studied at the University of Montreal for the degree of Bachelor of Arts graduating in June 1955. Four years later he received a Doctorate of Veterinary Medicine at the Ecole of Médecine Vétérinaire de la Province de Québec. In July 1959 he commenced studies towards the degree of Master of Veterinary Science at the Ontario Veterinary College

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INTRODUCTION

The limited amount of research on Vibrionic Dysentery in swine may be attributed to the misunderstanding of the enteric diseases of this species, the failure to recognize the etiologic agents of the enteric conditions which may result in fibrinonecrotic enteritis, the prevalence of hog cholera in certain countries, and the difficulties encountered in isolating Vibrio coli from affected animals and in experimentally transmitting this infection.

Vibrionic Dysentery is a specific, acute or chronic, infectious, transmissible disease of swine characterized by a severe inflammation of the large intestine and diarrhea. The feces may contain mucus, blood or particles of croupous exudate but real bloody diarrhea is encountered only in a few instances.

Hofferd (63) and Doyle (32) reported that the disease was first noticed in the United States in 1918. Vibrionic Dysentery was described in 1921 by Whiting, Doyle and Spray (133). Since then it has been reported from a number of different countries and has been regarded as a specific disease.

In Ontario, Vibrionic Dysentery is probably the most important enteric infection of swine. Its primary importance is economic in regards to the loss of body weight and the set-back of pigs, in addition to the mortality caused by the disease.

The obvious lesions encountered are situated in the gastrointestinal tract, almost exclusively in the large intestine. The lesions most frequently seen are those of catarrhal inflammation. Lesions of hemorrhagic and fibrinonecrotic inflammation are also frequently observed. Neither constant nor significant gross changes are found in the other organs.

Many names have been given to this condition by veterinarians and farmers throughout the years, including: hemorrhagic enteritis, hemorrhagic colitis, hemorrhagic dysentery, swine typhus, bloody dysentery, flux, bloody flux, bloody diarrhea, colitis, Dakota disease, bloody scours, black scours, necrotic enteritis, infectious enteritis, necro, swine vibriosis, vibrionic enteritis, swine dysentery and vibrionic dysentery.

For clarity, the following work has been divided in three parts:

The first part is a study of the gross and microscopic lesions of Vibrionic Dysentery as they are seen in natural cases in Ontario.

The second part deals with details concerning the isolation and identification of Vibrio coli from naturally or experimentally infected pigs.

The third part deals with the significance of Vibrio coli in the etiology of the disease in swine.

LITERATURE REVIEW

Enteritic diseases have long been recognized as important conditions in swine. Salmon and Smith (115) in 1885 published the first report on enteric diseases of swine in America. In the course of their work on the condition known as swine plague, they isolated and described a motile organism which they considered the etiological agent of the disease. They proposed the name Bacillus suispestifer for this organism and the name hog cholera for the disease to differentiate it from European swine plague. They reproduced experimentally a disease which resembled hog cholera by feeding per os or injecting intravenously large doses of cultures of this organism. The lesions of colitis and typhlitis resembled, in some aspects, hog cholera and for many years the Bacillus suispestifer organism, today known as Salmonella choleraesuis, was considered the causative agent of hog cholera.

In 1903, de Schweinitz and Dorset (120) discovered that hog cholera was caused by a filterable virus. They produced a disease in swine by subcutaneous inoculations of filtered body fluids free of bacteria. This discovery was later confirmed by Dorset et al (30) who proved that a filterable virus was the cause of hog cholera and concluded that S. choleraesuis was not the primary factor of the disease. Following the finding of de Schweinitz and Dorset (30) that hog cholera was caused by a filterable virus, S. choleraesuis was considered

as only a secondary invader until Glasser (52) in 1907, and Damman and Stedefeder (25) in 1910, showed that paratyphoid infection caused by S. choleraesuis occurred in swine in which no virus could be demonstrated.

In 1927 and 1928, Murray et al (92) continuing the work of Salmon and Smith (115) and Dorset et al (30) on infectious enteritis in swine, reported that S. choleraesuis was the primary etiological agent of infectious enteritis, so-called necrotic enteritis, and that Spherophorus necrophorus, long under suspicion, appeared consistently as a secondary invader of the intestinal tract. By feeding broth cultures, they reproduced necrotic enteritis and the organism was reisolated from the experimental animals. Salmonellosis in swine has also been reported from other European countries. From England by Blount (9) in 1933 and Breckett (13) in 1934, from Ireland by Shanks and Lamont (122) in 1939, from Australia by Hindmarsh et al (62) in 1939 and from New Zealand by Marshall (88) in 1938.

From 1927 to 1940, the hog cholera virus and S. choleraesuis were quite generally admitted to produce enteritis in swine. But following the reports of Chick et al (21) in England, Madison et al (87) in the United States, in which a condition characterized by diarrhea and dermatitis was described in pigs on a diet consisting largely of corn and deficient in nicotinic acid, Davis et al (26) in 1940 concluded that necrotic enteritis

in pigs was a secondary complication caused by intestinal invasion by S. suispestifer, and probably other organisms, after the symptoms of a deficiency in nicotinic acid have been developed. This again raised the question whether or not S. choleraesuis could be the primary etiological agent of necrotic enteritis.

On the other hand, Edgington et al (43), in 1942, concluded from their experiments that the data indicated that the protective value of nicotinic acid against S. choleraesuis infection was not sufficient to encourage its use as a specific preventive or curative measure for this type of bacterial infection in swine. Krider (31) considered that necrotic enteritis in swine was not solely a nutritional disorder. The same year, Breed (14) concluded that there were two types of necrotic enteritis, one being of the infectious type caused by S. choleraesuis and the other being of the nutritional type.

In 1947, McNutt and Dacorso (97) stated that necrotic enteritis has caused much confusion in the literature and noted that "were it understood that necrotic enteritis is a lesion and not a specific disease entity, a much better understanding would prevail, because it is certain that there are several causes for the lesion" and when "necrotic enteritis has been produced, a type of lesion has been reproduced which by itself is not a specific disease."

In 1944, Doyle (35) reported the isolation in apparently pure culture of a vibrio from pigs suffering from intestinal

disorders. To date many vibrio organisms have been isolated from various sources notably river and sea water, soil, cheese, fish, sewage, buccal and nasal cavities, abscesses of the large intestine of swine, etc (89).

However, even if most of these organisms are saprophytes and only few are considered pathogenic and important in medicine, the biochemical and serological relations between the organisms of the genus Vibrio are not perfectly known. Among the species considered pathogenic for man and animals are V. comma, V. fetus, V. jejuni, V. coli and vibrios isolated from cases of hepatitis in chicken. Most of the organisms enumerated above, as noted by Lecce (85), are morphologically similar, and are then impossible to differentiate on this basis alone.

V. comma was first isolated by Koch (79) in 1883 from the intestinal discharges of humans affected with Asiatic cholera. He described the morphological and biochemical characteristics of the organism.

The first report of a vibrionic infection in animals was presented by Gamaléia (50) in Russia in 1888. He isolated a vibrio from chickens affected with a condition characterized by a marked gastrointestinal infection accompanied by a severe diarrhea. He gave the name V. metchnikovii to this organism.

In 1909, McFadyean and Stockman (95) in England isolated a vibrio from the uterine discharges of aborting ewes. A few years later, in 1918, Theobald Smith (124) was the first in

the United States to isolate a vibrio in pure culture from the fetus and placenta of aborting cows. The next year, Smith and Taylor (125) described the morphological, cultural and biological characteristics and agglutination reactions of this organism. Smith named it V. fetus. Since then, vibrionic abortion in cattle and sheep has been extensively described and appears to be widely distributed. To date, however, some investigators have given evidence that vibrios, other than V. fetus, inhabit the genital tract of cattle and sheeps (29)(46) (47).

King (78) studied the public health significance of vibrios isolated from cases of gastroenteritis in man and found that these organisms were serologically related to V. fetus. She added that it was possible that the vibrios isolated from two children might be closely related to the organism described as V. jejuni.

Until recently, a vibrio has been accepted as the cause of an infection occurring in cattle. This condition was reported in 1919 by Steffen (127). Then, from 1931 to 1933 in a series of papers, Jones, Little and Orcutt (72 - 76) described the etiology, pathology and clinical aspects of winter dysentery in cattle. They proposed the name V. jejuni for the etiological agent of the disease and reported that, while resembling V. fetus morphologically and culturally, the antiserum prepared

against one culture of V. fetus, agglutinated slightly many cultures of V. jejuni in only the lower dilutions. However, because of the difficulty and inconsistency with which the disease can be reproduced in experimental animals with V. jejuni, workers are beginning to suspect that a virus is the cause of the infection or that two diseases exist concurrently (48)(86).

In 1958, Peckham (99) in New York State and Hofstad et al (64) in Iowa isolated a vibrio from chickens suffering from a disease characterized by a drop in egg production, light to fairly heavy losses, and a moderate to severe liver degeneration. The characteristics reported by Peckham for his isolate are closely similar to those described for vibrios isolated from cattle and sheep. In Canada, Whenham et al (132) also reported the isolation of vibrios from birds.

Vibrionic Dysentery, now thought to be caused by a vibrio organism, is another enteric disease of swine which has been recognized as a specific disease entity for many years. The earliest reports available concerning the occurrence of this disease in America date from 1918 and were recorded by Hofferd (63) in Iowa and Doyle (32) in Indiana. Hofferd stated that Dr. G.A. Kay informed him that he had encountered the disease in western Iowa in 1918. Kay said that the disease was very extensive then, particularly in hogs that passed through public

sale yards. Doyle (32) on the other hand noted that Indiana workers have experienced the disease in 1918 in hogs coming from stockyards in South St-Paul, South Dakota and Chicago.

Since then Vibrionic Dysentery has been reported from many other states: Illinois (91), Ohio (22), Michigan (41) (83), Wisconsin (97), and Texas (116). The disease has also been reported from a number of different countries: Australia (57) (62) (96) (105), Switzerland (117)(119)(130), Holland (131), Germany (66)(98), Hungary (24)(44), Poland (53)(69) (70)(129), Czechoslovakia (18), England (8), France (3), Sweden (111), and Scotland (28).

Vibrionic Dysentery was first described in 1921 by Whiting, Doyle and Spray (134). These authors described the symptoms, the bacteriological findings, the gross and microscopic features of the disease, and the experimental work done in Indiana. Both young and old pigs were affected. The incubation period in field outbreaks ranged from 7 to 60 days; in experimentally infected pigs it ranged from 4 to 12 days. Elevation of body temperature was the first symptom noticed together with twitching of the tail and depression followed by diarrhea. At first, the feces were thin and contained a large quantity of mucus and blood. Later, the intestinal discharge became darker and the animals showed weakness and emaciation. Forty to sixty per cent of the young pigs died, compared to 10 to 20 per cent of the feeder hogs,

with an average death loss of 25 per cent. The gross pathological findings in early cases revealed congestion and hemorrhage of the mucosa of the stomach, cecum, colon and rectum. In more advanced cases, necrosis of the mucosa, frequently extending into the submucosa and muscular layers, was the macroscopic picture of the stomach, cecum, colon and rectum. The microscopic sections taken from the colon in the early stages of the disease showed exudate on the mucous surface, marked hyperemia and hemorrhage in the mucosa and some hyperemia and hemorrhage in the submucosa. Sections taken later in the course of the disease showed that the marginal epithelium was practically all gone and the epithelium of the crypts was atrophied. Sections taken still later in the course of the disease showed necrosis of the superficial portion of the mucosa with leukocytic infiltration of the submucosa and atrophy of the cryptic epithelium. Following the experimental studies of the disease, Whiting et al (134) concluded that this type of dysentery in swine was distinct from hog cholera, that the etiological agent of the disease was in the intestinal discharge and was not S. choleraesuis.

In 1924, Whiting (133) confirmed his previous experimental findings. He stated that he was able to reproduce experimentally the disease in each pig by feeding colon and cecum contents and feces from affected swine to healthy pigs. Following the feeding of hearts, lungs, spleens and kidneys, none of the pigs developed the disease but were proved susceptible when exposed to dysenteric

colon. Blood samples were also taken from outbreaks of dysentery and administered intramuscularly, intravenously and per os to pigs not vaccinated against hog cholera. None of the pigs developed the disease but were susceptible to hog cholera on later challenge. He also failed to reproduce the disease by feeding of B. coli, B. paracoli, B. pyocyaneus, spirochetes or amoebae; but pigs surviving B. suispestifer infection were proved to be susceptible to dysentery. The incubation period in experimentally infected pigs fed colon, cecum or feces varied from 5 to 18 days. In pigs infected by pen contact, the incubation period was 5 to 39 days. Whiting (133) described the symptoms as emaciation, weakness and diarrhea which in typical cases became red colored. The gross lesions were chiefly seen in the cecum, colon, rectum and stomach, they consisted of congestion in early stages and diphtheritic exudate in later cases. The microscopic examination of sections taken in early cases showed hyperemia, extravasations of red blood cells, desquamation of the surface epithelium and goblet cells, distention. One of the illustrations shows what appears to be a vibrio. In later cases necrosis was observed frequently involving the submucosa. Balantidium coli were also frequently observed.

In 1927, Murray et al (92) in their work on "infectious enteritis" in swine, differentiated this condition from "bloody diarrhea". They noted that in one case of "bloody diarrhea" numerous "spirilla" were present in the intestinal lesions.

In 1936, it became apparent that the disease had increased considerably in incidence. Hofferd (63) gave his observations from field cases. He reported that Vibrionic Dysentery became an important disease in the last few years in Iowa and stated that the disease "had become so prevalent, menacing, destructive and difficult to control that it had caused alarm among the live stock raisers and veterinarians all over the state." The mortality varied greatly and was often 50 per cent, even though effort had been made to stop the losses. Pigs of all ages were susceptible, but it seemed that young pigs and recently vaccinated pigs were more susceptible. He was of the opinion that reinfection could occur following new importations of cattle in a farm. The incubation period varied between two and seven days. The disease occurred during the entire year but seemed to be less prevalent during the hot season. Diarrhea was an early symptom. At first, the feces were often dark becoming bloody with mucus and often accompanied by croupous exudate; sometimes free blood was passed. Fever was moderate running about 104.5 F. Usually the pigs showed weakness and great loss of body weight. Death might occur in two to seven days. The post-mortem findings revealed a hemorrhagic inflammation of the cecum and large intestine accompanied frequently by a severe gastritis and hyperemia of the mesenteric lymph glands. Diphtheritic membrane formations were seen in more chronic cases.

In 1937, Vibrionic Dysentery was observed for the first time outside the United States by Hindmarsh et al (62) in New South Wales, Australia.

In 1938, McLennan et al (96) reported the disease from South Australia. The disease they described was characterized principally by diarrhea affecting 33 out of 400 pigs of the herd. The duration of illness was from two to four days before death. Affected animals showed slight fever, were dull, and could hardly move; they passed diarrhetic brownish-colored feces containing blood clots and shreds of mucus. At autopsy, no lesions were seen in the small intestine but hemorrhages and inflammation of the large bowel were noticed; the lymph glands appeared normal. Bacteriological examination of the intestinal contents failed to reveal any causal agent and no growth occurred on cultures from the liver, spleen, kidneys, and lymph glands. Swine fever was eliminated as a possibility by subcutaneous injection of blood from affected pig to normal pig. Microscopic studies of the affected colon showed congestion but no extensive ulceration. In some areas, a fibrinous exudate containing erythrocytes and polymorphonuclear leucocytes was noted; in other areas, slight erosion of the epithelium was observed.

Two years later, Gray (57) in New South Wales and Victoria also reported the disease and described the clinical symptoms and the post-mortem lesions.

In 1939, Hidmarsh et al (62) investigated the etiology of a disease which appeared to be identical with the one reported by American workers in the United States. The lesions described by the authors were essentially those of necrotic enteritis and the first deaths encountered were thought to be due to a salmonella infection. However, serological studies indicated that the disease was not due to S. choleraesuis var. kunzendorf. The authors reported that experimental transmission of the disease by feeding feces of affected pigs was accomplished in only a few instances and the incubation period was 13 days.

In 1939, Koen (80) during his work for the hog cholera section of the Bureau of Animal Industry, stated that losses from Vibrionic Dysentery appeared to be heavier than those due to hog cholera in western Iowa.

One of the advances in the study of Vibrionic Dysentery was made by Doyle (32 - 38) who confirmed the observations of Whiting (133) and reported extensive studies on Vibrionic Dysentery. He described the symptoms, the gross and microscopic lesions, the prevalence, the transmission and the etiology of the disease.

Doyle stated that Vibrionic Dysentery may attack swine of all ages although it is more severe in younger pigs. The experimental incubation period varied from 4 to 12 days, while that of natural outbreaks varied from 7 to as long as 60 days.

The presence of blood and mucus in the feces was characteristic of the diarrhea but in mild cases mucus and blood were not seen in the bowel discharge. In young pigs, the death rate ranged from 10 to 20 per cent while in older pigs it varied from 40 to 60 per cent. The colon and cecum showed marked lesions of inflammation. In the early stages of the disease, the mucosa of the colon and cecum was congested, hemorrhagic and increased mucus secretion could be noted. Later on, flakes formed by the sloughed mucosa were found in the large bowel giving a "rice water" appearance to the contents. Feeding either the colon or the feces of affected pigs set up the disease in healthy pigs but feeding of other viscera did not.

Thus far, the causative agent of the disease was known to be present in the colon and in the bowel discharge of affected pigs; the disease had not been reproduced by feeding viscera other than the large intestine. In 1944, Doyle (35) reported the recovery in apparently pure culture, of a vibrio from the colon of swine affected with dysentery. This vibrio grew only in atmosphere containing 10 to 15 per cent carbon dioxide, and when fed to susceptible pigs, it produced diarrhea in six out of eight pigs; the incubation period being usually from three to five days. The diarrhea produced was less severe and usually less blood and mucus were observed than in cases induced by feeding colon. There was no mention of the recovery of the vibrio from experimentally infected pigs. Doyle named this

organism Vibrio coli while European workers (58)(70)(110) called it Vibrio suis or Spirillum suis.

Vibrionic Dysentery was discussed from the practitioner's point of view by Wilson (135), Truax (128), and Steenerson (126). Murphy (31) stated that the disease seemed to be less prevalent in summer.

In Russia in 1940, a disease named "swine dysentery" was reported by Agapov (1) and Andreev(2). This disease, however, was believed to be primarily due to a virus.

In 1946, Gorrie (54) in Victoria, reported the difficulties encountered to distinguish Vibrionic Dysentery from salmonellosis; however, as also stressed by Hindmarsh et al (62) salmonella organisms were not constantly isolated in cases of Vibrionic Dysentery. He reported the successful transmission of the disease by contact or by drenching the contents of the large bowel to susceptible pigs. He considered that about 10 per cent of normal pigs were resistant. The average incubation period was eight days when infected by drenching and fourteen days when infected by contact. Gorrie (54) also reported that a pig may remain a carrier of the infection even though it is perfectly normal.

In 1947, James and Doyle (71) confirmed the results of Doyle (35) in 1944, where a vibrio was thought to be the etiologic agent of swine dysentery. They recorded that a vibrio was isolated from the cecal submucosa of a pig suffering from dysentery. When the organism was suspended in gastric mucin and fed to susceptible animals, 50 out of 60 pigs developed the disease. They reported

that the symptoms and the gross and microscopic lesions were similar to the ones seen in naturally infected animals.

In 1947, McNutt and Dacorso (97) reviewing the different types of swine enteritis reported that vibrios were frequently seen in the intestinal contents of pigs affected with Vibrionic Dysentery. They reported the successful experimental transmission of the disease by feeding feces or intestines to healthy pigs, while they failed to reproduce the disease by feeding other viscera or bacteria free filtrates of feces. Vibrios were isolated in pure culture from feces using Berkefeld N filters. They also stated that deaths ceased to occur when sick pigs following feeder cattle were isolated, the disease reappearing when animals were again placed in contact with cattle.

Cole (38) reported, in 1949, that several outbreaks of Vibrionic Dysentery had been observed in Ohio. The disease was the most serious condition on several farms in the State. V. coli was isolated from the colon in one outbreak. Hemorrhagic and fibrinonecrotic, typhlitis, colitis, and proctitis were the outstanding lesions observed. Remissions and exacerbations were common.

In Switzerland, Vibrionic Dysentery appeared to be more severe than the disease described in America. The lesions seen at autopsy were far more extensive with degenerative lesions found in various organs outside the alimentary tract. Vibrios were isolated from the intestines in many cases.

Vibrionic Dysentery was first reported in Switzerland by Schmid and Klingler (119) in 1949. They noted that changes occurred in the stomach, large intestine, liver, kidneys, spleen, bladder, lungs, and heart. A vibrio was isolated from the alimentary tract. Schmid (117), describing the clinical symptoms, stated that Vibrionic Dysentery might occur in three forms: acute, characterized by rusty red diarrhea and a rapid course clinically resembling hog cholera; subacute, characterized by a yellow diarrhea; and chronic in which pigs were unthrifty. Vibrios were isolated in 150 cases at Berne. In 1950, van Balmoos (130) made extensive studies on Vibrionic Dysentery at Berne; he stated that the disease was encountered throughout Switzerland. The most constant findings were catarrhal, croupous or hemorrhagic gastroenteritis with enlargement of the local lymph nodes, degeneration of the myocardium and cloudy swelling of the liver and kidneys. Vibrios were seen in smears of the mucosa of the large intestine and grew on blood agar plates under carbon dioxide tension. Unweaned pigs and pigs 12 to 16 weeks of age were the most affected. During experimental infection, two ten-week old pigs remained healthy when they were fed vibrio cultures on alternate days for 49 days. However, when killed on the 50th day, the author reported that the pigs showed typical lesions of Vibrionic Dysentery.

