

THE BACTERIOLOGY OF THE INTESTINAL TRACT OF  
YOUNG CALVES WITH SPECIAL REFERENCE TO  
THE EARLY DIARRHEA ("SCOURS").\*

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PLATE 8.

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The studies to be described in the following pages were made to obtain some information concerning the common occurrence of diarrhea or cholera of very young calves which frequently terminates in death during the 1st week of life. This disease, known as scours, *Kälberruhr*, *diarrhée des veaux*, prevails wherever calves are raised. It has been the subject of extensive investigations. Inasmuch as the scope and course of the present work is somewhat different from that of preceding investigations, a consideration of existing published work will be taken up later.

The results obtained recently in experiments with colostrum have in a way shaped the trend of present studies. Hence a brief restatement of these results will be necessary.<sup>1-3</sup>

It was shown that new-born calves fed on milk in place of colostrum with few exceptions succumbed within a few days to a generalized infection with *B. coli*. Cultures from spleen, liver, kidneys, and joints from animals killed during the disease and immediately autopsied showed an abundance of this organism. More rarely other intestinal forms such as enterococci and spore-bearing anaerobes were found in such cultures. Among the calves which received colostrum there were a few that became manifestly ill, probably as a result of receiving not

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\*In a study of the intestinal bacteria, the senior author was assisted, in 1917, by E. W. Smillie, in 1918-20, by Marian S. Taylor. Assistance in the clinical observation of the animals was given throughout by R. B. Little.

<sup>1</sup> Smith, T., and Little, R. B., *J. Exp. Med.*, 1922, xxxvi, 181.

<sup>2</sup> Smith, T., and Little, R. B., *J. Exp. Med.*, 1922, xxxvi, 453.

<sup>3</sup> Smith, T., and Little, R. B., *J. Exp. Med.*, 1923, xxxvii, 671.

enough colostrum. These partly recovered and developed in the 2nd week swellings of one or more joints, chiefly the tarsal. When such animals were killed and cultured the organs and joints were found to contain a small number of bacteria, chiefly *B. coli*. These were evidently held in check and gradually destroyed by the partial immunity furnished by the colostrum. This latent or partly suppressed microbism was also responsible for a peculiar nephritis leading to the well known spotted kidney.<sup>4</sup> The tissues and organs of calves sufficiently protected by colostrum or cow serum were found sterile even when large bits of tissue were transferred to culture media. The liver proved an occasional exception to this rule. A few colonies of *B. coli* developed around the bit of tissue. The close relation between the liver and the digestive tract through the portal circulation serves to account for this occasional escape of *B. coli*.

In spite of the protection against invasion afforded by colostrum, the digestive tract frequently fails to show a similar resistance and after the 1st or 2nd day liquid discharges begin which may cease after a few days, become chronic, or grow in severity until the calf succumbs, usually within the 1st or 2nd week.

It is not within the scope of this article to consider the various clinical manifestations of this early choleric disease. The present study was directed chiefly towards the bacteriological picture as it develops during the first days of life. The material studied consisted of five groups of calves, all from the same large herd: (a) an early normal group killed within 24 to 72 hours of birth; (b) a group about 2 days old evidently in an early stage of scours but as yet without definite symptoms; (c) calves having diarrhea and symptoms of toxemia; (d) calves examined immediately after natural death from scours or within 12 hours after the bodies had been chilled with a stream of cold water and refrigerated; (e) normal calves up to 3 or more weeks of age.

Although calves which died of scours were drawn into the investigation, emphasis was placed on those killed and promptly examined. The method regularly followed was to stun the calf with a blow and then bleed to death by severing the large vessels of the neck. The calf was then tied out and the skin reflected from the ventral surface. The abdomen was next opened and the stomachs ligated and removed. The intestines were then rapidly cut from the mesenteries and 6 inch portions 10 to 12 feet apart, beginning with the duodenum, removed and examined immediately or placed in a refrigerator for 1 or more hours. The serous surface of such segments was seared, the wall incised, and a loop introduced to remove material for microscopic examination, plating, etc.

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<sup>4</sup>Pfenninger, W., and Krupski, A., *Schweiz. Arch. Tierheilk.*, 1923, lxxv, 1.

In some cases a small amount of bouillon was introduced to make a suspension of the contents. Five or six segments were removed in this way for study. The cecum was tied off and reserved. Contents of the fourth stomach were obtained by ligating the pyloric portion. The condition of the mucosa at the different levels of the small intestine was ascertained by snipping off minute bits which brought away a small number of villi and examining these fresh. The presence of bacteria determined by this examination was confirmed by dried films of contents and scrapings stained according to Gram. At the same time plain and blood agar plate cultures were made with contents or scrapings at the five or six different levels of the small intestine, and with contents of the fourth stomach and the cecum.

It is impossible to predict within the first 24 or 48 hours what the subsequent course of a calf normal when killed would have been. On the 3rd day, however, a prognosis is more likely to be correct. After the 3rd day certain changes have taken place. The colostrum has given way to the ordinary milk diet. A reducing substance present in the urine at birth has disappeared. The peculiar "dropsical" epithelium of the lower small intestine found in many cases during the 3 first days is not seen thereafter.<sup>5</sup> Finally, the urine is now free from protein, or all but traces of it, provided a nephritis due to *Bacillus coli* is not under way.

Petechiæ and petechial hemorrhages almost regularly present in the mucosa of the fourth stomach at birth, and presumably due to mechanical causes during parturition, may play a part in the decline of the calf. In rare cases they are very numerous and when the bleeding persists for several days, the drain upon the calf's vitality is sufficient when combined with scours to kill the animal.

Omphalitis following infection of the umbilical stump with progression of the inflammation along the umbilical vein into the liver has been very rare in the cases studied as well as in the calves kept alive for longer periods. There has therefore been little to complicate the intestinal disease.