Boley et al (60), in 1951, failed to reproduce the disease by feeding lyophilized cultures of V. coli whereas they had successful results by feeding intestinal contents from sick pigs.

Carpenter and Larson (20) reported that vibrios could usually be found, though in greatly reduced numbers, in the fecal material and colon contents of swine which have been treated and were apparently cured. They concluded that the pig may be a carrier even if the symptoms of the disease have disappeared.

In 1952, Gorrie (55) reported that a vibrio was isolated from affected pigs in Victoria, Australia, but he failed to reproduce the disease in pigs experimentally exposed.

The same year, Vibrionic Dysentery was reported in France by Bertrand (3) who described the clinical aspect and the macroscopic lesions of the disease. He was of the opinion that the infection was caused by a vibrio.

In Holland in 1953, van Ulsen (131) isolated a vibrio on blood agar plates incubated under 10 per cent carbon dioxide. The organism was isolated from ten cases of "hemorrhagic gastritis" in swine. He noted that this vibrio was morphologically similar to V. fetus and was successfully subcultured in chick embryos.

The same year, in Germany, Harms and Horter (58) described a method of cultivation of V. suis and reported that the organism was viable for 12 months in skim milk, at 37 C. Rolle and Mundt (109)(110) and Mundt (90) also described a method of cultivation of a spiral organism isolated from the intestine of pigs and compared it morphologically with V. fetus isolated from aborted fetuses. They named this organism Spirillum suis on the basis

of the distribution of its flagella which were always at both poles whereas all strains of V. fetus studied showed flagella at only one pole. S. suis also differed from V. fetus by the fact that it showed marked endoplasmic granulations. The authors also noted that V. fetus produced a yellowish-white pigment on solid media while colonies of spirilla appeared reddish-brown.

Hupka (66) in Lower Saxony described a highly infectious gastroenteritis in pigs. The disease was characterized by a severe diarrhea and an elevation of the body temperature. The disease was fatal in unweaned pigs but older pigs usually recovered after six days. The lesions seen at post-mortem examination were a severe gastroenteritis, myocardial degeneration, general venous congestion and hemorrhages on the kidneys and the serous membranes. The exact etiology of the disease was unknown but the author suspected a vibrio. Vibrionic Dysentery was also reported from Schleswig-Holstein, Germany in 1954 by Newmann and Steinborn (98).

Vibrionic Dysentery was reported from Hungary in 1955 by Csontos and Pesti (24) and by Erdős et al (44). Csontos and Pesti (24) reproduced successfully the disease by feeding feces and pure vibrio cultures to healthy pigs. Erdős et al (44) isolated vibrio organisms from the mesenteric lymph nodes of one diseased pig.

In Poland, Vibrionic Dysentery was first reported by Janowski (69)(70) in 1955. This worker isolated V. coli on two occasions but he failed to subculture the organism. The disease was experimentally transmitted to two out of six pigs by oral admin-

istration of parts of the large bowel and feces from affected pigs. The post-mortem findings were a thickening of the wall of the colon with diphtheritic deposits on its mucosa. Hemorrhagic gastritis and degeneration of the myocardium were also noted. V. coli was again isolated and, in a new series of experiments, the disease was reproduced in three out of four pigs by oral feeding of affected colon. Truazczynski (129) isolated V. coli from affected pigs on four farms. Forty-eight hour-blood agar cultures of the organism were fed to six pigs for 3 consecutive days; one pig developed dysentery after 8 days, three showed transient diarrhea, while two remained healthy. In another experiment, the author fed cultures of V. coli to four pigs two days after vaccination against hog cholera; two pigs developed typical symptoms of Vibrionic Dysentery after 16 to 18 days and one of them died while one of two contact pigs also showed dysentery. Golebiowski (53) concluded that Vibrionic Dysentery was more frequent in pigs 3 to 20 weeks of age. He isolated V. coli from the submucosa of the colon and the mesenteric lymph glands. The chief lesions were hemorrhagic gastritis and fibrinonecrotic colitis. Agglutination and complement fixation tests had no value for the diagnostic of the disease.

In 1956, Roberts (105) in New South Wales reported the isolation of a vibrio from an outbreak of dysentery in a farm where no pigs had been introduced for two years. The vibrio isolated reproduced the disease 6 to 7 days after feeding to susceptible

pigs. The disease produced experimentally, however, was less severe than that which occurred naturally but was comparable to that produced by feeding infected large bowel. In a following paper, Roberts (106) considered the cultural and biochemical characteristics of this vibrio. He failed to detect homologous antibody in the sera of convalescent pigs by agglutination, antiglobulin and complement fixation tests. He also described the gross and microscopic changes encountered in pigs affected with Vibrionic Dysentery. The principal lesion was an acute inflammation of the large intestine characterized by a leukocytic infiltration of the mucosa, a great activity of the goblet cells and, in some areas, necrosis and sloughing of the mucous membrane; hemorrhage occasionally accompanied the picture. The liver and kidneys showed lesions of cloudy swelling but no lesions were detected in the heart and spleen.

In Czechoslovakia, the morphology of V. coli was studied by Bystricky and Stricker (18) in 1957. These authors described the organism as amphitrichous. In old cultures, spore-like granules of 0.4 to 1.3 microns in diameter were seen.

Vibrionic Dysentery has been reported from England by Birrell (8) in 1957. He described an acute disease in pigs characterized by diarrhea, staggering gait, reduced feed consumption and stunted growth. The lesions at autopsy were principally seen in the cecum, and colon. The mucous membrane was thicker and softer than normal and showed inflammatory foci. The mesenteric lymph nodes were enlarged but normal in color. A vibrio was demonstrated in

smears made from the mucosa of the colon. The organism appeared as spiral, S- shaped, comma or seagull forms. Shand and Gitter (121) fed cultures of V. coli in gastric mucin employing different methods and keeping pigs under stress. A small percentage of the animals developed diarrhea but the authors concluded that V. coli was not highly pathogenic.

Florent (46)(47) compared strains of V. coli isolated from the intestines of pigs with strains of V. fetus causing abortion in cattle. He classified V. coli in two types on the basis of H₂S production; type 1 producing H₂S and type 11 not producing H₂S. On blood agar plates containing brilliant green, colonies of V. fetus and V. coli were identical. Morphologically both organisms were also identical. Biochemically V. fetus and V. coli were both catalase-positive, carbohydrates-negative, did not liquefy gelatin, reduced nitrates, did not produce H₂S except V. coli type 11. Agglutination tests showed that V. fetus and V. coli type 1 were serologically related. Florent was of the opinion that V. coli type 1 may enter the blood stream from the intestine and colonize in the placenta causing sporadic abortion of a type so far attributed to V. fetus. He fed a mixture of V. coli type 1 and 11 to six cows in their sixth month of pregnancy. Vibrios were demonstrable for several weeks in the feces but did not cause abortion.

In Canada, Roe and Drennan (108) obtained good results in treating Vibrionic Dysentery with nitrofurazone given in the

drinking water during 7 days.

Miliello et al (29), studying the biochemical and serological reactions of 68 vibrio cultures from various sources, noted that "the isolation of V. fetus - like organisms from swine indicates the possibility of these animals being carriers for V. fetus infection in cattle, sheep, goat, and possibly in man."

Nordus (111) reported Vibrionic Dysentery in Sweden in 1959. The death loss attributed to the disease was about 10 per cent.

In Scotland, Deas (28) reported the isolation of two types of vibrios from the colon and mesenteric lymph nodes of weaned pigs affected with acute or subacute colitis. The vibrios isolated were classified according to Florent (46) in two types. Type 11 was the most frequently observed type and type 1 was morphologically and biochemically similar to V. fetus. On the basis that he failed to reproduce the disease in susceptible pigs using both types of vibrios and on the basis that he isolated one type of vibrio from apparently normal animals, he concluded that vibrios play a secondary role and are of "minor importance and that the cause of this type of dysentery must be sought elsewhere."

In France, the results of a serological investigation conducted by Jacotot and Vallée (68) on pigs, calves, sheep, fowls, monkeys, pigeons, rabbits, and human beings indicated that antibodies agglutinating V. fetus were found in over

90 per cent of 594 adult pigs and 316 fowls. No antibodies were found in unweaned pigs. The titers, however, were considered significant only in 2.5 per cent of the pigs and 13 per cent of the fowls.

PART 1

STUDY OF VIBRIONIC DYSENTERY AS IT OCCURS IN ONTARIO

MATERIALS AND METHOD

The data presented in this section are derived from 275 pigs which came from 249 different outbreaks of Vibrionic Dysentery (table 1). These pigs were presented to the Ontario Veterinary College for diagnostic purposes from January 1958 to January 1960. They were submitted by clinicians, veterinarians and farmers and came mainly from southern Ontario.

The cases were diagnosed as Vibrionic Dysentery on the clinical, pathological and bacteriological basis. In each case, a history was recorded when the pigs were admitted. Living animals presented were killed by electrocution. A complete autopsy was performed and a suspected case of Vibrionic Dysentery was confirmed by direct smears made from the mucous membrane of the large intestine and stained by Gram's method.

Specimens of tissue from the alimentary tract were immediately fixed in Zenker's fluid and specimens from other organs were fixed in 10 per cent formalin. Fixed tissues were embedded in paraffin; sections were cut at approximately six microns and stained with hematoxylin and eosin.

OBSERVATIONS

A. Clinical aspects and incidence

Four distinct clinical syndromes were usually observed in an outbreak in a herd.

TABLE 1

Relationship Between the Number of Pigs Affected with Vibrionic Dysentery and the Number of Pigs Autopsied.

	1958	1959	1960
January	8(82)*	6(97)	9(64)
February	1(51)	11(84)	8(52)
March	4(84)	3(61)	9(59)
April	3(68)	12(88)	10(57)
May	7(100)	6(71)	6(38)
June	7(116)	7(63)	11(34)
July	7(81)	4(47)	7(48)
August	5(60)	9(55)	4(46)
September	6(73)	11(64)	5(47)
October	6(88)	12(73)	12(72)
November	10(117)	7(62)	12(91)
December	10(77)	5(57)	15(79)
TOTAL	74(997)	93(822)	108(687)
PERCENTAGE	7%	11%	16%

* Figures in parentheses represent the number of pigs autopsied including those suffering from Vibrionic Dysentery.

In the peracute cases, the pigs could die without showing any symptoms and the first evidence of any trouble in the herd was the finding of one or more dead pigs: However, in most of these cases, if the pigs were found alive or were more closely observed, many of them were seen to have diarrhea.

In the acute form, the pigs were usually submitted with a history of diarrhea, loss of appetite, and rapid shrinkage of the flank region. The diarrhea developed early in the course of the disease and the feces varied in color depending on whether or not they contained blood. However, the feces were usually yellowish or muddy gray in color, watery in consistency, and of fetid odor. Shreds of white, inspissated mucus or mucoid material were mixed with the liquid fecal material. The hind legs and tail were soiled with bowel discharges mainly due to tenesmus or to the relaxation of the sphincter and permitting feces to ooze out. Affected animals were usually off feed or at least ate less than normally; in most cases an increased thirst was noticed. If the course of the disease was prolonged for only a few days, the pigs became gaunt, emaciated, dehydrated, weak, and showed great prostration with a disinclination to move.

In subacute infections, the predominant signs were loss of body weight and unthriftiness. Diarrhea was frequent in most outbreaks but in some it was not noted until the disease had been present in the herd for about a week. Feed consumption dropped markedly.

In chronic infections, the predominant sign was a fluid diarrhea containing necrotic membranes which sloughed off from the mucosa and gave the feces the characteristic "rice water" appearance. The diarrhea could last for weeks and was accompanied by a progressive emaciation and dehydration. Even though the animals continued to eat, feed consumption dropped markedly.

The disease spread rapidly through a herd and the morbidity was usually around 75 per cent.

The duration of the disease in individual animals varied from a few days in acute infections to a week or more in sub-acute and chronic infections. No immunity seemed to develop since exacerbations were often noted in untreated as well as in treated pigs. In untreated animals, mortality was extremely variable ranging from 5 per cent to as high as 25 per cent.

Elevation of body temperature was not constant and the temperature was usually within the normal range; but variations from a low of 96 F. to a high of 105 F. were noted.

Vibrionic Dysentery was uncommon in suckling pigs and in the breeding stock. The age incidence ranged from 3 weeks (1 case) to 24 weeks (1 case), but the disease was most commonly seen in pigs between 7 and 12 weeks of age (Plate 1). In many instances, diseased animals presented were feeder pigs which had been bought from stock yards or sales barns or, less frequently, they were breeding stock which had been added to the farm herd without an isolation period. However, in many

cases, pigs presented with the disease had been raised on the farm where no addition had been reported for the last years. In most of the cases, no history of disturbance in the management such as weaning, change in feed or a recent change of environment were recorded.

Vibrionic Dysentery was diagnosed during every month of the year and the disease did not seem to be more prevalent in a particular season (table 1).

From the 275 cases diagnosed, Vibrionic Dysentery was complicated by a variety of unrelated lesions on a number of occasions (43 per cent). Table 2 summarizes these conditions; enzootic pneumonia was the most prevalent of these.

B. Pathological findings

(1) Macroscopic findings

In the peracute cases presented, the pigs were usually in fair state of nutritional condition. In acute infections, wasting was evident when the pigs were presented with diarrhea of more than a few days duration. In subacute and chronic infections, however, affected pigs were usually stunted, dehydrated, emaciated, showed a hunched back, protruding ribs and eyes sunken into the orbits. The skin was dry and wrinkled. Most of the tissues, especially the subcutaneous tissues, the muscles and the peritoneum, were dry and sticky. Some pigs showed a bluish-red discoloration of the skin of the venter and extremities.

Superficial lymph nodes - The superficial lymph nodes appeared normal in size, color, and consistency.

Stomach - The gross findings in the stomach varied somewhat. In many cases, the stomach was normal and more or less filled with concentrated feed or extraneous material such as straw or wood shavings. In other cases, gastric atony was indicated by the presence in the stomach of a large amount of undigested food in a sour state. In acute cases, the fundic portion of the gastric mucosa was frequently hyperemic, bright red or dark red in color; however, no hemorrhage was seen into the lumen and the ingesta was not blood-stained. In these cases, the mucosa was usually covered with excess of white, slightly tenacious mucous or, less frequently, the mucosa showed a tendency to slough-off. The slough material then adhered to the mass of food. The severe congestion of the mucous membrane could be seen through the serous surface about the greater curvature. The stomach wall was thicker than normal.

Small intestine - The small intestine was usually empty and dilated by considerable volume of gas. Sometimes a watery, bile-stained material filled the small intestine right down to the ileocecal valve. In most cases, the mucous membrane was normal; however, an increased mucoid exudate and a mild congestion were sometimes seen throughout the entire length of the small intestine or more frequently in the last portion of the ileum. No evidence of any of the more severe inflammatory changes seen in the large intestine were encountered in the small intestine.

Parenchymatous organs - No gross characteristic lesions were generally seen in the parenchymatous organs and in severe cases,

TABLE 2

Summary of the Conditions Complicating
Vibrionic Dysentery.

Complicating conditions*	No. of pigs
Enzootic pneumonia	54
Salmonellosis	18
Atrophic rhinitis	9
Sarcoptic mange	9
Mulberry heart disease	6
Glässer's disease	3
Gastric ulceration	3
Coliform enteritis	2
Erysipelas	2
Pericarditis	2
Peritonitis	1
Salt poisoning	1
Glomerulonephritis	1
Swine pox	1
Verminous pneumonia	1
Streptococcal endocarditis	1
Inclusion bodies rhinitis	1
Torsion of the intestine	1
Muscular hypertrophy of ileum	1

* Number of cases not complicated 157 (57%)
Number of cases complicated 118 (43%)

the changes seen in the liver, spleen, and kidneys were generally those observed in mild toxic conditions.

The liver was sometimes congested, swollen, with rounded edges. It was soft in consistency and ordinarily friable with small, pale, grayish-brown foci. The cut surface usually bulged. Bluish or gray ascarid scar formations were frequently seen.

The spleen was normal in size and color.

The kidneys were ordinarily normal. In only a few cases, they were swollen, had a cooked appearance and their cut surface bulged. Congestion of the medulla was sometimes encountered. The capsule peeled off easily and no petechial hemorrhages were seen on the cortical surface. The remainder of the urogenital tract was normal.

A small amount of clear or straw-colored fluid was sometimes encountered in the pericardial cavity. In two cases complicated by virus pneumonia, gross lesions of fibrinous pericarditis were observed.

Internal lymph nodes - The internal lymph nodes draining the intestines were usually normal. In a few cases, the lymph nodes draining the affected portion of the large intestinal tract were enlarged, edematous and, at times, hemorrhagic. The cut surface was moist, reddened but very rarely dark red. All of the other internal lymph nodes were normal in size and color.

Large intestine - The disease was chiefly an infection of the large intestine and the obvious lesions were seen in the cecum, colon and rectum. Upon opening the abdominal cavity, the serous coat and the mesentery connecting the loops of the large intestine usually showed congestion or cloudiness and had a "ground glass" appearance. In many cases, the presence of lesions could be determined before incising the large intestine, which was usually flaccid and distended with fluid or semifluid material. Small diverticula, 0.5 to 3.0 mm. in diameter, were sometimes visible on the serous coat of the colon and less frequently of the cecum (Plate 13). Doyle (31) described these diverticula in the wall of the colon as "circular, clear, hyaline areas resembling, in their early stage, grains of cooked tapioca." Later, these diverticula resembled minute abscesses containing in their center a gray or whitish caseous material.

The lesions were usually sharply demarcated from the neighbouring healthy small intestine at the ileocecal junction. The mucosa of the terminal part of the ileum was apparently normal while the lips of the valve and its adjacent region showed marked inflammatory changes.

In the large intestine, different types of inflammation were encountered depending on the duration and probably on the intensity of the infection. Catarrhal, hemorrhagic and necrotic inflammations were the forms usually observed. Sometimes, however, mixed types of inflammation occurred simultaneously

and there was no sharp line of demarcation between these divisions; areas of focal diphtherësis frequently accompanied catarrhal or hemorrhagic inflammations.

Fifty-five per cent of the cases studied microscopically presented lesions of catarrhal inflammation. Seven per cent presented lesions of hemorrhagic inflammation. Sixteen per cent presented lesions of necrotic typhlitis, colitis and proctitis; while twenty-two per cent of the sections examined showed lesions of catarrhal or hemorrhagic inflammations with foci of necrosis.

In acute catarrhal inflammation, the intensity of the lesions varied somewhat. In mild cases the intestinal wall was swollen, its mucous membrane inflamed, velvety and the cut surface showed that the subserous and submucous layers were thickened and edematous. The mucous membrane was irregularly reddened and covered by a varying amount of mucus. The reddening of the mucosa was ordinarily streaked, the summits of the rugae appearing more congested than the rest of the mucosa which in mild cases was seldom red throughout.

In more severe cases, the large intestinal wall was swollen and the mucous membrane was more uniformly and severely red than in milder cases. The mucosa occasionally presented a slightly rough or eroded appearance. Scattered petechial hemorrhages were sometimes visible on the mucous membrane.

In catarrhal inflammation, the cecum, colon, and to a lesser degree, the rectum usually contained large quantities of mucus. In many cases, however, an increased mucus production was not grossly observable. The fecal material was soft in consistency, yellowish in color and only infrequently the ingesta was blood-stained.

Real hemorrhagic inflammation of the cecum, colon and rectum was the less commonly encountered form of inflammation. The large intestinal wall was thick, the mucosa edematous, hemorrhagic and covered by mucus. The fecal material was dirty, reddish-brown to chocolate-colored, fluid in consistency and mixed with mucus. In no case was clotted or fluid blood seen in the lumen of the large intestine.

In some cases of catarrhal and hemorrhagic inflammations, the mucous membrane had a rough or eroded appearance. After a more or less long period of time these eroded foci were covered by varying thicknesses of yellowish or gray necrotic material. Between these necrotic foci, the intestinal surface was rough, reddened or hemorrhagic.

If the pig did not succumb early in the course of the disease, when the inflammation was of longer duration, a uniform hyperemia of the cecum, colon and rectum occurred, necrosis ultimately resulted and the large intestinal wall became still more thickened and edematous.

In the early stages of necrotic inflammation, necrosis was focal with yellow or gray diphtheritic accumulations on the surface of the mucosa. As the disease progressed, the necrotic patches increased in size and number and coalesced to form a diffuse necrotic membrane affecting a very large portion of the cecum, colon and less frequently the rectum (Plate 12). The rectum usually contained necrotic material passed down from the cecum and colon. The intestinal wall was thick, edematous and the inflammatory reaction extended through the intestinal wall; fibrin covered the serous coat which, from smooth and shiny, became dull and rough.

Usually the superficial portion of the necrotic exudate sloughed off and mixed with the fecal material giving the intestinal contents the typical "rice water" or barley gruel appearances described in human cholera. The exudate could be scraped off easily leaving a moist, dull, reddened granular surface with scattered hemorrhages. In no case did the ulceration seem to penetrate very deeply into the large intestinal wall nor did it form into the classical "button ulcers."

(2) Microscopic findings

Stomach - In the great majority of cases, no microscopic lesions were seen in the stomach. The marginal epithelium and that of the crypts were intact. The mucus production

was normal and hyperemia or edema were not evident. In some cases however, infarction of the fundic region was encountered. This type of lesion is frequently referred to as a gastritis but histologically it was a severe congestion with formation of hyaline thrombi in the capillaries and veins. Frequently secondary necrosis and ulceration of the superficial part of the mucosa was encountered. It is not a specific lesion for Vibrionic Dysentery since it is common in many of the acute infectious diseases of swine.

Duodenum, jejunum, ileum - Histologically the small intestine was usually normal. The epithelium of the villi and that of the glands of Lieberkühn were intact. No evidence of hyperemia and edema was noted in most of the sections examined. In a few instances, catarrhal inflammation of the last portion of the ileum was noted. The capillaries and lymphatics were distended and the interstitial tissue was separated by exudated fluid. In these cases, degeneration and desquamation of the epithelium was evident. Inflammatory cells collected in the lamina propria and were chiefly lymphocytes or less frequently macrophages and plasma cells. Eosinophils were sometimes predominant. The goblet cells showed a moderate increase in mucus production.

Parenchymatous organs - Cloudy swelling of the muscles fibres of the heart was noted in only two cases. Microscopic lesions of fibrinous pericarditis were seen twice in cases complicated by virus pneumonia.

In the liver, cloudy swelling with its characteristic picture or enlarged cells, granular cytoplasm and indistinct nuclei was seen in almost every section examined. Lesions were more severe around the portal space.

Cloudy swelling of the renal tubules was noted in almost every section examined. Hyaline casts were sometimes seen in the collecting tubules.

Cecum, colon and rectum - Microscopically the lesions of catarrhal inflammation were acute or chronic. In acute catarrhal inflammation, the different coats of the affected portions of the alimentary tract did not react to the same extent, the mucosa and submucosa predominantly showing microscopic reactions. The blood vessels of the mucosa were definitely congested and the interstitial tissue was separated by exudated fluid from the lymphatics and the blood vessels. At such points, the epithelium was destroyed or pushed away from the mucosa by extravasated fluid (Plate 2). Sometimes the epithelium was not entirely gone, it appeared loosened, was still more or less in position but was no longer in contact with the lamina propria (Plate 3). The epithelial desquamation was either focal or extensive and generally over these foci of desquamation, the penetrating action of the bacteria could be observed. The mucosa and less frequently the submucosa were infiltrated by leukocytes, chiefly lymphocytes. In few instances, a great number of neutrophilic polymorphonuclear leukocytes

was also noted (Plate 4). The muscular layers were not generally infiltrated by leukocytes and did not show edema nor early lesions of degeneration. The serous coat was often infiltrated by lymphocytes and edema was frequently encountered between the serous coat and the outer longitudinal fibres of the muscularis externa.

Occasionally solitary lymphoid follicles and Peyer's patches were swollen. A mild proliferation of the reticular cells in the germinal centers or a decrease in mature lymphocytes were also noted.

The goblet cells of the glands of Lieberkühn were more prominent and numerous than in the normal state and, in many occasions, were so distended with a palely basophilic mucus that the other epithelial cells of the gland had become atrophic by pressure. The lumens of the glands were also gorged with mucus and in many instances the mucus was pushed up from the gland into the intestinal lumen forming the major component of the exudate coating the mucous membrane (Plate 3). This exudate also contained cellular debris, colonies of bacteria and to a lesser extent neutrophils, lymphocytes, erythrocytes and fibrin (Plate 4). In the early stages of catarrhal inflammation, a decrease in mucus secretion was sometimes noted.

The histological appearance of the diverticula, as described by Doyle (31), varied with the stage. In the early stage, the epithelial cells of the glands showed mucous degeneration

and a large amount of mucus distended the diverticulum. In a later stage, diverticula were usually filled with neutrophils and cellular debris.

In general, the histopathological changes seen during the early mucoid stage consisted chiefly of engorgement with blood, epithelial desquamation, excessive mucus production and, at times, focal hemorrhages and diphtheritic exudate.

In chronic cases of catarrhal inflammation, proliferation of the connective tissue of the mucosa and less frequently of the submucosa was frequently seen. The epithelium was dysplastic, especially at the tips of the glands. The epithelial cells showed variation in their size, shape and orientation. The columnar cells of the epithelial layer had lost their orientation, they were much larger but flatter than normal, their nuclei were dark and increased in size.

The interstitial tissue of the lamina propria was infiltrated by leukocytes, chiefly macrophages, plasma cells and few lymphocytes. Lymph follicles and Peyer's patches were surrounded by a hyperemic zone. Mucous degeneration and atrophy of the epithelium of the crypts was also a common picture. In mucous degeneration, the cytoplasm of the cell was replaced by mucin and the nuclei were pushed at the base of the cells so that the cellular outline was lost. Closure of the mouth of the glands resulted in formation of cysts.

The same histopathological findings as in severe acute catarrhal inflammation were seen in hemorrhagic inflammation of the large intestine; but the lesions were more severe. The large amount of extravasated red blood cells with leukocytes and fibrin in the lumen and in the superficial portion of the lamina propria was the characteristic picture of this type of inflammation. The epithelial layer was practically all gone, destroyed by the bacterial action or pushed away by the extravasated blood and lymph. Injured, distended capillaries were exposed on the intestinal surface. Goblet cells were greatly increased in number and size and the glands were filled with mucus. Necrosis and sloughing of the mucous membrane could be seen in some areas (Plate 5). Thrombosis of the mucosal veins were seen in some cases.

Microscopic sections taken from necrotic type of inflammation showed lesions of coagulation necrosis of the mucosa. The cells passed through the various changes characteristic of necrotic tissue; the nucleus became pyknotic and eventually disappeared.

The epithelial cells were eroded and the lamina propria showed coagulation necrosis. In the underlying healthy tissue there was a reaction zone of hyperemia, hemorrhage and leukocytic infiltration chiefly by neutrophils (Plate 6).

Over the mucosa, the necrotic material was more or less adherent to the healthy tissue. This necrotic material was formed chiefly by cellular debris, mucus, fibrin, leukocytes,

and red blood cells (Plate 7). In no case did the necrotic zone pass beyond the muscularis mucosae; it was only superficial in nature even if it was extensive. Blood vessels of the mucosa and submucosa not infrequently showed thrombosis.

Balantidium coli were frequently seen over the intestinal mucosa in all types of inflammation. These ciliated protozoans in only a few occasions were seen penetrating deeply into the mucosa or submucosa. Their significance in the etiology of enteritis in swine is not perfectly understood but they are not usually considered pathogenic.

C. Bacteriological methods

A suspected case of Vibrionic Dysentery on the basis of clinical symptoms and gross lesions was confirmed when a large number of comma shaped or spiral filaments with two, three or more curvatures were found in smears made from the mucous membrane of the large intestine.

In most cases, isolation of the vibrio was attempted but the diagnosis was not based on its isolation.

Other enteritic diseases such as colibacillosis, coliform enteritis and salmonellosis were excluded by inoculating specimens onto tryptose blood and MacConkey agar plates.

D. Differential diagnosis

Enteritis in swine may result from a variety of causes and the diseases from which Vibrionic Dysentery must be

differentiated include chronic necrotic and acute septicemic salmonellosis, coliform enteritis, non-specific colibacillosis associated with iron-deficiency anemia, and the so-called nutritional necrotic enteritis.

Pigs dying of acute and subacute coliform enteritis may present the same clinical appearance as pigs dying of acute or subacute Vibrionic Dysentery. However, the body temperature is usually higher in coliform enteritis. The condition is also usually seen in the immediate post-weaning period or is associated with a recent change in diet. A differential diagnosis can be made at autopsy and on the basis of bacteriological findings. In coliform enteritis, the small intestine is usually the site of catarrhal inflammation; it contains watery material and yellow floccules of catarrhal exudate and fibrin. In Vibrionic Dysentery, lesions are usually limited to the large intestine and inflammation of the small intestine is not a frequent lesion; when present however, the inflammation is usually less severe than in coliform enteritis and affects chiefly the ileum. Bacteriologically Richards and Fraser (125) observed that ninety-one per cent of their strains of hemolytic E. coli were of the same serotypes as those isolated by Kelen et al (122) and Campbell (120) from pigs dying from edema disease in Canada. Hemolytic E. coli was isolated from pigs with Vibrionic Dysentery in a few cases. However, the

organisms were usually in small numbers and were not of the specific serotypes seen in coliform enteritis.

Subacute Vibrionic Dysentery may be differentiated from non-specific colibacillosis associated with iron-deficiency anemia at autopsy and on the basis of bacteriological findings. In non-specific colibacillosis, pigs are anemic and if the course of the condition has been prolonged, catarrhal inflammation of the small intestine is to be expected. The infection is septicemic and E. coli can be isolated from all organs. As noted above, lesions of Vibrionic Dysentery are usually limited to the large intestine and smears made from the mucous membrane of the cecum, colon or rectum show a large number of vibrio organisms.

The chronic form of Vibrionic Dysentery bears a marked resemblance to the chronic form of salmonella enteritis and to the condition described as niacin deficiency.

Clinically the difference between Vibrionic Dysentery and necrotic enteritis due to salmonella infection has long been based on the fact that in Vibrionic Dysentery bloody diarrhea was observed in the beginning of the infection. Since blood is rarely seen in the condition observed in Ontario and diagnosed as Vibrionic Dysentery, the differential diagnosis depends upon post-mortem and bacteriological examinations. In the enteric form of salmonellosis, the necrotic inflammation often extends into the ileum; the spleen is usually dark blue in color and soft in consistency, the mesenteric lymph nodes are greatly enlarged, edematous,

congested, and dark in color. In Vibrionic Dysentery, necrotic lesions do not usually extend into the ileum. The spleen is normal in size and color and the lymph nodes draining the affected portions of the intestinal tract are less severely congested. Microscopically in salmonellosis, typical typhoid nodules consisting of focal necrosis and lymphocytes, plasma cells and mononuclear cells infiltration are usually seen in the liver, spleen, lymph nodes, Peyer's patches, lymphoid follicles and occasionally the kidneys. These microscopic lesions are not observed in Vibrionic Dysentery. Bacteriologically salmonella organisms are usually easily isolated from the intestinal tract and associated lymph nodes. Salmonella organisms were isolated from diseased pigs in eighteen cases of Vibrionic Dysentery (Table 2). One condition can certainly complicate the other, however, salmonella organisms are probably not isolated more frequently from pigs suffering from Vibrionic Dysentery than they are from apparently normal pigs.

Gross lesions presumably due to niacin deficiency in swine have been described by a number of authors, namely: Birch et al (7), McEwen (94), Chick et al (21), Davis and Freeman (26), Braude et al (12), and Powich et al (102).

Most of the authors working on that subject described gross lesions of fibrinonecrotic inflammation involving principally the cecum and colon. The diagnosis was

mainly based on the facts that blood was not present in the feces and that S. choleraesuis was not isolated from these pigs. In 1949, Dunne et al (42) described the microscopic lesions of the colon in niacin deficient pigs. The lesions encountered were an extensive mucoid degeneration characterized by a distention of all goblet cells. The glands of Lieberkühn were markedly enlarged, as the result of the increased mucus production. The mucus exudate with enmeshed debris accumulated about the orifice of the glands. In most instances, lymphocytes and macrophages were the only important inflammatory cells present but neutrophils were also frequently seen. Areas of focal necrosis were also a common picture together with hyperemia. These authors stated that bacterial invasion of the tissues was frequently noted. They ruled out the possibility of salmonella infection since cultures were negative for S. choleraesuis but they did not, by any means, rule out the possibility of a vibrio infection. The lesions described in this report have a marked similarity with the ones encountered in naturally infected pigs with Vibrionic Dysentery in Ontario.

It is probable that V. coli as well as S. choleraesuis are likely to assume a pathogenic role if the pig's resistance is lowered by poor environmental conditions such as unsanitary quarters or intercurrent disease. Environmental conditions which may reduce the resistance of pigs to infection are damp, humid piggeries and dietary deficiencies such as lack of proteins, vitamins and minerals.

Field (45) stated that although the chronic form of salmonellosis is generally more common than the acute form the latter may be more frequently encountered in some areas. The cases encountered here during this investigation and diagnosed as salmonellosis were usually of the acute septicemic form. These cases must be differentiated from peracute Vibrionic Dysentery. Clinically septicemic salmonellosis is characterized by a high body temperature ranging from 105 to 107 F. In Vibrionic Dysentery such a rise in body temperature is not usually observed. In salmonellosis, the skin of the venter and extremities has usually a severe purpish discoloration, the spleen is dark blue in color, greatly enlarged throughout and is of soft consistency. Frequently the lungs show lobar pneumonia and pleuritis. When the lungs are not pneumonic, they are cyanotic, edematous and focal hemorrhages are seen throughout. Frequently the kidneys show varying numbers of petechial hemorrhages beneath the capsule. Epicardial, myocardial and endocardial hemorrhages are also frequently seen. The lymph nodes are usually greatly enlarged, edematous, and either very red or bluish black. Their cut surface is moist and a varying degree of hyperemia or hemorrhage are observed. Microscopically lesions of focal necrosis and hemorrhages are observed. Peracute Vibrionic Dysentery can be differentiated from the acute septicemic form of salmonellosis at autopsy and by bacteriological examination. In uncomplicated cases of

Vibrionic Dysentery, lesions of septicemia are not encountered and the spleen is normal in size and color. Bacteriologically salmonella organisms are usually easily isolated from various organs.

The peracute form of coliform enteritis in which pigs are found dead may bear resemblance to peracute Vibrionic Dysentery and definite diagnosis should await post-mortem examination. In coliform enteritis, a severe catarrhal inflammation of the small intestine is a characteristic feature. The contents are watery and usually contain floccules of catarrhal exudate and fibrin. Bacteriologically hemolytic E. coli of specific serotypes, as noted above, are usually isolated from the intestinal tract. In Vibrionic Dysentery, the lesions are confined to the large intestine and smears made from that part of the alimentary tract show the presence of a large number of vibrios.

Microscopic lesions of acute catarrhal inflammation of the digestive tract must also be differentiated from the physiological changes occurring during the phenomenon of digestion and from post-mortem changes. To eliminate post-mortem changes, sections were taken from pigs killed immediately prior to autopsy. Care was taken in manipulating the intestinal sections not to damage the epithelial layer. Tissues were fixed in Zenker's fluid because this solution had the advantage of giving more rapid and more even penetration than formaldehyde solution. During the phenomenon

of digestion, one observes a distention of blood vessels and lymphatics together with an increased amount of leukocytes in the lamina propria; but the epithelial layer of the mucous membrane remains intact.

DISCUSSION

The enteric disease syndrome of swine has been and is still today one of the most important economic problem of the swine industry. In Ontario, one member of this group, Vibrionic Dysentery, has been recognized since 1958 and is at the present time probably the most important of these enteric conditions. The disease may affect pigs of any age but it is most frequent in pigs 7 to 12 weeks of age.

The nearly constant clinical observations are diarrhea and a rapid sinking of the flank region. Diarrhea is characterized by the presence of mucus and at times by the presence of sloughed necrotic material mixed with the fluid intestinal discharges. The passage of dark-colored feces as described in the literature was rarely noticed, while the passage of fluid blood or clotted blood was never encountered. In this instance, the disease was probably less severe than the one usually described by American workers.

Microscopic examination of the feces showed enormous numbers of vibrios. These vibrios were isolated in pure culture in approximately 50 per cent of the attempts.

The gross pathological findings were that of acute or chronic inflammation localized to the large bowel with lesions

beginning at the ileocecal junction. The most frequent type of inflammation encountered was a catarrhal typhlitis, colitis and proctitis. In only a few instances, hemorrhagic inflammation of the large intestine was noted. This hemorrhagic type of inflammation may be due to a more virulent strain of the organism.

Necrotic inflammation of the large bowel was not infrequently observed in advanced cases of Vibrionic Dysentery. This type of inflammation is not grossly distinguishable from that found in advanced cases of hog cholera and salmonella enteritis. The action of Spherophorus necrophorus on the damaged mucous membrane is probably similar in Vibrionic Dysentery, salmonellosis and hog cholera. The intensity of the lesions in the large intestine presumably vary depending upon the length of time between the primary infection and the death of the animal.

Venous infarction of the fundic region of the stomach was not infrequently seen but is not characteristic of Vibrionic Dysentery, since it may be encountered in many other infectious diseases namely: erysipelas, salmonellosis, hog cholera, coliform enteritis. In the literature, this gastric lesion is usually referred to as a gastritis, but histologically it is a severe congestion especially of the gastric mucosa, with the formation of hyaline thrombi in the capillaries and veins and infarction.

In the large intestine, the histopathological lesions agree rather closely with the gross findings. In most instances an acute catarrhal inflammation was observed with engorgement of blood vessels and lymphatics, edema of the interstitial tissue, excessive mucous production by goblet cells and formation of a diphtheritic exudate over the mucous membrane. In more chronic cases, coagulation necrosis with an inflammatory reaction zone between the necrotic layer and the healthy tissue was usually seen.

Vibrionic Dysentery appears then as a specific enteric disease of swine distinguishable from the other enteric conditions of swine on the basis of clinical, pathological and bacteriological findings.

PART 11

ISOLATION AND IDENTIFICATION OF VIBRIO COLI

To date, vibrios are isolated from various sources and many of these organisms are considered saprophytes (89). Strains of vibrios were isolated from pigs suffering from Vibrionic Dysentery and attempts were made to characterize the organisms associated with the disease and to compare with other members of the genus.

MATERIALS AND METHODS

A. Isolation of Vibrio coli

Culture media: The media used for the isolation or maintenance of V. coli were blood agar and Albimi brucella broth **. Tryptose blood agar * was used as the base medium for the addition of five per cent sterile citrate sheep blood in the preparation of the blood agar plates and slants. In the early stage of the work, citrated horse blood in a volume of five per cent was used for the isolation of V. coli in conjunction with sheep blood but was later discontinued in favor of the latter. Agar in the concentration of 0.1 per cent was added to Albimi burcella broth in screw cap tubes.

Techniques: For the isolation of V. coli from the large intestinal tract of dead pigs, a modification of the methods described by Doyle (35) and Deas (28) was followed. The serous coat of the cecum or colon was seared with a hot spatula and

* Difco Laboratories, Detroit, Michigan.

** Albimi Laboratories, Brooklyn, New York.

an oval piece of the intestinal wall, approximately 5 by 10 mm. including the mucous membrane, was snipped out with sterile scissors and removed with sterile forceps. The mucous membrane was lightly seared with a hot spatula in order to remove the superficial mucosa, exudate, and necrotic material and serially streaked onto four blood plates. Serial inoculations were employed to insure well isolated and identifiable colonies of V. coli from other enteric organisms normally or pathologically present in the intestine.

For the isolation of V. coli from the lymph nodes a different procedure was adopted. The lymph node to be cultured was dipped into 70 per cent alcohol and passed in the flame of a bunzen burner. The lymph node was cut with sterile scissors and the cut surface streaked onto a blood plate.

Incubation: After inoculation, all of the media used to isolate and maintain V. coli were placed in a wide mouth jar sealed with a roll of warm plasticene. Fifteen per cent of the air in the jar was replaced by carbon dioxide from a commercial CO₂ cylinder. The jars were then incubated at 37 C. for 48 hours.

Aerobic cultures: In every case, aerobic cultures at 37 C. were carried out on blood and MacConkey agar plates in an attempt to isolate any organisms associated with specific diseases such as salmonellosis and coliform enteritis.

B. Maintenance of cultures

After isolation, the stock cultures of V. coli were maintained in the lyophilized state until shortly before use. When necessary, cultures were reconstituted from lyophilized organisms in Albimi brucella semisolid medium and incubated at 37 C. under 15 per cent CO₂ for 48 hours. They were then left at room temperature. For maintenance during the period of the experiments, cultures were transferred at intervals of 5 to 7 days.

C. Identification of Vibrio coli

(1) Morphological and colonial characteristics

Morphological characteristics of V. coli from the mucous membrane of naturally or experimentally infected pigs and from the growth on blood plates were studied in direct smears. Smears were made from various types of colonies and stained with Gram's method or with diluted fuchsin. Morphological characteristics were also studied with the electron microscope. Electron micrographs were prepared from blood agar slants and were taken from young and old cultures.

Colony characteristics were studied under the dissecting microscope after cultivation on tryptose blood agar. The method used for colonial differentiation was taken largely from Wilson and White (136) and Bond (11). The plates were flooded with a 1:2000 solution of crystal-violet for 15 seconds; excess stain was poured off and the plates were examined under the dissecting microscope using

obliquely transmitted light, as suggested by Henry (60).

Stability of V. coli cells in acriflavine was determined by suspending the cells in a drop of 1:1000 neutral acriflavine and observing whether or not clumping occurred.

Motility was observed by the hanging drop technique.

(2) Biochemical properties

The study of the physiological activities of vibrios isolated from pigs suffering from Vibrionic Dysentery was performed in order to obtain information on the biochemical characteristics of those vibrios found associated with the disease.

Cultures of Vibrio coli: Physiological characteristics were studied on forty-six cultures of vibrio isolated from the digestive tract of affected pigs with Vibrionic Dysentery. Seed cultures were prepared by reconstitution from the lyophilized stock cultures in Albimi brucella semisolid medium. The purity of the cultures was ascertained by subcultivation onto blood plates and by microscopic examination of smears stained with Gram's method.

Incubation: Media were incubated as outlined. The incubation period, however, varied somewhat with the different tests performed. When cultures were incubated for a period of time longer than 72 hours, the jars were removed from the incubator every other day, and the cultures were allowed to stand at room temperature for about two hours in order to give the inside of the jars and the cotton plugs

a chance to dry and thereby preventing growth of fungi (6). After this time, cultures were reincubated under the usual conditions.

Unit of inoculum: The various media used for determining the biochemical characteristics were each seeded with one drop of a 48-hour Albimi brucella semisolid medium. Simmon's citrate agar and gelatin medium were exceptions in that they were inoculated by stab.

Tests performed: Physiological activity studies were carried out using the following tests:

Catalase production

Hydrogen sulfide production

Indole production

Citrate utilization

Nitrates reduction

Salt tolerance

Growth in nutrient broth

Gelatin liquifaction

Action in litmus milk

Final pH in Albimi brucella broth

Carbohydrates fermentation and gas production

Catalase activity: The growth layer of a 72-hour Albimi brucella semisolid medium was measured and pipetted into a clean screw cap tube. An equal amount of a 3 per cent commercial hydrogen peroxide solution* was added and the

* Hydrogen peroxide solution U.S.P. (10 volume, 3% H₂O₂, Stevens Companies.

tube plugged and inverted a few times. The immediate evolution of gas bubbles denoted the presence of catalase. No attempt was made to measure the amount of gas produced.

Hydrogen sulphide production: The lead acetate test strip technique was used for the detection of hydrogen sulphide. Whatman No. 1 filter paper saturated with a 10 per cent solution of lead acetate was dried and cut into strips. A strip was inserted in the mouth of the culture tube containing Albimi brucella semisolid medium. Blackening of the edges of the filter paper after 72 hours was considered evidence of H₂S production by the organism. Control test tube was used in each experiment using uninoculated medium over which test strip was suspended.

Indole production: One per cent Bacto-tryptose* was added to Albimi brucella broth containing 0.1 per cent agar. This medium served for indole test. Indole production was determined using Ehrlich-Bohme's technique (23). One ml. of ether was added to a 5-day broth culture and the tube shaken. After allowing the ether to rise to the top, 0.5 ml. of Ehrlich's reagent was carefully added so that it spread out as a layer between the medium and the ether. The appearance of a red color at the junction and into the ether layer was considered positive.

Nitrates reduction: The culture medium used for this test was Albimi brucella broth containing 0.1 per cent agar to which 0.1 per cent sodium nitrate had been added. Inoculated tubes were incubated for 5 days. Reduction of nitrates to

* Difco Laboratories, Detroit, Michigan.

nitrites was detected by adding a few drops of the standard reagents, sulphanilic acid and alphanaphthylamine (23). A pink to red color was considered as a positive reaction. Uninoculated medium incubated under similar conditions served as control.

Final pH in Albimi brucella broth: The pH of a 5-day Albimi brucella semisolid medium was determined by electrometric procedures on the Coleman (model 18a) pH meter*. Sterile tube incubated under the same conditions served as control.

Gelatin liquefaction: Bacto nutrient gelatin** to which agar had been added to make a 0.1 per cent concentration was used to determine whether cultures had the ability to liquify gelatin. After 14 days of incubation, tubes were placed in the refrigerator at 4 C. to determine whether or not the gelatin would solidify. Uninoculated control tubes incubated under similar conditions served as controls.

Carbohydrates fermentation and gas production: Fermentation and gas production studies were carried out on the following substrates: maltose, lactose, trehalose, salicin, sucrose, xylose, mannitol, dextrose, galactose and inositol. These carbohydrates were added in the concentration of one per cent to Albimi brucella broth containing 0.1 per cent agar. Phenol red was used as indicator. Production of gas was detected

* Coleman Instruments Inc., Maywood, Illinois.

** Difco Laboratories, Detroit, Michigan.

by placing inverted Durham tubes into the tubes containing the medium. Inoculated tubes were incubated for 14 days. Tubes were observed periodically over that period of time. Suitable controls were checked simultaneously with the performance of each test.

Salt tolerance: Sodium chloride tolerance was determined in Albimi brucella broth containing 0.1 per cent agar to which salt in the concentrations of 1, 3, and 5 per cent had been added. Tubes were incubated for 5 days and examined daily for growth. Control Albimi brucella semisolid medium without salt was also inoculated and examined for growth.

Methyl Red - Voges - Proskauer Tests and Citrate utilization: Bacto-methyl red - Voges - Proskauer medium* and on Simmon's citrate agar were inoculated and the tubes were incubated for 5 days.

Nutrient broth: Difco nutrient broth* was inoculated and incubated for 5 days.

Action in litmus milk: Agar in the concentration of 0.1 per cent was added to Bacto-litmus milk* in 16 x 150 mm. tubes. Tubes were incubated for 14 days and examined periodically. After that period of time, actions on milk and on litmus were recorded.

(3) Serological characteristics

The objective of this limited investigation was twofold. First, to determine whether strains of V. coli isolated from

* Difco Laboratories, Detroit, Michigan.

pigs are likely to be antigenically homogeneous or whether they fall into certain more or less defined groups. Second, to compare antigenically these strains of V. coli to vibrios isolated from a case of avian hepatitis and from an aborted bovine fetus.

a) Whole cell cross agglutination reactions of vibrios isolated from porcine, avian and bovine sources.

Vibrio cultures: Thirty-nine vibrio cultures isolated from the mucosa of swine affected with Vibrionic Dysentery were used together with one vibrio culture isolated from the gall bladder of a chicken suffering from hepatitis and one vibrio culture isolated from the stomach of an aborted calf.

Preparation of antigens: Whole cell vibrio antigens were prepared from 48-hour growth on blood plates and removed with normal saline. The cells were washed according to Roberts' method (106) by centrifugation for 20 minutes at 3500 r.p.m. The supernatant was removed and the cells were resuspended in normal saline. This solution was centrifuged for 2 minutes at 2000 r.p.m., the supernatant removed and kept as No. 1 supernatant. The deposit was resuspended in normal saline and centrifuged again at 2000 r.p.m. for 2 minutes. The supernatant removed and mixed with No. 1 supernatant. The mixture was standardized to Brown's tube No. 1 and was used for agglutination tests and the preparation of antisera.

Preparation of antisera: Whole cells V. coli H - O antisera were prepared in fourteen adult rabbits whose sera tested negative to the antigen they were about to receive. These rabbits were given intravenous injections of living vibrio cells. The antigen was injected in 0.5 ml., 1.0 ml., 1.0 ml., and 1.5 ml. amounts for four consecutive days. After a 4-day lapse, the antigen was administered in 1.5 ml., 1.5 ml., 2.0 ml., and 2.0 ml. amounts for four consecutive days. The rabbits were bled by heart puncture approximately ten days after the last injection. The antiserum from each rabbit was tested for agglutinability with homologous and heterologous antigens.

Technique of the test: The macroscopic method was employed for agglutination tests. Twofold serial dilutions of serum were made and mixed with an equal amount of antigen giving final serum dilutions commencing at 1:20. The last tube was left without serum and served as control on the stability of the antigen. The tubes were examined for evidence of agglutination after incubation at 37 C. for 24 hours. The highest serum dilution in which there was a complete agglutination was recorded as the agglutination titer.

b) Latex agglutination tests for the serologic diagnosis of Vibrionic Dysentery.

The purpose of the investigation was to obtain data on the possibility of detecting V. coli agglutinins in the blood of infected pigs which would be helpful in the diagnosis of Vibrionic Dysentery.

Antisera: Blood samples were secured from 6 pigs naturally infected with Vibrionic Dysentery. In addition 15 samples of serum were collected from convalescent or affected pigs with Vibrionic Dysentery in a herd of swine where a large number of pigs had died from the disease.

Vibrio cultures and antisera: The vibrio strains isolated from the intestinal tract of infected pigs were tested against their own sera. In addition, strains 4365A and 4365B isolated from fatal cases of Vibrionic Dysentery were tested against the serum obtained from affected or convalescent pigs on the same farm.

Preparation of antigens: Antigens were prepared as outlined and the mixture adjusted to Brown's tube No. 8.

Technique of the test: A modification of the method described by Singer and Plotz (123) was used. One part of polystyrene latex* particles, 0.81 microns in size, was diluted with 4 parts of distilled water. An antigen-latex-buffer mixture was prepared by adding 0.5 ml. of the vibrio cell suspension and 0.2 ml. of diluted latex to 4.3 ml. of a borate-saline solution buffer at pH 8.2. Twofold serial dilutions of serum were made starting with a 1:10 dilution in borate-saline solution buffer at pH 8.2. To each tube of diluted serum, 0.5 ml. of the prepared antigen-latex-buffer was added. The tubes were shaken and incubated for 90 minutes in 56 C. water bath. The tubes were then centrifuged at 2300 r.p.m. for 3 minutes and read by the naked eye. Appropriate controls without serum and with known positive rabbit sera

* Difco Laboratories, Detroit, Michigan.

were included.

D. Sensitivity tests

The following tests were conducted in order to determine in vitro the susceptibility or resistance of V. coli to various chemotherapeutic agents and antibiotics.

Cultures of Vibrio coli: Forty-six V. coli strains were used for these tests. From the lyophilized stock cultures, the organisms were transferred into Albimi brucella broth containing 0.1 per cent agar and incubated for 48 hours under 15 per cent carbon dioxide.

Technique of the tests: Tryptose blood agar plates were inoculated with one loopful, 5mm. in diameter, taken from the broth culture. The inoculum was carefully spread over the entire surface of the plate with a bent sterile pipe cleaner. Immediately following inoculation, sensitivity disks were placed on the surface of the plate, about 3 to 4 cm. apart. Not more than five disks were placed on a plate. Excessive moisture was avoided by allowing the plates to stand at room temperature for an hour; at the end of which time the cultures were incubated at 37 C. under 15 per cent carbon dioxide for 48 hours.

Chemotherapeutic agents and antibiotics: BBL* disks impregnated with the following nine therapeutic agents were used:

Aureomycin	5 mcg.
Terramycin	5 mcg.
Chloromycetin	5 mcg.

* Baltimore Biological Laboratory Inc., Baltimore, Maryland.

Dihydrostreptomycin	10 mcg.
Hibitane	0.25 mg.
Tetracycline	5 mcg.
Bacitracin	2 units
Furacin	100 mcg.
Triple sulfas**	0.25 mg.

Interpretation of the results: Cultures were recorded as "sensitive" or "resistant" to the various therapeutic agents on the following basis: "Sensitive" when a definite zone of inhibition of growth of a minimum width of 1 mm. was noted around the disks and "resistant" when either no zone of inhibition or a zone of inhibition of less than 1 mm. was observed around the disks.

OBSERVATIONS AND RESULTS

E. Morphological and colonial characteristics

The strains of V. coli examined showed considerable pleomorphism, were motile and gram-negative. The morphology also varied somewhat depending on whether it was observed from fecal material or from artificial media.

(1) Fecal material

Different forms were encountered in smears made from the mucous membrane of the large intestine or from fecal swabs. The types most frequently observed were the spirally curved vibrios with two and a half to four turns. The S-shaped, the

** Sulfadiazine, sulfamethazine and sulfamerazine.

"flying seagull" type, and the comma-shaped forms were also frequently seen. The organism measured about 0.2 to 0.5 microns by 1.5 to 15 microns. It stains well with Gram's method. (Plate 17)

(2) Artificial media

On primary isolation growth usually appeared in 48 hours when the oxygen tension was reduced with 15 per cent of carbon dioxide. Colonies were irregular, spreading, translucent, and bluish gray. Their diameter varied from 1 to more than 20 mm. Subcultivation on blood plates produced abundant growth. Two forms of growth were noted with V. coli. A moist continuous spreading growth was more frequently encountered than distinct colonies. These colonies were usually circular, low convex with entire edge, 0.5 to 1 mm. in diameter and, as noted by Doyle (35), had a tendency for elongating along the line of streak (Plate 10). In many instances, however, certain cultures yielded flat, dull colonies with less regular edges and with a rough or mucoid texture (Plate 11). With the crystal-violet method, smooth type colonies were stained with an outer purple ring and a clear yellowish center. The rough type colonies stained with pale to dark blue color, lacking rings of stain. The mucoid type colonies usually appeared yellowish in color, flat and slightly irregular in shape.

In cultures adapted to artificial media satisfactory growth appeared in 24 hours.

In young cultures, the cells were S-shaped or spiral formed with one and a two to four turns, 1.5 to 2 microns. Comma shaped forms were sometimes encountered in small number. The vibrios stained well with basic fuchsin diluted and were gram-negative. In older cultures, coccal forms 0.5 to 0.7 microns appeared in large number and after a 96-hour incubation period, most of the forms were coccoid. In a few strains, however, very long spiral forms up to 15 microns with five and more turns were sometimes encountered in old cultures. Coccal forms tend to develop more slowly in semisolid medium than on solid medium.

Electron micrographs showed that V. coli usually possess-
es one flagellum attached to one pole or two flagella one
attached to each pole (Plates 14 and 15). Occasionally, though
rarely, two flagella were seen at one pole (Plate 15). Coccal
forms were usually seen with only one flagellum (Plate 16).

Discussion

Once V. coli has been isolated it is easily propagated
by subcultivation on blood plates or in Albimi brucella broth
containing 0.1 per cent agar. On this point, the organism
differs somewhat from V. fetus which has been described by
several authors (65)(101)(113)(125) as being relatively
difficult to cultivate immediately after isolation.

In smears made from the mucous membrane of the large
intestine or from fecal swabs, the morphology of the organism

differs somewhat from the one observed after cultivation on artificial media. The length and the amplitude of the spirals were usually longer and larger in smears made from the mucous membrane or from fecal swabs.

The morphology of V. coli varies from predominantly S-forms and spirally curved rods with two and a half to four turns in young cultures to coccid forms in older cultures (Plates 14,15,16). These variations in shape have also been reported with V. fetus (101) and vibrios isolated from chickens (64)(99). Whatever the real explanation for these variations may be, they are regularly reproduced when V. coli is grown in vitro for a prolonged period of time. However, the S-forms or spirally curved rods reappear when the organism is transferred to a fresh medium and incubated for 24 hours. For this reason, the variations may be considered as temporary variants resulting from conditions which are relatively unfavorable and may interfere with the normal growth of the organism. On improvement of the conditions, the coccid form is transformed into a spiral-shaped rod.

The variations in shape do not seem to be related with the phase of dissociation of the organism. Smooth, rough and mucoid cultures were seen to yield indifferently normal spiral forms, coccid forms or very long spiral forms. However, smooth cultures yielded more frequently spiral forms. V. coli appears then as a pleomorphic organism which in some occasions may not be recognized or which may be easily taken for

another organism.

Herzberg and Ristic (61) and Bond (11) observed several types of colonial variations with V. fetus cultures of human, ovine and bovine origins. In most of the cultures studied here, V. coli appeared to be very instable and dissociation occurred readily even after a very short period of incubation. Albimi brucella semisolid medium did not seem to prevent this phenomenon and smooth to rough variation also took place very rapidly in this medium. The rough forms, however, were more stable, and the reverse change from rough to smooth forms rarely occurred under the conditions in which V. coli was grown.

Electron micrographs were taken in order to determine the taxonomic status of V. coli. Rolle and Mundt (109) pointed out that the organism isolated from the intestinal tract of pigs did not belong to the genus Vibrio but was a spirillum. Spirilla are described in Bergey's manual (89) and by Myers (93) as cells forming either long screws or portions of a turn, and usually motile by means of a tuft of five to twenty polar flagella. Not more than two polar flagella were observed with the strains examined here. On this basis, the organism isolated here from the intestinal tract of swine does not appear to be a spirillum.

F. Biochemical properties

Results

Vibrio cultures isolated from pigs were submitted to the

biochemical tests already described. Tables 3,4,5, and 6 show a summary of the results obtained. All of the strains tested produced catalase and hydrogen sulfide, reduced nitrates to nitrites, and did not produce indole when grown in tryptose broth. Despite good growth, none of the strains produced acid or gas in the presence of the carbohydrates used. All of the strains studied failed to grow in nutrient broth. Since no growth occurred in Simmon's citrate and Voges - Proskauer methyl red media, it was impossible to determine those reactions. The vast majority of the strains isolated, with the exception of two strains, reduced litmus milk. With the exception of one strain, all of the strains tested grew in presence of 1 per cent NaCl but all of the strains failed to grow in media containing 3 and 5 per cent NaCl. After a 5-day incubation period, the pH of Albimi brucella broth was only slightly elevated.

Discussion

The attempt made to characterize the different V. coli cultures on a biochemical basis shows that these vibrios follow a quite definite biochemical pattern and are undistinguishable physiologically. The results obtained are, in general, well supported by the reports of other workers. Bergey's manual (89) reports that V. coli does not liquefy gelatin, does not reduce nitrates, does not produce indole, does not grow in litmus milk, and does not utilize glucose, sucrose, lactose, maltose, and mannitol.

TABLE 3

Summary of the Biochemical Characteristics of Forty-Six Strains of V. coli.

	No. of Strains	
	Positive	Negative
Catalase production	46	0
H ₂ S production	46	0
Nitrates reduction	46	0
Gelatin liquefaction	0	46
Indole production	0	46
Growth in nut. broth	0	46

Hauduroy (59) reported that V. coli had no action on litmus milk, did not liquefy gelatin, produced indole, reduced nitrates and did not utilize glucose, lactose, saccharose, maltose, and mannitol.

Doyle (38) reported that V. coli did not liquefy gelatin, reduce nitrates, nor produce indole. No change was observed in litmus milk or in dextrose, lactose, saccharose, maltose, and mannitol.

Characterizing a vibrio associated with Vibrionic Dysentery, Roberts (106) stated that it produced catalase but not H_2S . It reduced nitrates to nitrites, did not liquefy gelatin, grew but produced no change in litmus milk, produced neither acid nor gas from arabinose, glucose, fructose, sucrose, galactose, maltose, mannitol, lactose or trehalose. Voges - Proskauer and methyl red tests were negative.

Eighteen vibrio cultures isolated from cases of dysentery in swine were studied biochemically by Davis (27). These vibrios were H_2S and catalase-positive, indole-negative, and failed to liquefy gelatin. No reaction was noted in litmus milk containing 0.1 per cent agar and little or no growth occurred in that medium. No fermentation took place in the seventeen carbohydrates and related compounds tested.

TABLE 4

NaCl Tolerance of Forty-Six
Strains of V. coli.

	Salt concentration					
	1%		3%		5%	
	Tol.*	Sen.**	Tol.	Sen.	Tol.	Sen.
No. of Strains	45	1	0	46	0	46

*Tolerant
**Sensitive

TABLE 5

Action on Litmus Milk of Forty-
Five Strains of V. coli.

	Reduction	No change
No. of Strains	43	2

TABLE 6

Final pH of Albimi Brucella Broth
after 5 Days of Incubation.

	pH					
	6.8	6.9	7.0	7.1	7.2	6.7-6.9
No. of Strains	6	3	18	14	5	controls

Two types of V. coli were isolated by Florent (46) from the intestinal tract of pigs. One type produced H₂S whereas the other type did not. Both types were catalase-positive, did not produce indole, did not liquefy gelatine, did not reduce nitrates, and did not ferment carbohydrates.

Deas (28) classified his vibrios isolated from swine, according to Florent, in two types. One type did not change litmus milk, did not produce indole, did not liquefy gelatin, reduced nitrates to nitrites, was methyl red-negative, grew in nutrient broth, did not produce change in lactose, glucose, sucrose, xylose, maltose, mannitol, and sorbitol and was catalase and H₂S - negative.

Diliello (29) studied biochemically six vibrio cultures of swine origin. These cultures were catalase and H₂S - positive and were salt-tolerant.

Kuzdas and Morse (82), studying the physiological activities of different vibrio cultures, found that one vibrio isolated from a case of dysentery in pig produced catalase, did not produce indole, failed to liquefy gelatin, did not reduce nitrates to nitrites and reduced litmus milk containing 0.1 per cent agar.

G. Serological studies

(1) Cross agglutination tests

A series of cross agglutination tests employing whole cells and antisera were used to determine the relationships existing between different vibrio cultures isolated from

TABLE 7

Cross Agglutination Reactions of 37 Strains of Vibrios Isolated from Swine, Cattle, and Poultry Tested against 14 Prepared Antisera

Antigens	Antisera													
	2A1	2A3	4156	4228	4365A	4365B	4622	4692	4711	4883	4954	5055	Chic-26	V. fetus
2A1	5120*	0	0	0	0	0	0	0	0	40	160	0	0	0
2A2	80	320	2560	640	40	40	0	0	0	0	40	0	160	0
2A3	320	<u>2560**</u>	80	2560	0	0	0	0	0	0	0	80	0	0
2B1	1280	0	0	0	320	0	0	40	160	1280	320	0	0	40
192	0	0	0	0	20	0	0	160	20	320	0	0	0	40
1053	1280	20	0	0	0	0	0	0	0	0	320	0	0	0
2244	0	0	0	0	0	0	0	0	40	0	0	0	0	0
2383	0	0	0	0	0	0	0	640	0	0	0	0	0	0
2754	160	320	0	320	320	0	0	640	160	320	0	0	0	160
3363	80	0	0	1280	1280	80	0	0	160	40	40	0	40	0
P4134	0	0	0	0	40	0	80	0	0	0	0	320	0	0
P-4156	320	2560	5120	0	0	80	80	0	160	C	40	5120	0	0
4156	640	320	<u>1280</u>	5120	160	0	80	0	20	0	0	160	80	0
P4200	80	0	0	0	0	0	0	0	0	0	0	0	0	0
4228	640	80	640	<u>81920</u>	0	0	0	0	0	0	0	0	80	0
P4228	0	320	5120	5120	0	0	0	0	0	0	0	0	0	0
4365A	160	0	0	0	<u>2560</u>	320	0	1280	320	640	80	40	160	0
4365B	20	0	0	0	0	<u>320</u>	0	160	320	640	0	0	0	0
4376	0	0	0	0	0	0	0	0	0	0	320	160	0	0
4389	0	0	0	0	80	0	0	0	0	20	0	0	0	0
4622	320	0	40	0	0	0	<u>1280</u>	0	0	0	0	320	0	0

4365B	20	0	0	0	0	<u>320</u>	0	1280	320	640	0	0	0	0
4376	0	0	0	0	0	0	0	0	0	0	320	160	0	0
4389	0	0	0	0	80	0	0	0	0	20	0	0	0	0
4622	320	0	40	0	0	0	<u>1280</u>	0	0	0	0	320	0	0
4622	0	1280	320	320	0	0	0	0						
4711	80	0	320	0	640	0	0	160	<u>1280</u>	640	0	0	0	80
4755	640	0	0	0	320	0	0	40	320	640	40	0	160	0
4876	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4883	160	0	0	0	80	0	0	0	0	<u>640</u>	0	0	0	0
4905-1	80	0	0	0	1280	320	0	0	80	160	0	0	0	0
4905-2	0	0	0	0	160	0	0	0	0	0	320	0	0	0
4954	1280	0	0	0	0	0	0	0	0	0	<u>1280</u>	0	0	0
4976	0	0	640	160	0	80	0	0	0	0	0	0	0	0
4986	1280	0	0	0	0	0	0	0	0	0	0	0	0	0
5055	0	40	0	40	0	0	80	0	0	0	0	<u>2560</u>	0	0
5068	0	0	320	5120	0	0	80	0	0	0	0	320	0	0
5372	320	320	20	0	640	320	40	320	80	160	160	320	160	40
5400	1280	20	0	0	0	0	0	0	0	0	320	0	0	0
5444	20	0	320	0	0	0	0	160	0	0	0	0	0	0
5511	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5705	80	0	0	80	0	80	0	0	40	0	0	0	40	0
5950	0	0	0	0	2560	0	0	0	0	0	0	0	0	0
Chic-26	640	0	0	0	160	0	0	0	0	160	320	0	<u>1280</u>	0
V. fetus	20	1280	2560	160	320	320	80	0	80	0	0	0	80	<u>2560</u>

* The reciprocal of the highest dilution showing complete agglutination.

** Homologous antiserum.

swine and to compare these vibrios with vibrios of other animal sources. The reciprocals of the highest dilution showing complete agglutination are given in table 7. Titers of less than 1:80 were considered insignificant. Rabbits were found satisfactory for the preparation of antisera against vibrios from porcine, avian and bovine origins. The rabbits immunized yielded antisera which agglutinated homologous antigens to titers ranging from 1:320 to 1:81920.

As noted previously, vibrio cultures dissociated readily on artificial media and antigens tended to settle out of solution. By picking smooth cultures for antigen production, stable antigens were usually obtained. The method of centrifugation described by Roberts (106) also contributed to give stable antigen suspensions consisting almost entirely of short or S-shaped cells; the instable cells which usually agglutinate spontaneously being removed by this method.

The highest titers were obtained with homologous antisera. There was a great deal of variation in the cross reactions of the porcine strains. In this group, response of antigens to heterologous antisera varied from no titer to titers as high as those obtained with homologous antisera. Most of the thirty-nine porcine strains tested appeared to

be antigenically separated and none of the antigens gave cross agglutination with all antisera prepared. Strain 5372, however, gave cross reactions with all the antisera tested except with one antiserum prepared from strain 4156. Strains 2244, 4876 and 5511 failed to agglutinate in significant titer any of the fourteen sera tested.

Strain 4954 reacted to only one serum in addition to its homologous serum while strains 2383, 2244, P4200, 4389, 4986, and 5950 also reacted to only one antiserum.

Strains 2A1, 4376, 4389, 4692, 4883, and 4905-2 reacted to only two heterologous antisera.

It is interesting to note that V. fetus reacted in significant titer to seven antisera of porcine origin and to the antiserum of avian origin. The antigen of avian origin, strain Chic-26, reacted to four antisera prepared from swine vibrios. On the other hand, V. fetus antiserum agglutinated to low titers only five vibrio antigens of porcine origin while the chicken vibrio antiserum agglutinated seven swine antigens in addition to V. fetus antigen.

In many cases, unilateral reactions were observed. In these cases, an antigen was agglutinated by heterologous antiserum while the homologous antiserum failed to react to the corresponding heterologous antigen. For example, table 7 shows that antigen 2A3 was agglutinated by antiserum 2A1 to a titer of 1:320 while antiserum 2A3 failed to agglutinate antigen 2A1 in any dilution.

Discussion

The serological relationships of vibrios of porcine origin were investigated; and these vibrios were compared with vibrios of bovine and avian sources. In the literature, serological studies on V. coli are very scant. DiLiello et al (29) noted that six vibrio cultures isolated from swine were serologically heterogeneous. Florent (46) reported that V. coli type 1 was antigenically similar to V. fetus. On the other hand, Price et al (103) failed to detect antigenic relationships between one strain of vibrio isolated from a case of dysentery in pig and vibrios isolated from bovine and ovine fetuses, bovine vaginal mucus, bovine uterus and from bull semen.

On the basis of the cross agglutination tests performed, it appears that strains of V. coli isolated from cases of Vibrionic Dysentery are not antigenically homologous. However, further studies with a larger number of strains should be undertaken in order to determine the extent of this heterogeneity. On the other hand, antigenic comparison with other members of the genus Vibrio should be carried out in order to investigate the possibility of one animal species being a carrier of a vibrio infection for another animal species.

Although only a limited amount of work has been done on the serological relationships of whole cell V. coli antigens, the results obtained indicate that there seem to be a great deal of variation in the antigenic structure of the porcine vibrio strains. However, some of these vibrios

seem to have antigenic components in common with other members of the genus Vibrio.

(2) Latex agglutination tests

Results

Latex agglutination technique failed to detect V. coli agglutinins in the serum of six pigs suffering from Vibrionic Dysentery and in the serum of fourteen sick or convalescent pigs.

Discussion

The latex agglutination technique has been employed with excellent results in the diagnosis of many infectious diseases and in detecting V. coli agglutinins from the sera of hyperimmune rabbits. Since the latex particles are thought to make more apparent a precipitinogen - precipitin reaction (49), they were used here in an attempt to find a useful method of diagnosis of Vibrionic Dysentery. The data obtained indicate that this test does not seem to be satisfactory for the diagnosis of Vibrionic Dysentery. Negative results have also been reported by Roberts (106) and Golebiowski (53) with agglutination and complement fixation tests. However, due to the small number of sera tested, and the small amount of work done on the subject, the absence of antibodies in affected pigs is not established beyond question. It should be noted also that antibodies have been found in the intestinal contents of man and guinea pigs during experimental studies of Asiatic cholera by Burrows et al (16)(17). These coproantobodies seemed to play

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a more important role than serum antibodies in the defense of the organism and were not related to serum antibodies in an immediate apparent manner. The presence or absence of coproantibodies in affected pigs with Vibrionic Dysentery was not investigated in this study.

H. Sensitivity tests

Results

Table 8 summarizes the results of the tests conducted to determine the sensitivity of V. coli isolated from pigs to various therapeutic agents. From this table, it is seen that these organisms conform to a fairly definite sensitivity pattern. All of the strains of V. coli were sensitive to furacin and hibitane and resistant to bacitracin. Most of the strains were sensitive to terramycin, tetracycline, aureomycin, and chloromycetin. Only slightly more than half of the forty-four strains tested were sensitive to triplesulfas and dihydrostreptomycin.

Discussion

The results were read as "sensitive" or "resistant". The size of the zone of inhibition was not considered since the size of this zone may vary with a number of factors unrelated to the degree of sensitivity of the organism. Among these factors, Pelczar (100) lists the following: the rate of diffusion of the drug through the agar medium, the number of organisms contained in the inoculum, the

depth of agar layer in the plate, and the amount of moisture.

From the results recorded, it appears that the drugs of choice in the prevention of treatment of Vibrionic Dysentery would be furacin and hibitane.

Since there is only one previous report on the in vitro sensitivity of these organisms to chemotherapeutic agents, it is difficult to determine whether their sensitivity to these agents was altered. In his report on the in vitro sensitivity of V. coli, Schmid (118) noted that V. coli was sensitive to sulfamethazine and resistant to aureomycin. Many reports, however, have been published in which satisfactory to good results were reported in treating Vibrionic Dysentery clinically with a variety of chemotherapeutic agents and antibiotics. Among these, bacitracin (4)(20)(39)(56)(112), streptomycin (3)(4)(39)(67)(114), and arsenicals (4)(10)(20)(51)(67)(107)(112) appeared to be the most effective drugs. Good results were also observed with aureomycin (3)(15)(20)(39)(114), terramycin (20), nitrofurazone (108), and sulfamethazine (84).

From that point of view, it appears that the sensitivity of the organism was somewhat altered. None of the strains tested were sensitive to bacitracin and only slightly more than half of the strains were sensitive to dihydrostreptomycin. Unfortunately no method was found satisfactory to test in vitro the sensitivity of V. coli to arsenicals.

TABLE 8

Summary of the Sensitivity of Forty-Four V. coli Strains to Various Chemotherapeutic Agents and Antibiotics.

	No. of Strains	
	Sensitive	Resistant
Aureomycin	43	1
Bacitracin	0	44
Chloromycetin	34	10
Dihydrostreptomycin	26	18
Furacin	44	0
Hibitane	44	0
Terramycin	38	6
Tetracycline	41	3
Triplesulfas	23	21

PART III

EXPERIMENTAL TRANSMISSION OF VIBRIONIC DYSENTERY

Since Vibrio coli is not unanimously accepted as the causal agent of Vibrionic Dysentery in swine, it seemed desirable to investigate the role played by this organism in the etiology of this infection.

MATERIAL AND METHODS - GENERAL

Only pigs in good physical condition and coming from a source known to be exempt from Vibrionic Dysentery were used for experimental purposes. Before experimentally infecting them, the absence of vibrios in the feces was determined by smears stained by Gram's method. Smears were made from fecal swabs taken every day for 3 or 4 consecutive days. These facts reasonably assured us that the pigs were not infected with or carrying V. coli. Furthermore, in each experiment, healthy, uninfected pigs were kept separately from the exposed animals and served as controls.

The pigs were placed in a cement-floored pen that was frequently cleaned. Before use, all of the units were cleaned and disinfected with creolin. When entering and leaving the pen, boots were disinfected in creolin solution. Pigs were fed twice a day with a commercial concentrate feed mixed with skim milk. This will be referred to as the normal regimen.

In every instance where cultures of V. coli were given per os, the organisms were mixed with a 5 per cent solution

of gastric mucin prepared by adding 5 grams of powdered mucin* to 100 ml. of distilled water. The solution of gastric mucin was mixed for three minutes in a Waring blender and the pH adjusted to 7.4 with normal sodium hydroxide. Sterilization was done by autoclaving at 15 lbs. pressure for 20 minutes. Gastric mucin was used because it apparently protects against digestive enzymes in a similar way to that of mucus material found in the stools of naturally infected pigs with V. coli (71).

From the lyophilized stock cultures or from blood plates after primary isolation, V. coli was subcultured onto blood plates and incubated at 37 C. for 48 hours under 15 per cent carbon dioxide. The growth was then washed with physiological saline and the suspension standardized to an opacity equivalent to Brown's tube No. 10. Motility was ascertained by hanging drop preparation while purity and morphology were determined by smears stained by Gram's method.

Pigs that remained alive at the end of each experiment were killed by electrocution and specimens from the stomach, duodenum, jejunum, ileum, cecum, colon, and rectum were fixed in Zenker's fluid for 12 hours. Specimens from the myocardium, liver, kidney, spleen, mesenteric and colic lymph nodes were fixed in 10 per cent formalin for at least 24 hours. Tissues were embedded in paraffin and stained with hematoxylin and eosin.

* General Biochemicals, Inc., Chagrin Falls, Ohio.

The procedure used in part 1 was employed for the re-isolation and identification of V. coli from experimental pigs. The mucosa of the colon was cultured on blood plates incubated at 37 C. under 15 per cent carbon dioxide. In addition, aerobic cultures at 37 C. of the liver, spleen, small and large intestines were carried out on blood plates for the isolation of possible pathogens. Cultures of the small and large intestines and mesenteric lymph nodes were carried out on MacConkey agar plates for the possible isolation of Salmonella sp.

EXPERIMENT 1

EFFECT OF THE ADMINISTRATION OF VIBRIO COLI TO HEALTHY PIGS

(1) Materials and Methods

Animals: Six healthy pigs (334R, 350R, 367R, 653, 654, 655) 6 to 8 weeks of age and averaging 30 pounds in weight were obtained from the Ontario Agricultural College. The animals were separated into two groups. One group of 4 pigs (334R, 350R, 367R, 653) was experimentally exposed to V. coli and two pigs (654, 655) served as controls. The pigs were starved during 48 hours before the beginning of the experiment.

Infective material: A saline suspension of a V. coli culture, strain 4156, isolated from the colonic mucosa of a pig naturally infected with Vibrionic Dysentery was mixed in gastric mucin as outlined and administered to the experimental pigs. The culture had been kept in the lyophilized state for 115 days.

Pigs 653 and 367R were given, via stomach tube, 63.5 ml. of the bacterial suspension in 63.5 ml. of gastric mucin.

Pigs 334R and 350R were given, per rectum, 63.5 ml. of the bacterial suspension. Twelve days after the initial exposure, these pigs received orally 50 ml. of the same bacterial suspension together with 50 ml. of gastric mucin mixed in one pint of milk. The pigs were killed 10 days after the second exposure i.e. 22 days after the first exposure.

Pig 654 was given, via stomach tube, 63.5 ml. of gastric mucin and pig 655 was given, per rectum, 63.5 ml. of normal saline. These pigs were killed on day 22.

(2) Results - Table 9 summarizes these results.

Pig 653

The daily pre-exposure temperature of this animal was 103.6, 102.6 and 102.6 F. Three days after the feeding of the infective material, the temperature rose to 104.2 F but dropped to within the normal range the next day and remained so throughout the rest of the experiment. The pig did not at any time exhibit any abnormal behavior and was killed 12 days after the exposure.

a.- Necropsy

Gross findings

No macroscopic lesions were visible in the alimentary tract and the parenchymatous organs.

Microscopic findings

Stomach, duodenum, jejunum, and ileum - The epithelium was intact. No evidence of hyperemia edema or excessive mucus production was observed.

Cecum, colon, and rectum - The marginal epithelium was mostly intact but presented foci of exfoliation especially where colonies of bacteria were in contact with the mucosa. The rest of the epithelial layer was mostly dysplastic. The mucus production was apparently normal.

Spleen, myocardium, liver, and colic lymph node - No microscopic lesions were observed.

Kidney - Mild swelling and increased granularity affecting principally the cells of the proximal convoluted tubules were noted together with few hyaline casts in distal convolutions and collecting tubules.

b.- Bacteriological findings

Smears made from fecal swabs taken daily after the exposure showed a few vibrios on the 6th day. These vibrios were seen for 2 days and disappeared thereafter. At the time of the necropsy, no vibrios were seen in smears made from the mucous membrane of the cecum and colon. Aerobic cultures of the liver at 37 C. were negative and cultures of the small and large intestines and mesenteric lymph node yielded E. coli. Culture of the colon under reduced oxygen tension was negative for V. coli.

Pig 367R

The daily pre-exposure temperature of this animal was 104.6, 102.8 and 104 F. Three days after the feeding of the infective material, the temperature rose to 105.2 F. and dropped to within the normal range for the next two days. It rose again to 104.2 F. on the 6th day and finally dropped to within the normal range on the 7th day. The pig did not at any time exhibit any abnormal behavior and was killed 12 days after the exposure.

a.- Necropsy

Gross findings

Stomach, small intestine and parenchymatous organs - No visible lesions were observed.

Cecum, colon, and rectum - The mucous membrane, especially that of the cecum was congested and edematous. The walls were thicker than normal and small depressed foci surrounded by an elevated border were noted on the mucosa.

Microscopic findings

Stomach - The blood vessels of the mucosa and submucosa were distended and the lamina propria was infiltrated by inflammatory cells chiefly lymphocytes and few macrophages. The marginal epithelium was still present but was lifted up by extravasated blood and appeared necrobiotic.

Duodenum, jejunum, and ileum - The epithelium of the crypts was intact but the marginal epithelium was slightly eroded. The interstitial tissue showed marked infiltration mostly by lymphocytes and few eosinophils. Hyperemia and edema were also evident.

Cecum, colon, and rectum - The mucosa was edematous, most of the marginal epithelium was intact but many foci of epithelial desquamation were observed. The crypts were markedly distended with mucus. An increased number of lymphocytes was observed in the mucosal interstitial tissue. In addition, the ileocecal valve showed focal necrosis. A severe neutrophilic infiltration was observed in the lamina propria of the rectum.

Spleen, myocardium and colic lymph node - No microscopic lesion were observed.

Kidney - Nephrosis and hyaline casts were seen in the collecting tubules.

Liver - Cloudy swelling was observed.

b.- Bacteriological findings

Smears made from fecal swabs taken daily after the exposure revealed a few vibrios on the 7th day and every day thereafter until the pig was killed. A few vibrios were also visible in smears made at the time of the necropsy from the mucous membrane of the cecum and colon. Aerobic cultures at 37 C. of the liver were negative. Cultures of the small and large intestines, mesenteric lymph node and spleen yielded a mucoid E. coli. Cultures of the colon and cecum at 37 C. under reduced oxygen tension were negative for V. coli.

Fig 334R

The daily pre-exposure temperature of this pig was 103.6,

103.4, and 103.8 F. Five days after the feeding of the infective material, the temperature rose to 104.2 F. and dropped to within the normal range for the next two days. It rose to 105.3 F. on the 8th day, dropped to normal for the next two days and rose again to 104 F. on the 11th and 12th days. Two days after the second exposure, the feces were moderately soft and contained large quantity of mucus without grossly recognizable blood. At this time, the animal's temperature was 104.8 F. but was normal by the next morning and remained so until the pig was killed 5 days later. On the 11th and 12th days, the animal appeared listless and ate less than usually.

a.- Necropsy

Gross findings

Stomach - The fundic portion of the stomach was slightly congested.

Small intestine and parenchymatous organs - No visible macroscopic lesions were observed.

Cecum and colon - The mucous membrane was congested and velvety. Small hemorrhages were visible on the mucosa. The walls were thicker than normal.

Rectum - The mucous membrane was less severely congested and did not have the velvety appearance seen on the mucosa of the cecum and colon. No hemorrhages were observed grossly.

Microscopic findings

Stomach - The marginal epithelium and that of the crypts were normal. The blood vessels of the mucosa were distended.

Duodenum, jejunum, and ileum - The epithelium was intact. No evidence of hyperemia, edema, abnormal cellular infiltration or increased mucus secretion was observed.

Cecum, colon and rectum - The blood vessels of the mucosa and submucosa were distended. The submucosa was edematous. The lamina propria was infiltrated by leukocytes, chiefly lymphocytes, and few macrophages. The marginal epithelium was mostly eroded; where the epithelium was still present, it was dysplastic. Goblet cells showed an increased mucus production. Diphtheritic membrane was present in the intestinal lumen and consisted mainly of desquamated epithelial and inflammatory cells but mucus and fibrin were also present. Numerous colonies of bacteria were found in the debris.

Spleen and myocardium - No microscopic lesions were observed.

Kidney - Mild nephrosis and focal interstitial nephritis with infiltration of mononuclear and fibroblast cells were seen.

Liver - Cloudy swelling was observed.

Colic lymph node - Collection of neutrophils was seen in the connective tissue of the trabeculae.

b.- Bacteriological findings

Smears made from fecal swabs taken daily after the exposure

showed many vibrios on the 7th day. Vibrios were also visible on days 8, 9 and 10 but were not visible thereafter. Many vibrios were observed in smears made from the mucous membrane of the cecum and colon at the time of necropsy. Aerobic cultures at 37 C. of the liver, spleen, mesenteric lymph node, small and large intestine yielded E. coli. Cultures of the colon and cecum at 37 C. under reduced oxygen tension were negative for V. coli.

Pig 350R

The daily pre-exposure temperature of this pig was 104, 104.6 and 104.8 F. The day following the feeding of the infectious material the temperature was 104.6 F and it rose to 105.2 F. one day later. During the rest of the experiment the temperature varied between 102.6 and 104.2 F. Four days after the initial exposure, the feces were softer than normal and the 2nd, 3rd and 4th day following the second exposure, the feces were very soft almost watery and contained a great quantity of mucoid material. During that period of time, the animal was depressed, did not eat as much as usually, but its thirst was increased. On the 5th day after the second exposure, its behavior became normal and the animal was killed three days later.

a.- Necropsy

Gross findings

Stomach - The fundic portion of the stomach was slightly congested.

Small intestine and parenchymatous organs - No visible

macroscopic lesions were noted.

Cecum, colon and rectum - The mucous membrane was congested and velvety. Small hemorrhages were visible on the mucosa. The walls were thicker than normal.

Microscopic findings

Stomach - The marginal epithelium and that of the crypts were normal.

Duodenum, jejunum and ileum - The epithelium was intact. No evidence of hyperemia, edema, abnormal cellular infiltration or increased mucus secretion was present.

Cecum, colon and rectum - The mucosa and submucosa were edematous. The blood vessels and lymphatics were distended. Many of the crypts were dilated with mucus. The marginal epithelium was in great majority eroded or when present appeared to be breaking up. An increased lymphocytic infiltration was observed in the mucosa and submucosa.

Spleen, myocardium, liver, kidney, and colic lymph node - No lesions were observed.

b.- Bacteriological findings

Smears made from fecal swabs taken daily after the exposure showed a few vibrios on the first day after the second exposure. Vibrios were seen daily until the animal was killed. Many vibrios were visible in smears made, at the time of necropsy, from the mucous membrane of the cecum and colon. Aerobic cultures at 37 C. of the liver and spleen were negative. Cultures of the small and large intestines and mesenteric lymph node yielded E. coli. Cultures of the colon and cecum at 37 C. under reduced oxygen tension were negative for V.

coli.

Pigs 654 and 655

The daily temperature of pigs 654 and 655 before the beginning of the experiment was 102.8, 102.2 and 101.2 F. and 102.6, 102.4 and 102 F. respectively. During the experiment the temperature of these pigs remained within the limits of the normal range and their behavior was never noted to be abnormal. The animals were killed 22 days after the beginning of the experiment.

a.- Necropsy

Gross findings

Alimentary tract and parenchymatous organs - No lesions were noted.

Microscopic findings

Stomach, duodenum, jejunum, and ileum - The epithelium was intact. No evidence of hyperemia, edema or excessive mucus production was observed.

Cecum, colon, and rectum - The marginal and glandular epithelia were intact. No evidence of hyperemia, edema, cellular infiltration or excessive mucus production was observed.

Spleen, myocardium, colic lymph node, liver and kidney - No lesions were observed.

b.- Bacteriological findings

No vibrios were seen in the smears made from fecal swabs

during the experiment and from the mucous membrane at the time of the necropsy. Aerobic cultures at 37 C. of the liver and spleen were negative. Cultures of the small and large intestines and mesenteric lymph nodes yielded E. coli. Cultures of the colon at 37 C. under reduced oxygen tension were negative for V. coli.

EXPERIMENT 2

EFFECT OF STARVATION, CHILLING AND GASTROINTESTINAL IRRITATION AS STRESS FACTORS ON SUSCEPTIBILITY TO VIBRIO COLI INFECTION.

(1) Materials and Methods

Animals: Nine healthy pigs (2A1, 2A2, 2A3, 2B1, 2B2, 2B3, 2B4, 5-1, 5-2) 6 to 8 weeks of age and averaging 25 pounds in weight were obtained from the Ontario Reformatory Farm. The pigs were separated into three groups. One group of 4 pigs (2B1, 2B2, 2B3, 2B4) was exposed to starvation and cooling. One group of three pigs (2A1, 2A2, 2A3) was exposed to starvation, cooling and gastrointestinal irritation. Two pigs (5-1, 5-2) served as controls.

Infective material: A saline suspension of two V. coli cultures, strain 5372 kept in the lyophilized state for 20 days and strain 5400 subcultured only once, were used as infective material. Both strains were isolated from the colonic mucosa of pigs naturally infected with Vibrionic Dysentery.

Pigs 2A1, 2A2, 2A3 were chilled by being placed in a pen with open windows. At this time, the average outside temperature was around the freezing point. The pigs were starved for 48

TABLE 9

Effect of the Administration of
V. coli to Healthy Pigs.

Pig No.	Route of Exposure	Clinical Signs	Inflammatory Reaction of Large int.	Bacteriological Findings		
				Smears Fecal Swabs	Smears From Mucosa	Cultures Under CO ₂
334R	# *	104.8-105.3F. Feces soft Mucus Listless	/	P	N	N
350R	# *	104.2-105.2F. Feces soft Mucus Depressed	/	P	PP	N
367R	#	Normal behavior	/	P	P	N
653	#	Normal behavior	/	P	N	N
654	control	Normal behavior	-	N	N	N
655	control	Normal behavior	-	N	N	N

Per os
* Per rectum
/ Present
- Absent
N Negative for V. coli
P V. coli present
PP V. coli present in large number

hours and given 2 teaspoons of castor oil without food. Twenty-four hours later they were given 50 ml. of a bacterial suspension of strain 5372 in 100 ml. of gastric mucin mixed to one pint of skim milk. In the evening, the pigs received a small amount of concentrated feed mixed with skim milk. They were again starved for 24 hours and, at the end of this period, they received 50 ml. of a bacterial suspension of strain 5400 in 50 ml. of gastric mucin mixed with one pint of skim milk. Twelve hours later, the pigs were placed on normal diet.

Pigs 2B1, 2B2, 2B3, and 2B4 were chilled as described above. The pigs were starved for 72 hours and were given 50 ml. of a bacterial suspension of strain 5372 mixed in 100 ml. of gastric mucin and added to one pint of skim milk. In the evening, the pigs received a small amount of concentrated feed mixed with skim milk. They were again starved for 24 hours and, at the end of this period, they received 50 ml. of a bacterial suspension of strain 5400 with 50 ml. of gastric mucin mixed with one pint of skim milk. Twelve hours later, the pigs were placed on normal diet.

Pigs 5-1 and 5-2 were chilled as described above. The pigs were starved for 72 hours and, at the end of this time, they were given 50 ml. of gastric mucin mixed in one pint of skim milk. In the evening and from then on, the pigs were placed on normal diet.

(2) Results - Tables 10 and 11 summarize these results.

Pig 2A1

The pre-exposure temperature of this pig was 104, 102, and 101.4 F. Approximately 12 hours following the administration of castor oil, the pig's feces were watery in consistency but became normal after 24 hours. Six days after the feeding of the first culture of V. coli, the pig became prostrated and developed a violent diarrhea. By the next morning, the bowel discharge contained a great quantity of mucus. The diarrhea persisted until the pig was killed 12 days after the initial exposure. Throughout the experiment the temperature ranged from 97 to 103 F.

a.- Necropsy

Gross findings

Stomach, small intestine, and parenchymatous organs -
No microscopic lesions were observed.

Cecum, colon, and rectum - The mucous membrane was congested and velvety. Few hemorrhages were present on the surface but no diphtheritic exudate was noted. The intestinal wall was appreciably thickened and the intestinal contents were watery in consistency.

Microscopic findings

Stomach, duodenum, jejunum, and ileum - The epithelium was intact. No evidence of edema or hyperemia was observed.

Cecum, colon, and rectum - The blood vessels of the mucosa and submucosa were distended. The submucosa was extremely edematous and the mucosa was infiltrated by a large number of neutrophils.

Spleen, myocardium, and colic lymph nodes - No microscopic lesions were observed.

Kidney and liver - Mild cloudy swelling was noted.

b.- Bacteriological findings

Smears made from fecal swabs taken daily after the exposure revealed a few vibrios on the 6th day. By the next day, a large number of vibrios were present in the smears and vibrios were seen throughout the experiment thereafter. Many vibrios were also visible in smears made from the mucous membrane of the cecum and colon at the time of the necropsy. Aerobic cultures at 37 C. of the liver, spleen, mesenteric lymph nodes, small and large intestines yielded E. coli. Cultures of the colon and cecum at 37 C. under reduced oxygen tension yielded V. coli.

Fig 2A2

The pre-exposure temperature of this pig was 103, 102.6 and 101.2 F. About 12 hours following the administration of castor oil, the pig's feces were watery in consistency for approximately 24 hours and became normal thereafter. Six days after the feeding of the first culture of V. coli, the pig started passing soft feces. The feces remained soft until the animal was killed 12 days after the initial exposure. Throughout the experiment the pig's temperature ranged from 99 to 101.2 F.

a.- Necropsy

Gross findings

Stomach, small intestine, and parenchymatous organs -
No gross lesions were observed.

Cecum, colon, and rectum - The mucous membrane was congested and velvety. No hemorrhages or diphtheritic exudate were noted. The large intestinal contents were fluid in consistency and the wall was thicker than normal.

Microscopic findings

Stomach, duodenum, jejunum, and ileum - The marginal epithelium and the epithelium of the crypts were normal. No evidence of hyperemia, edema or increased mucus production was observed.

Cecum, colon, and rectum - The mucosa was extremely edematous and the goblet cells showed an increased mucus production. The mucosa was infiltrated with leukocytes, chiefly neutrophils, and the epithelium showed foci of desquamation.

Spleen, myocardium, colic lymph node, liver, and kidney -
No histological lesions were observed.

b.- Bacteriological findings

Smears made from fecal swabs taken daily after the exposure revealed a few vibrios on the 7th day and during the rest of the experiment. Many vibrios were visible in smears made from the mucous membrane of the cecum and colon at the time of the necropsy. Aerobic cultures at 37 C. of the liver

spleen, and mesenteric lymph nodes were negative. Cultures of the small and large intestines yielded E. coli. Cultures of the colon and cecum at 37 C. under reduced oxygen tension yielded V. coli.

Pig 2A3

The pre-exposure temperature of this pig was 99, 98.2, and 99.6 F. Four days following the administration of castor oil, the pig developed a severe watery, grayish diarrhea, lost weight rapidly and became very weak. It showed an incoordinate gait and a severe depressed general attitude. The pig's temperature was 94 F. and the animal died the next morning. The abdominal skin was bluish in color.

a.- Necropsy

Gross findings

Stomach - The fundic portion was severely congested and dark red in color.

Duodenum, jejunum, and ileum - The mucous membrane was severely congested. The small intestinal wall was thickened and the intestinal lumen was filled with a watery, yellowish exudate.

Cecum, colon, and rectum - The mucous membrane was slightly congested and velvety. No hemorrhages or diphtheritic exudate were noted. The large intestinal contents were semi-solid in consistency.

Parenchymatous organs - No macroscopic lesions were observed.

Microscopic findings

Stomach - The marginal epithelium and that of the crypts were normal. The blood vessels of the mucosa were distended.

Duodenum, jejunum, and ileum - The mucus production was markedly increased and numerous lymphocytes were observed in the interstitial tissue of the mucosa. The marginal epithelium showed some exfoliation particularly at the tips of the villi. The lesions were less severe in the duodenum than in the rest of the small intestine.

Cecum, colon, and rectum - The blood vessels of the mucosa and submucosa were distended. The marginal epithelium showed foci of desquamation. Goblet cells were increased in number and size and the crypts were distended with mucus. The mucosa was infiltrated with neutrophils.

Spleen, myocardium, colic lymph node, liver, and kidney - No lesions were observed.

b.- Bacteriological findings

Smears made from fecal swabs taken daily after the exposure did not reveal vibrios but smears made from the mucous membrane of the cecum and colon after the animal's death showed a large number of vibrios. Aerobic cultures at 37 C. of the liver, spleen, mesenteric lymph node, and small intestine yielded hemolytic E. coli. Cultures of these organs on MacConkey agar plates were negative for Salmonella organisms. Cultures of the cecum, colon, and colic lymph nodes at 37 C. under reduced oxygen tension yielded V. coli.

Fig 2Pl

The pre-exposure temperature of this pig was 99, 99.6 and 99.2 F. Throughout the experiment the temperature ranged from 98.8 to 101.4 F. The pig did not at any time exhibit any abnormal behavior and was killed 20 days after the initial exposure.

a.- Necropsy

Gross findings

Stomach, small intestine, and parenchymatous organs -
No visible lesions were observed.

Cecum, colon, and rectum - The mucous membrane was congested, edematous and presented few hemorrhages. The intestinal wall was appreciably thicker than normal.

Microscopic findings

Stomach, duodenum, jejunum, and ileum - The epithelium was intact. No hyperemia, edema or excessive mucus production was observed.

Cecum, colon, and rectum - The mucosa and submucosa were hyperemic. The marginal epithelium was destroyed and the interstitial tissue of the mucosa was infiltrated with lymphocytes. The mucous membrane was covered with diphtheritic exudate. The crypts were distended with mucus.

Spleen, myocardium, and colic lymph node. No histological lesions were observed.

Kidney - Cloudy swelling was seen in the proximal convoluted tubules.

Liver - Fatty degeneration was observed.

b.- Bacteriological findings

Smears from fecal swabs made daily after the exposure did not show vibrios. A few vibrios were seen in smears made from the mucous membrane of the large intestine at the time of the autopsy. Aerobic cultures at 37 C. of the liver, spleen, and small intestine yielded a mucoid E. coli. Cultures of the cecum and colon at 37 C. under reduced oxygen tension yielded V. coli.

Pig 2B2

The pre-exposure temperature of this pig was 100.6, 100.6, and 100 F. Throughout the experiment the temperature ranged from 100 to 102.8 F. Six days after the initial exposure, the pig developed a mild diarrhea which contained mucus. The feces were semisolid for 3 days and thereafter became gradually normal in consistency. The animal was killed 20 days after the initial exposure.

a.- Necropsy

Gross findings

Stomach, small and large intestines, and parenchymatous organs - No gross lesions were observed.

Microscopic findings

Stomach, duodenum, jejunum, and ileum - The marginal epithelium and that of the crypts were normal. The mucosa was slightly edematous.

Cecum, colon, and rectum.-- A mild increased mucus production was observed, but the epithelium was intact and no hyperemia or edema was observed.

Spleen, myocardium, colic lymph node, liver, and kidney -
No histological lesions were found.

b.- Bacteriological findings

Smears made from fecal swabs taken daily after the exposure revealed a few vibrios on the 6th day. Vibrios were seen for 3 days and disappeared thereafter. No vibrios were seen in smears made from the mucous membrane of the large intestine at the time of the autopsy. Aerobic cultures at 37 C. of the liver, spleen, and mesenteric lymph nodes were negative. Cultures of the small intestine yielded E. coli and cultures of the colon and cecum at 37 C. under reduced oxygen tension were negative for V. coli.

Fig 2B3

The pre-exposure temperature of this animal was 101.6, 100.2 and 99.6F. Throughout the experiment the temperature ranged from 99.5 to 102.6 F. The pig did not at any time exhibit any abnormal behavior and was killed 20 days after the initial exposure.

a.- Necropsy

Gross findings

Stomach, small intestine, and parenchymatous organs -
No visible lesions were observed.

Cecum, colon, and rectum - The intestinal wall was apparently normal. The mucosa was congested and the lumen contained an increased amount of mucus.

Microscopic findings

Stomach, duodenum, jejunum, and ileum - The marginal epithelium and that of the crypts were normal. No evidence of hyperemia, edema or cellular infiltration was evident.

Cecum, colon, and rectum - Some exfoliation of the marginal epithelium was observed. The mucus production was increased and many of the crypts were distended with mucus. Edema and hyperemia were noted in the mucosa and submucosa. The interstitial tissue of the mucosa was infiltrated with leukocytes, chiefly neutrophils.

Spleen, myocardium, colic lymph node, liver, and kidney - No histological lesions were observed.

b.- Bacteriological findings

Smears made from fecal swabs taken daily after the exposure were negative for vibrios. No vibrios were seen from the mucous membrane of the large intestine. Aerobic cultures at 37 C. of the liver, spleen and mesenteric lymph node were negative. Cultures from the small intestine yielded E. coli and cultures from the cecum and colon were negative for V. coli.

Fig 2B4

The pre-exposure temperature of this pig was 102.4, 99.6 and 98.6 F. Throughout the experiment the temperature ranged from 96 to 102.6 F. The pig did not at any time exhibit any abnormal behavior and was killed 19 days after the initial exposure.

a.- Necropsy

Gross findings

Stomach, small intestine, and parenchymatous organs -
No lesions were observed.

Cecum, colon, and rectum - The mucus membrane was congested and covered with an increased amount of mucus. The intestinal wall was apparently normal.

Microscopic findings

Stomach, duodenum, jejunum, and ileum - The marginal epithelium and that of the crypts were normal. No evidence of hyperemia, edema or cellular infiltration was evident.

Cecum, colon, and rectum - The mucosa and submucosa were slightly edematous, and the goblet cells were distended with mucus. The marginal epithelium was eroded and the interstitial tissue was infiltrated with neutrophils.

Spleen, myocardium, colic lymph nodes, liver, and kidney -
No histological lesions were observed.

b.- Bacteriological findings

Smears made from fecal swabs taken daily after the exposure were negative for vibrios. No vibrios were seen from the mucous membrane of the large intestine. Aerobic cultures at 37 C. of the liver, spleen and mesenteric lymph node were negative. Cultures from the small intestine yielded E. coli and cultures from the colon were negative for V. coli.

Figs 5-1 and 5-2

Throughout the experiment the temperature of these pigs remained within the normal limits and their behavior was never noted to be abnormal. The pigs were killed 21 days after the beginning of the experiment.

a.- Necropsy

Gross findings

Alimentary tract and parenchymatous organs - No lesions were observed.

Microscopic findings

Stomach, small and large intestines - The marginal epithelium and that of the crypts were normal. No evidence of hyperemia, edema, or excessive mucus production was evident.

Spleen, myocardium, colic lymph node, liver, and kidney - No lesions were observed.

b.- Bacteriological findings

Smears made from fecal swabs taken daily after the exposure were negative for vibrios. No vibrios were seen from the mucous membrane of the large intestine. Aerobic cultures at 37 C. of the liver, spleen, and mesenteric lymph nodes were negative. Cultures from the small intestine yielded E. coli and cultures from the cecum and colon were negative for V. coli.

TABLE 10

Effect of Starvation and Chilling as Stress
Factors on Susceptibility to V. coli Infection.

Pig No.	Route of Exposure	Clinical Signs	Inflammatory Reaction of large int.	Bacteriological Findings		
				Smears Fecal Swabs	Smears From Mucosa	Cultures Under CO ₂
2B1	#	Normal behavior	+	N	P	P
2B2	#	Diarrhea 6-9th day	-	P	N	N
2B3	#	Normal behavior	+	N	N	N
2B4	#	Normal behavior	-	N	N	N
5-1	control	Normal behavior	-	N	N	N
5-2	control	Normal behavior	-	N	N	N

Per os
+ Present
- Absent
N Negative for V. coli
P V. coli present

TABLE 11

Effect of Starvation, Chilling, and Gastrointestinal Irritation as Stress Factors on Susceptibility to V. coli Infection.

Fig No.	Route of Exposure	Clinical Signs	Inflammatory Reaction of large int.	Bacteriological Findings		
				Smears Fecal Swabs	Smears From Mucosa	Cultures Under CO ₂
2A1	#	97-103 F. Severe diarrhea Mucus	+	P	PP	P
2A2	#	99-101.2F Feces soft	+	P	PP	P
2A3	#	Severe diarrhea Listless Died 5th day	+	N	PP	P

Per os
 + Present
 N Negative for V. coli
 P V. coli present
 PP V. coli present in large number

EXPERIMENT 3

EFFECT OF ESCHERICHIA COLI ENDOTOXIN AS A STRESS FACTOR ON SUSCEPTIBILITY TO VIBRIO COLI INFECTION.

(1) Materials and Methods

Animals: Six healthy pigs (44,45,46,47,48,49) 6 to 8 weeks of age and averaging 25 pounds were obtained from the Ontario Reformatory Farm. The pigs were separated into two groups. In one group of three pigs (44,47,49), two were stressed by injection of endotoxin before receiving the infective material while the third pig was not stressed and received only the infective material. In the other group of three pigs (45,46,48), two were stressed by injections of endotoxin while the third one did not receive endotoxin. The pigs in this group were not exposed to V. coli.

Infective materials: E. coli endotoxin* was used to produce a generalized Shwartzman reaction. The "preparing" injection of endotoxin was given intravenously and after a 24-hour period, the "provoking" injection of endotoxin was also administered intravenously into the marginal ear vein.

A saline suspension of a V. coli culture, strain 2A3 was used as infective material. This strain was isolated during the preceding experiment and kept in the lyophilized state for 15 days.

Pig 44 was fed 35 ml. of the bacterial suspension and 35 ml. of gastric mucin mixed with one pint of skim milk.

*Difco Laboratories, Detroit, Michigan.

The mixture was given for two successive days. Pig 45 served as control and was not stressed nor infected. Pig 46 received 0.36 mg. of endotoxin per kilo. for two successive days. Pig 47 was given 0.36 mg. of endotoxin per kilo.; the next day the animal received the same amount of endotoxin and 35 ml. of the bacterial suspension with 35 ml. of gastric mucin mixed in one pint of skim milk. The third day the pig was fed 35 ml. of bacterial suspension and 35 ml. of gastric mucin mixed in one pint of skim milk. Pig 48 was given 1 mg. of endotoxin per kilo. for two successive days. Pig 49 was given 1 mg. of endotoxin per kilo. and received the same amount of endotoxin plus 35 ml. of the bacterial suspension and 35 ml. of gastric mucin mixed in one pint of skim milk. On the third day, the pig was fed 35 ml. of the bacterial suspension in 35 ml. of gastric mucin mixed with one pint of skim milk.

(2) Results - Table 12 summarizes these results.

Following each injection of E. coli endotoxin, there was a latent period, lasting from 15 to 30 minutes, during which the pigs appeared to be well. They then became less active, developed vomiting, showed a profuse salivation and generalized weakness. Respiratory distress was indicated by a rapid labored breathing. After a period of about 30 to 60 minutes, the pigs had a profuse, fluid, grayish diarrhea. The signs were less severe in pigs 46 and 47 which received a smaller dose of endotoxin.

Pig 44

This pig did not at any time exhibit any abnormal behavior and was killed 9 days following exposure.

Necropsy

Gross findings

Stomach, small and large intestines - No gross lesions were observed.

Heart - Fibrinous pericarditis was noted. The pericardium was adherent to the epicardium over the entire part of the cardiac surface.

Peritoneal cavity - The entire peritoneum showed lesions of inflammation with exudation, fibrin deposits and adhesions.

Pleural cavity - Fibrinous adhesions between the parietal and visceral pleurae were noted.

Microscopic findings

Stomach, small and large intestines, parenchymatous organs - No lesions were observed.

Figs 45 and 47

Necropsy

Gross and Microscopic findings

Stomach, small and large intestines, parenchymatous organs - No lesions were noted.

Pig 48

Necropsy

Gross findings

Stomach, small and large intestine, and parenchymatous

organs - No gross lesions were observed.

Ileum - The ileum, extending to about 20.0 cm. anterior to the ileocecal valve, was markedly thickened and hardened, and the muscularis layer was greatly enlarged. The mucous membrane appeared normal.

Microscopic findings

Stomach, duodenum, jejunum, large intestine, and parenchymatous organs - No histopathological lesions were observed.

Ileum - The mucosa was normal but the muscularis showed marked hypertrophy of both layers.

Fig 46

Necropsy

Gross lesions

Stomach and parenchymatous organs - No gross lesions were observed.

Small and large intestines - The mucosa was reddened and swollen and showed diffuse roughened foci simulating desquamation. An increased mucus production was noted over the mucosa.

Microscopic findings

Stomach, spleen, myocardium, colic lymph node, liver and kidney - No histological lesions were noted.

Small and large intestines - The epithelial layer was eroded so that a raw surface was left over these eroded foci. A diphtheritic exudate composed chiefly of mucus, cellular debris, leukocytes, fibrin, erythrocytes, and bacteria was

observed in the intestinal lumen. Goblet cells were increased in size and number. Leukocytes, chiefly neutrophils, infiltrated the mucosa. The blood vessels of the mucosa and submucosa were distended.

Fig 49

Necropsy

Gross lesions

Stomach, small intestine, and parenchymatous organs - No gross lesions were observed.

Cecum, colon, and rectum - No gross lesions were noted on the mucous surface. The serous coat of the cecum showed lesions of localized fibrinous peritonitis and adhesions to the peritoneum.

Microscopic findings

Stomach, small and large intestines - The marginal epithelium and that of the crypts were normal. No evidence of hyperemia, edema or increased mucus production was observed. The serous coat of the cecum was infiltrated with leucocytes and fibroblasts.

Spleen, myocardium, colic lymph node - No histological lesions were observed.

Liver and kidney - Hyaline droplets in hepatic cells and in the cells of the proximal convoluted tubules were observed in histological sections.

Bacteriological findings

Smears made from fecal swabs taken daily after the exposure were negative for vibrios. No vibrios were seen in smears made from the mucous membrane of the large intestine at the time of the necropsy. Aerobic cultures at 37 C. of the liver, spleen, and mesenteric lymph nodes were negative. Cultures from the small and large intestines yielded E. coli.

DISCUSSION

The result of the first experiment in which three out of four pigs developed lesions of enteritis and excreted variable numbers of vibrios indicates that Vibrionic Dysentery can be produced experimentally by feeding pure cultures of V. coli. As also observed by Doyle (35) and Roberts (105), the symptoms and lesions produced were less severe than the ones occurring in natural infection.

In this first experiment, however, one of Koch's postulates was not fulfilled since V. coli, even if present in the large intestine, was not isolated in pure culture. The methods can be criticized in that only one portion of the colon was streaked onto blood agar plates. It has been observed later that more than one portion of the colon or cecum should be cultured in order to isolate V. coli. However, as noted in part 11, even if this procedure is used and if V. coli is present in large number, the organism is not always successfully isolated from the infected large intestine. It is a

fact that the diagnosis of Vibrionic Dysentery in swine should not be based on the presence of vibrios alone; but when suggestive symptoms and lesions are present together with a large number of vibrios, these organisms should not be regarded as insignificant since they have been shown to be capable of reproducing the disease.

The experimental transmission of Vibrionic Dysentery was first reported by Doyle (35) and later confirmed by James and Doyle (71), Roberts (105), Csontos and Pesti (24), and Truazczynski (129). On the other hand, Boley et al (10), Shand and Gitter (121), Deas (28), Gorrie (54) failed to reproduce the disease by feeding pure cultures of V. coli and concluded that this organism probably play only a secondary role in the etiology of the disease.

It is a fact that predisposing conditions such as nutritional or metabolic disturbances, sudden changes in feed, fatigue, poor sanitation, exposure to cold, low grade bacterial infection or parasitic irritation of the alimentary tract act as predisposing factors in many swine diseases, particularly enteric conditions. Some of these conditions have been investigated in the second and third experiments to determine if they have a predisposing affect on the inability of the intestinal mucosa to resist invasion by V. coli.

In the second experiment, all of the pigs exposed to pure cultures of V. coli following gastrointestinal irritation, starvation and chilling, developed severe symptoms and lesions of Vibrionic Dysentery and one of these pigs died. V. coli was

TABLE 12

Effect of E. coli Endotoxin as a Stress Factor on Susceptibility to V. coli Infection.

Pig No.	Route of Exposure	Clinical Signs	Gross Findings	Bacteriological Findings	
				Smears from Fecal Swabs and Mucosa	Cultures Under CO ₂
44	V.c. #	Normal behavior	Pericarditis Pleuritis Peritonitis	N	N
45	control	Normal behavior	-	N	N
46	E.c.i/v	Normal behavior	Mild Enteritis	N	N
47	E.c.i/v V.c. #	Normal behavior	-	N	N
48	E.c.i/v	Normal behavior	Terminal Ileitis	N	N
49	E.c.i/v V.c. #	Normal behavior	Localized Fibrinous Peritonitis	N	N

V.c. # V. coli per os
 E.c. i/v E. coli endotoxin intravenous
 - Normal
 N Negative for V. coli

isolated from each of these pigs. On the other hand, only one out of four pigs developed lesions of Vibrionic Dysentery following the feeding of V. coli when starvation and exposure to cold, without gastrointestinal irritation, were imposed as stress factors.

The results of the second experiment suggest that some predisposing factors, especially intestinal irritation, should not be discarded as one of the possible predisposing causes in V. coli infection in pigs.

In the third experiment, stress imposed on the pigs by E. coli endotoxin did not seem to play a predisposing role in the etiology of the disease. None of the pigs in this experiment developed the disease after exposure to V. coli.

The pathological signs observed in these experiments were very similar to those occurring in field cases of Vibrionic Dysentery. The only consistent lesion observed was confined to the large intestine and consisted of an acute inflammation with infiltration of the mucosa by numerous leukocytes, including a variable proportion of neutrophils.

The incubation period varied from 4 to 13 days. These results are consistent with those obtained by Boyle (35) who found that after feeding pure cultures of V. coli, diarrhea appeared at the end of 3 to 5 days. Roberts (105) reported an incubation period of 4 to 13 days following the oral exposure of susceptible pigs.

In the first experiment, an increased body temperature was observed in three pigs. The highest degree recorded in these pigs was 104.8, 105.2 and 105.8 F. This increase in body temperature may be imputed to the high external temperature of the environment, which was around 95 F. at the time. The rise of temperature may also be attributed to the restraint of the pigs. However, as noted in part 1, an elevated body temperature is sometimes recorded in naturally infected pigs. No remarkable changes in body temperature were recorded in the other experimental pigs.

The difficulties encountered in transmitting experimentally Vibrionic Dysentery may also, in part, be attributed to the loss of virulence of the organism after cultivation on artificial media. V. coli may acquire virulence under conditions of growth in the pig's alimentary tract which may be rapidly lost upon subcultivation on artificial media, or on storage in the lyophilized state. On the other hand, it is known with many bacterial species that a variation from the smooth to the rough form is associated with a loss of virulence. In the second part of this work, the rapid dissociation of V. coli cultures has been noted; unfortunately, before feeding V. coli to susceptible pigs, the degree of dissociation of the cultures has not been determined and most of the cultures were probably rough.

SUMMARY AND CONCLUSIONS

- (1) The study of Vibrionic Dysentery as it occurred in Ontario from 1958 to 1960 is reported. The data presented are derived from 249 outbreaks.
- (2) The disease was observed in pigs 3 to 24 weeks of age but is more frequent in pigs 7 to 12 weeks of age.
- (3) Four clinical syndromes are observed. In the per-acute cases, the pigs are usually in fair state of nutritional condition. In acute infections, wasting is evident when the pigs are presented with diarrhea of more than a few days duration. In subacute and chronic cases, however, affected pigs are generally stunted, dehydrated, emaciated, showed hunched back, protruding ribs and eyes sunken into the orbits.
- (4) The pathological findings show that Vibrionic Dysentery is chiefly an infection of the large intestine and obvious lesions are seen in the cecum, colon, and rectum. Different types of inflammation are encountered depending on the duration and probably on the intensity of the infection.
- (5) Catarrhal, hemorrhagic, and necrotic inflammations are the forms usually observed. Sometimes, however, mixed types of inflammation are seen and there is no sharp line of demarcation between these divisions. Fifty-five

per cent of the cases studied microscopically presented lesions of catarrhal inflammation. Seven per cent presented lesions of hemorrhagic inflammation. Sixteen per cent presented lesions of necrotic inflammation while twenty-two per cent of the sections examined showed mixed types of inflammation.

- (6) V. coli organism isolated from the mucosa of diseased pigs is a motile, gram-negative organism which shows considerable pleomorphism. S-shaped, "flying seagull" type, comma-shaped and coccal forms are also seen.
- (7) The organism grows well on blood plate and in Albini brucella broth containing 0.1 per cent agar when the oxygen tension is reduced with 15 per cent carbon dioxide. On solid medium, a continuous spreading growth is more frequently encountered than distinct colonies.
- (8) Electron micrographs show that V. coli usually possesses one flagellum attached to one pole or two flagella one attached to each pole. Not more than two polar flagella were observed.
- (9) The biochemical tests conducted on forty-six strains of V. coli show that there appears to be no difference in the biochemical properties of these strains.
- (10) Serological investigations were carried out on thirty-nine vibrio strains of porcine origin, one vibrio strain of bovine origin and one vibrio strain of avian origin. It appears that the strains of V. coli isolated

from cases of Vibrionic Dysentery show a great deal of variation in their antigenic structures. On the other hand, V. fetus and the chicken vibrio show antigenic relation with some of the porcine strains.

- (11) Latex agglutination technique failed to detect agglutinins in the sera of pigs affected with or convalescing from Vibrionic Dysentery.
- (12) The sensitivity of forty-four strains of V. coli isolated from pigs was investigated with respect to various chemotherapeutic agents and antibiotics. All of the strains proved to be sensitive to furacin and hibitane and resistant to bacitracin. The majority of the strains proved to be sensitive to aureomycin, terramycin and tetracycline.
- (13) Enteritis was produced in three out of four pigs when pure cultures of V. coli were fed to healthy pigs. The clinical signs and lesions however, were less severe than in natural infection.
- (14) Two out of four pigs showed signs and lesions of Vibrionic Dysentery after starvation and chilling were imposed as stress factor. When gastrointestinal irritation was added to these factors, severe symptoms and lesions of Vibrionic Dysentery developed in the three pigs exposed and one of these died.

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Plate 1 - Age distribution of pigs affected
with Vibrionic Dysentery.

AGE DISTRIBUTION OF 264 PIGS
AFFECTED WITH VIBRIONIC DYSENTERY

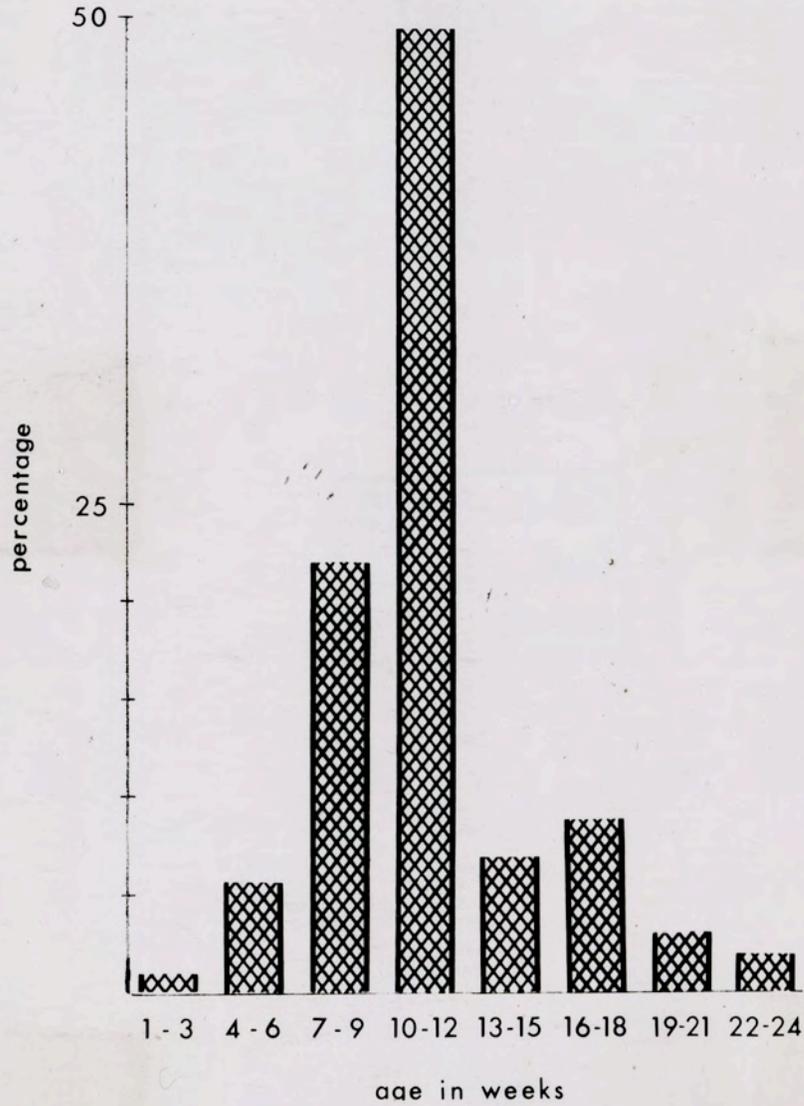


Plate 2 - Section of the colon showing epithelial desquamation and infiltration of the mucosa by round cells and neutrophils. Fig 2A1. (H. & E.)

Plate 3 - Section of the colon showing an increased mucus production. The mucus is pushed up from the gland into the intestinal lumen forming the major component of the exudate coating the mucous membrane. Naturally occurring case. (H. & E.)

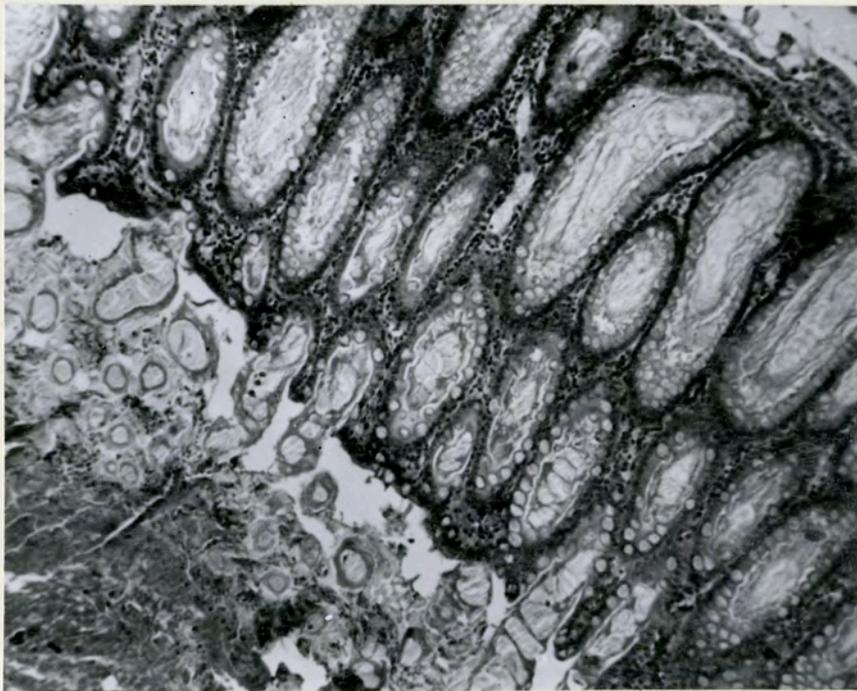
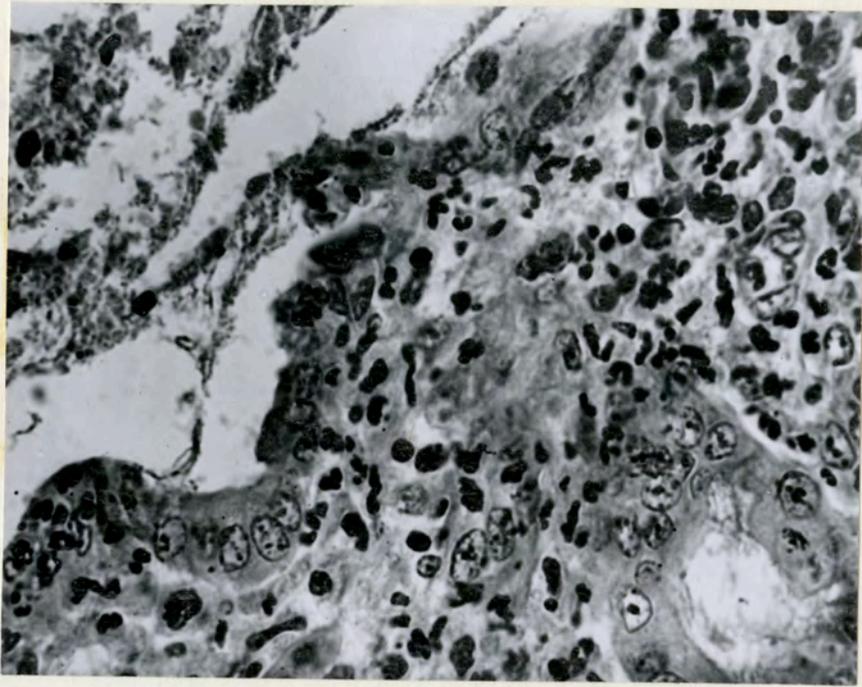


Plate 4 -

Section of the colon showing exudate in the lumen composed of cellular debris, mucus, and leukocytes. A severe leukocytic infiltration of the mucosa is also visible. Naturally occurring case. (H. & E.)

Plate 5 -

Section of the cecum showing epithelial desquamation and leukocytic infiltration of the mucosa. Fig 2A3. (H. & E.)

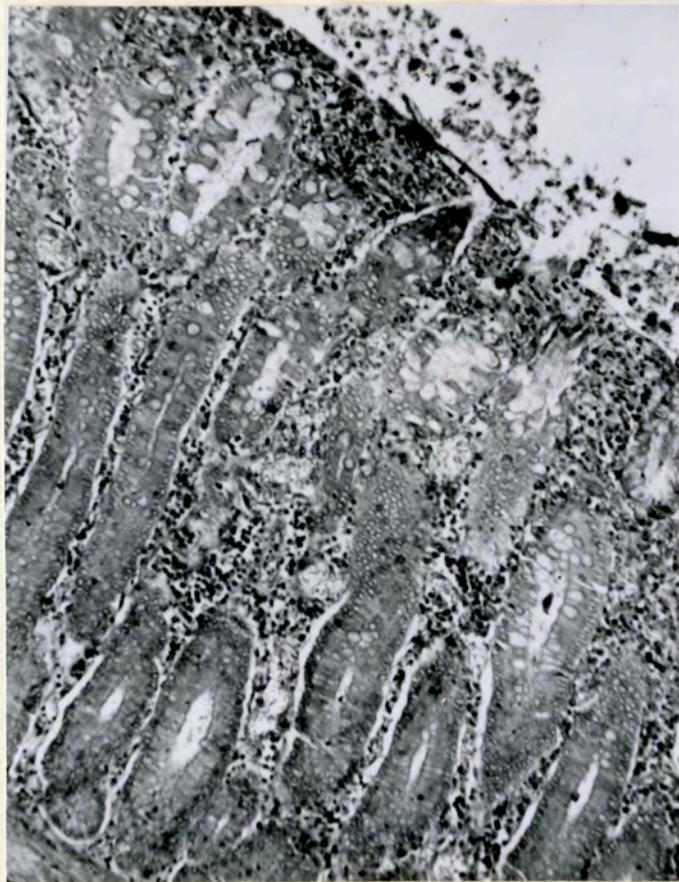
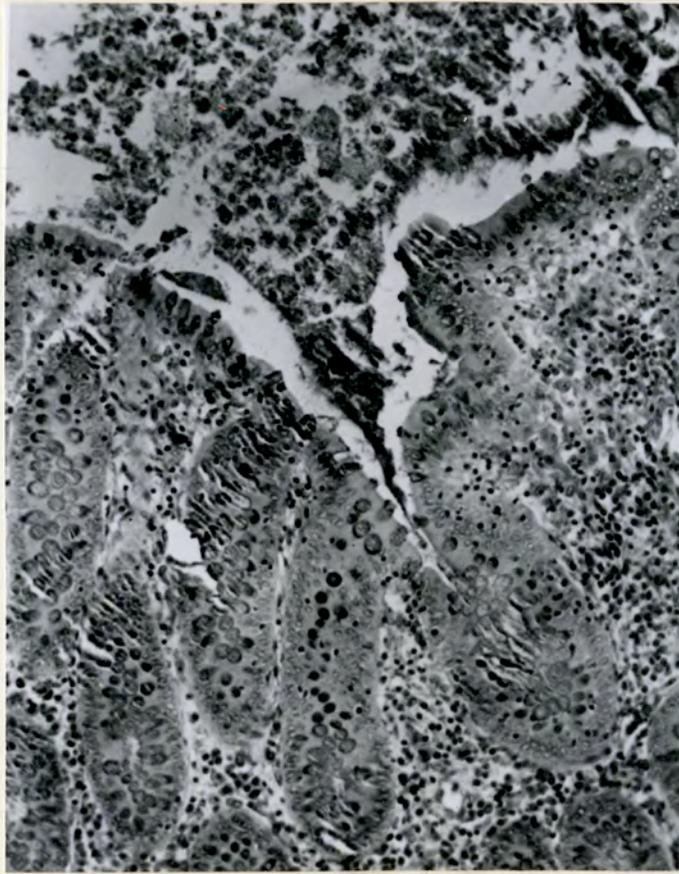


Plate 6 - Section of the cecum showing necrosis
and sloughing of the mucous membrane.
Naturally occurring case. (H. & E.)

Plate 7 - Coagulation necrosis of the superficial
portion of the mucosa. The necrotic
material is still more or less adherent
to the underlying tissue in which a zone
of hemorrhage and leukocytic infiltration
is visible. Naturally occurring case.
(H. & E.)

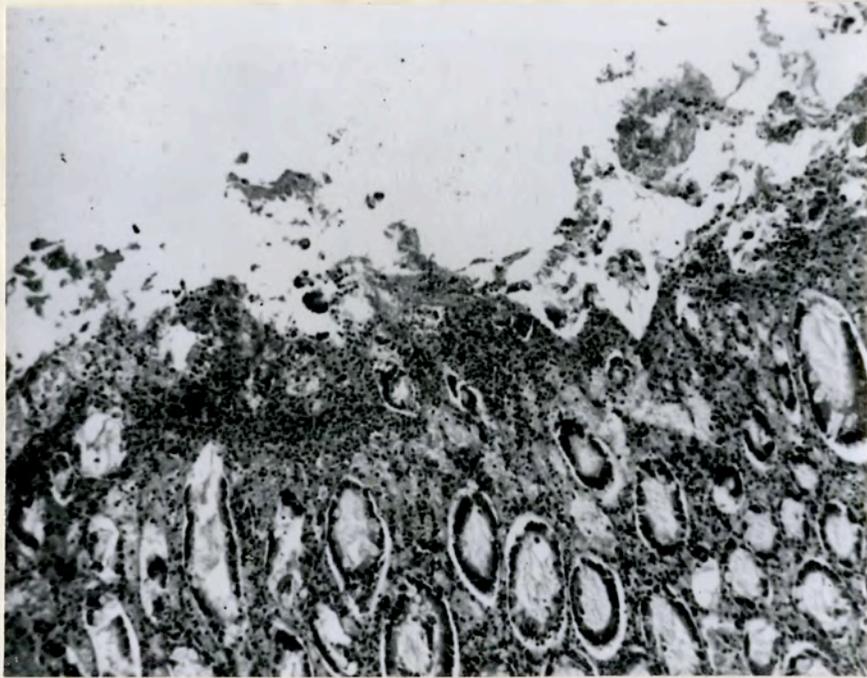


Plate 8 - Section of the colon in which the epithelium is not entirely gone; it is more or less in position but is pushed away by extravasated fluid from blood vessels and lymphatics. Fig 350R. (H.& E.)

Plate 9 - Dull V. coli colonies with a rough texture; 1 to 5 mm. in diameter. Not frequently encountered.



Plate 10 - Low convex V. coli colonies, bluish gray in color, having a tendency for elongating along the line of streak. Frequently encountered.

Plate 11 - Circular, bluish gray V. coli colonies, about 1 mm. in diameter. Not frequently encountered.

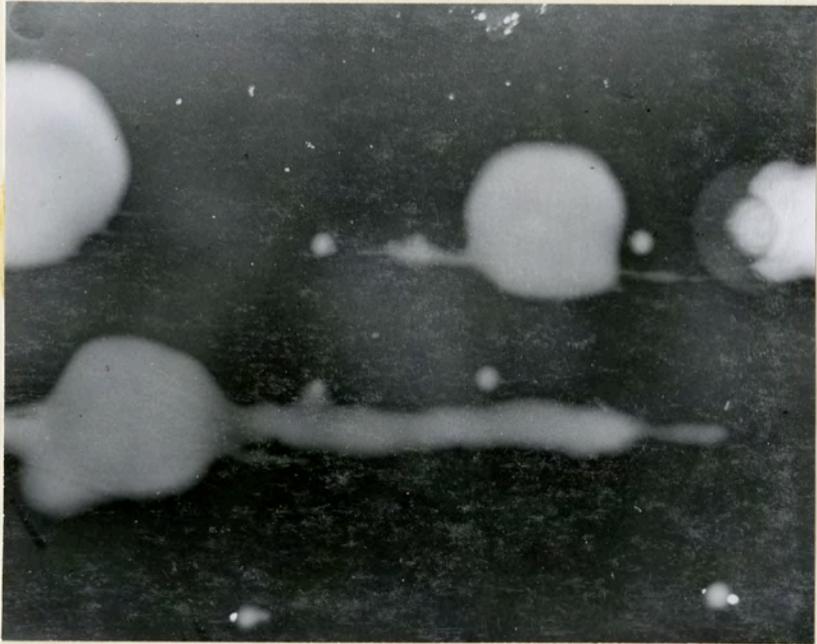


Plate 12 -

Necrotic inflammation - Necrotic patches have coalesced to form a diffuse necrotic membrane affecting a large portion of the colon. When these diphtheritic accumulations slough off, they give the feces the "rice water" appearance.

Plate 13 -

Small diverticula, 0.5 to 3.0 mm. in diameter sometimes visible on the serous coat of the colon and less frequently of the cecum. These diverticula, however, are not specific of Vibrionic Dysentery.



Plate 14 - Spirally shaped V. coli cells.

In the lower right corner is a V. coli
cell with bipolar flagella.

(Magnification: 14,300)

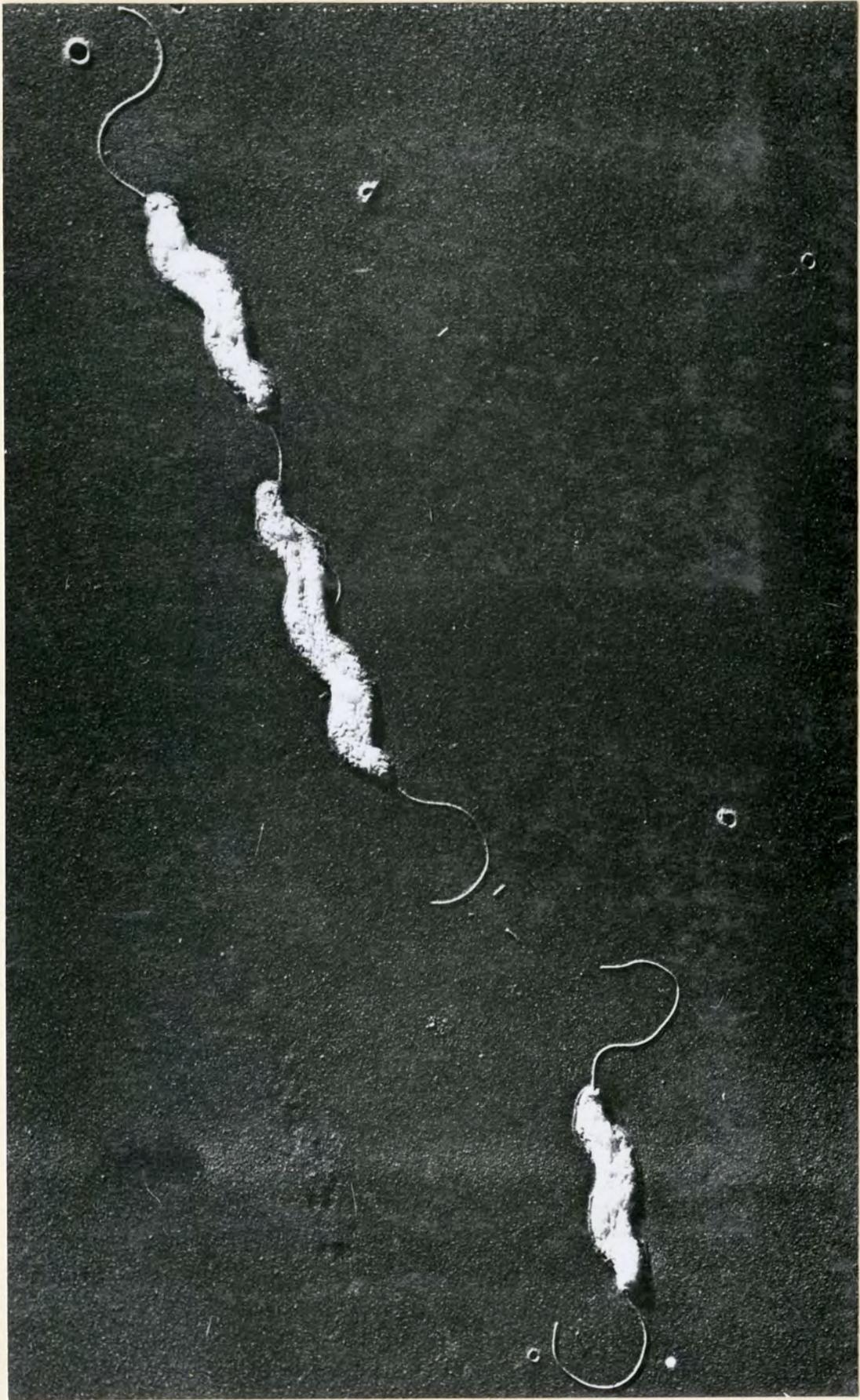


Plate 15 -

In the upper right corner, a comma-shaped V. coli cell. The other two V. coli cells are S-shaped. All the cells possess bipolar flagella and the cell in the lower left corner possesses two flagella at one pole.

(Magnification: 25,300).

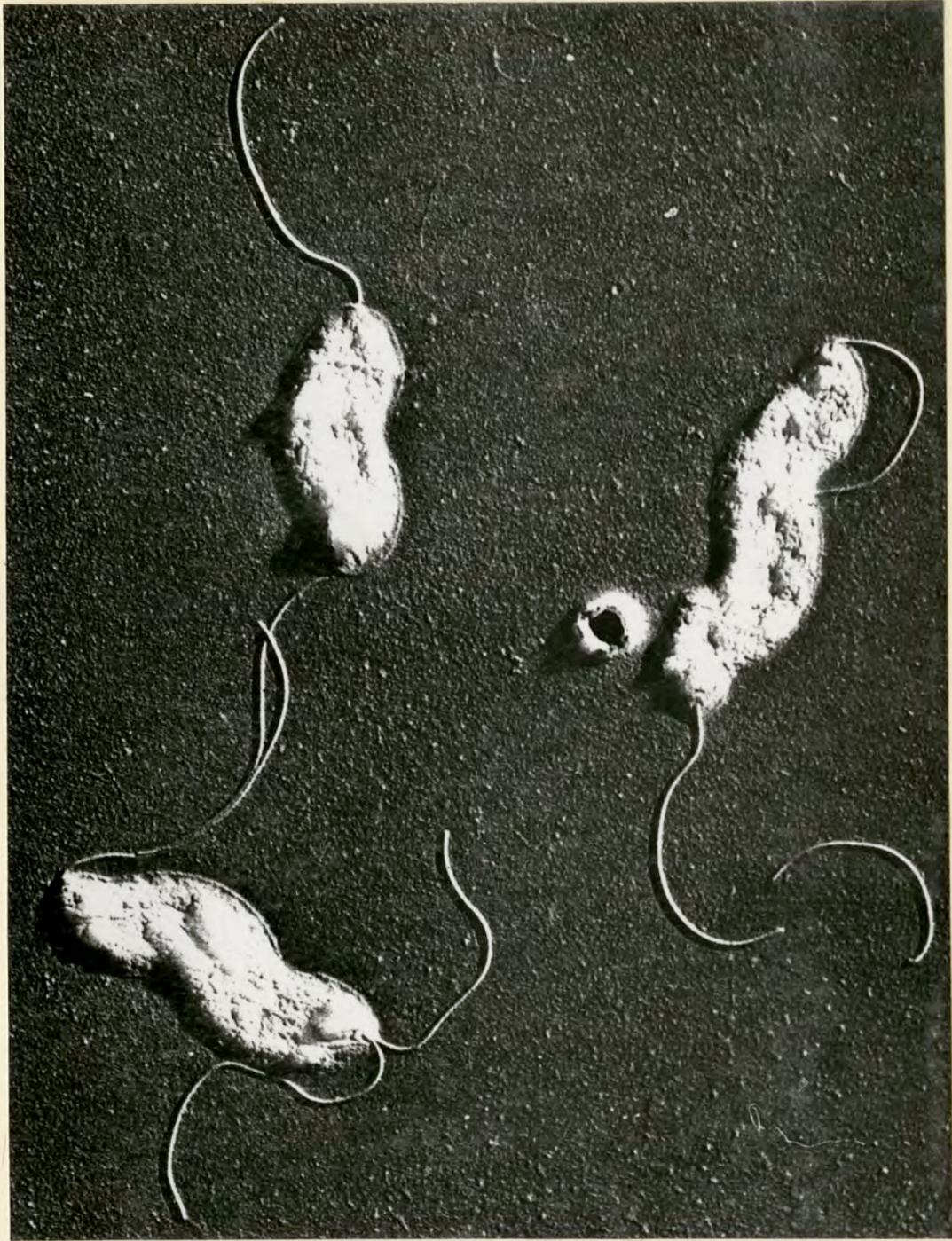


Plate 16 - Coccoid forms of V. coli cells with a
single flagellum.
(Magnification: 21,000).

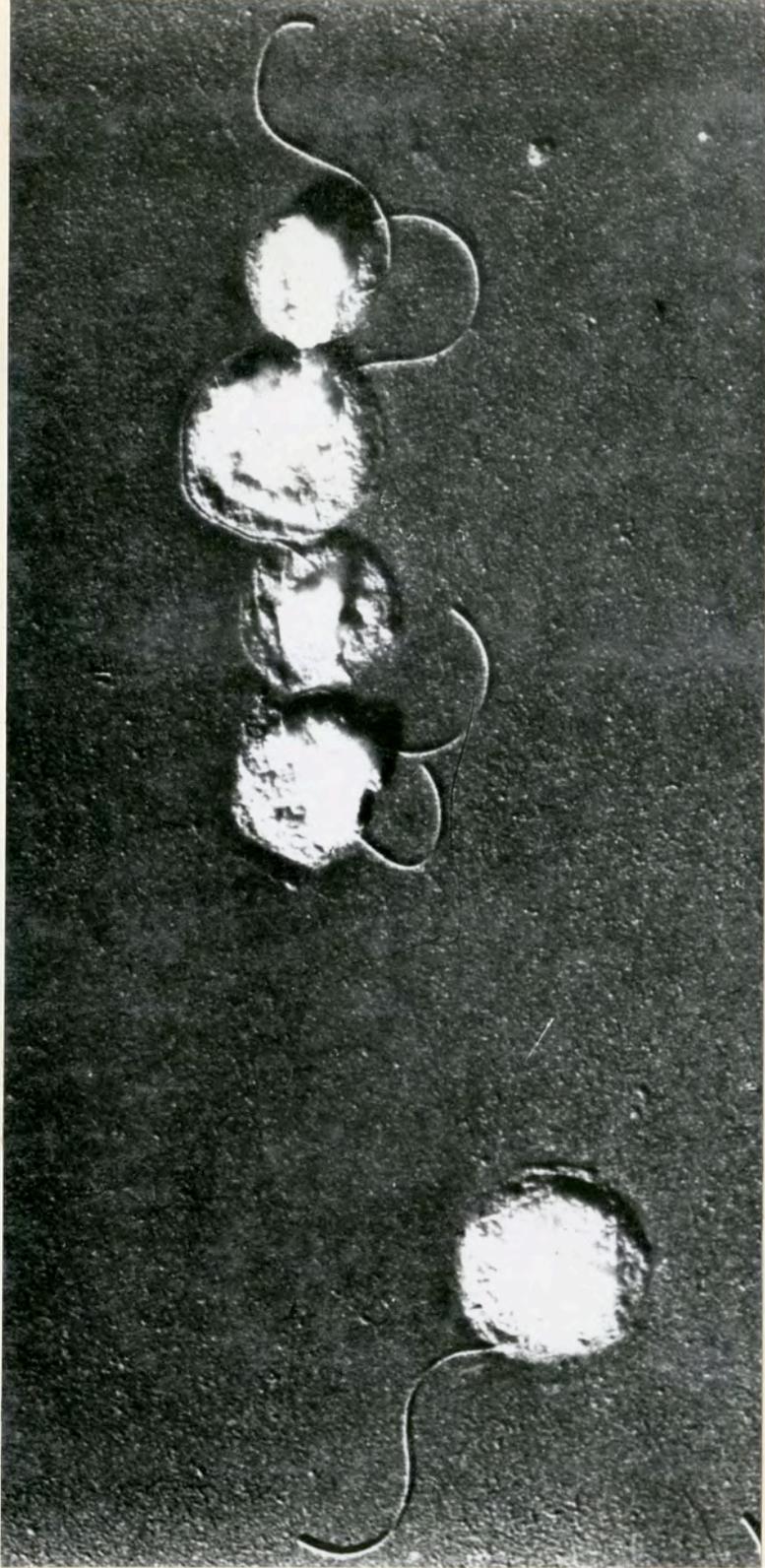
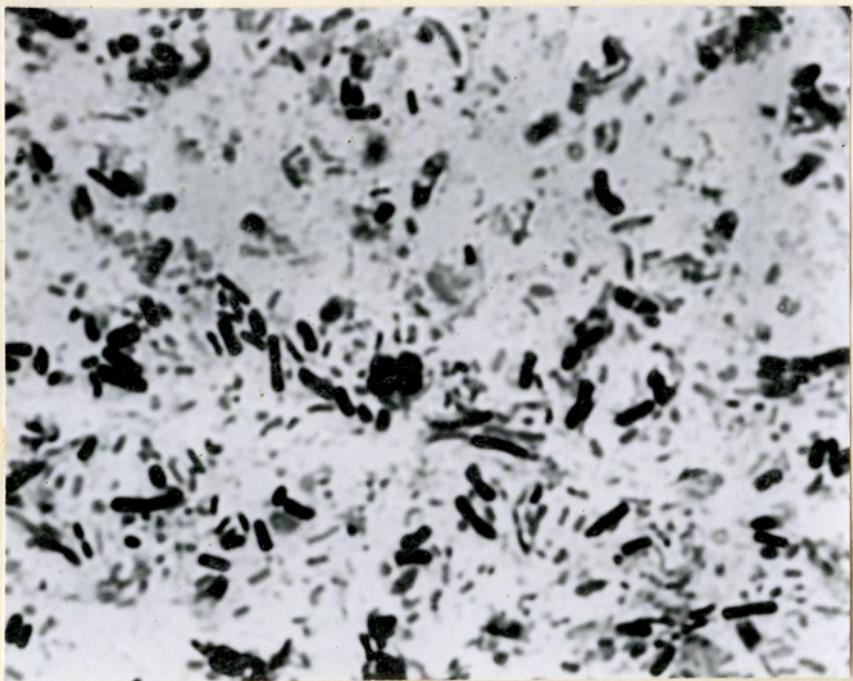
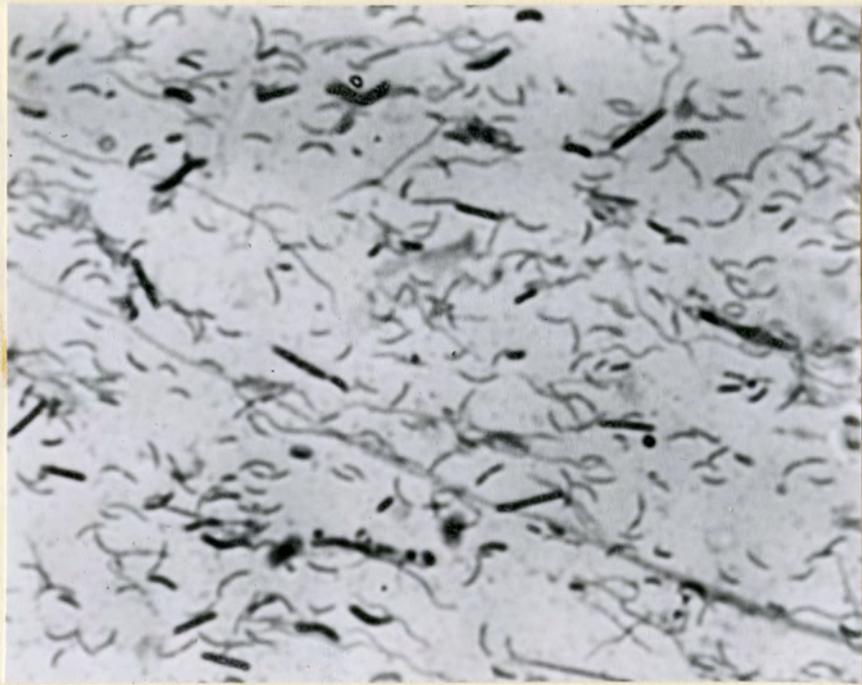


Plate 17 -

Smear made from the mucous membrane
of the colon of a pig affected with
Vibrionic Dysentery.

Plate 18 -

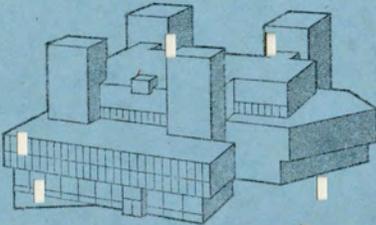
Smear made from the mucous membrane
of the colon of an apparently normal
pig.



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