The invasion of the digestive tract by bacteria after birth has been the subject of a number of papers in the past. It has been possible in this investigation to examine the intestinal tract at different hours after birth in calves prematurely born and without food in the stom-

<sup>5</sup> Smith, T., *J. Exp. Med.*, 1925, xli, 81.

achs. In general the invasion is by the mouth rather than *per rectum*. Bacteria penetrate as far as the cecum within 12 hours in calves not suckling. To this point the meconium is liquid and favorable to a rapid diffusion of bacteria; below this it is consistent and much drier. Cultures made from various segments of the digestive tract in premature but living calves infected with *Bacillus abortus* have contained miscellaneous bacteria until the rectum was reached. The contents at this point still yielded pure cultures of *Bacillus abortus*.

*Group a.*—Data upon young calves when free from symptoms of scours or diarrhea have been gathered from cases killed from time to time since 1917. The meat of such animals was used for the preparation of culture media. The results were quite uniform and indicated a fairly stable flora. Although a number of different species may succeed in reaching the small intestine, they are not in evidence because so few in numbers unless special methods are used to reveal them. The bacteria that are significant are those that are capable of multiplying and appearing in sufficient numbers to be detected in fresh and stained films. Two species occupy the field in the normal calf, in the fourth stomach, *Bacillus acidophilus* and Gram-positive cocci, which will be called enterococci. The same species are found at the different levels of the small intestine until the cecum is reached. In the ileum *Bacillus coli* appears in small numbers in plates, rarely abundant enough to be recognized in films. It may be isolated by incubating in bouillon one or more loopfuls of scrapings or contents overnight and plating next day.

In general the bacterial content of the fourth stomach, aside from *Bacillus acidophilus*, is more varied than that of the small intestine, probably because it depends on the condition of the ingested food. In the duodenum it may be higher than in the next lower levels where it falls to a minimum. It gradually increases again towards the ileocecal valve. No approximate figures are available because the stage of intestinal digestion, or, in other words, the particular level where the chyme happens to be when the animal is killed, governs the number. The chyme carries with it living and dead bacteria whereas the empty intestine is relatively sterile. Bacteria when present are usually found in small groups or colonies, multiplying where opportunity presents. In some cases a loopful of contents of the ileum transferred

to agar failed to produce any growth. In others a few colonies of *Bacillus coli* appeared. Among the many cases more or less carefully studied the following may be cited.

*No. 1070.*—Holstein heifer calf, weighing 94 pounds when 1 day old. Killed at this time. Stomachs rather large. They contain about 2,600 cc. of a milky fluid in which are both yellowish and white curds. In the small intestine the mucosa feels velvety. "Dropsical" epithelium extensive in lower half and partially or slightly cephalad, and well into duodenum. Mucosa of large intestine normal. Contents, yellowish, pasty, rather doughy masses.

In films from six different levels of the small intestine the predominating forms are Gram-positive rods and cocci. In the three lowest segments a few forms resembling *B. coli* are present. In the cecum both Gram-positive and negative forms are present, the former most numerous. Plate cultures contained a few colonies of *B. coli* at all levels, with slight increase in the three last segments.

Cultures containing bits of tissue from spleen and kidneys remained sterile. In the liver tube growth appears only around the tissue, made up of *B. coli*.

*No. 1086.*—Holstein female. 2 days, 20 hours old. Weight 70½ pounds. Has not scoured since birth. Killed and autopsied. No abnormalities found. The urine taken from the bladder is slightly alkaline, free from proteins, and containing some reducing substance. The calf had received milk 4 to 5 hours before it was killed. The rumen contained about 500 cc., the fourth stomach 1,600 cc. of milky fluid with curds. The bacteriological examination showed about 10,000 Gram-negative rods, like *B. coli*, about 7,000 enterococci, and 200 *B. acidophilus* colonies per cc. of stomach contents.

The duodenum contained large numbers of enterococci, a moderate number of *B. acidophilus*, and a few *B. coli*. The 2nd segment (10 feet lower down) showed in films some enterococci and *B. acidophilus* and some Gram-negative rods. In the 3rd segment, enterococci and *B. acidophilus* scarce in films; in plates, enterococci are numerous, Gram-negative rods few. In the 4th segment, enterococci and *B. acidophilus* increased in the films; in plates, enterococci numerous, Gram-negative rods few. In the 5th segment (ileum), films show very few bacteria. In plates, colonies also not numerous. They are chiefly *B. coli*. In the cecum, both films and plates show large numbers of bacteria of the types found in the small intestine and some spore formers.

*Group b.*—In a certain number of calves during the 2nd day when normal conditions still prevail, *Bacillus coli* may be found in fairly large numbers in the ileum, and in certain cases in one or more segments higher up.

*No. 1085.*—This calf when 2½ days old began to scour but it still appeared normal and took its food. It was killed at this time. The urine from the bladder

was normal. *B. coli* made its appearance in the 4th segment of the small intestine and was abundant in the 5th and 6th (ileum) and in the cecum.

*No. 1032.*—Holstein bull calf, fed colostrum from a bottle until 1 day old. Killed when 2 days old. In the lowest levels of the small intestine there is some "dropsical" epithelium. Bacteria are present, both Gram-negative and positive, in small numbers only. Cultures were made from contents of the lowest three levels of the small intestine. There are very few bacteria present except in the lowest segment (ileum). The contents here contain about 9,000 *B. coli* per loopful. Spleen, liver, and kidneys sterile.

*Group c.*—When the feces had become fluid the multiplication of *Bacillus coli* in the lower segments of the small intestine was pronounced in all animals examined. The following cases, one from the 1917 group, the rest from the 1923 and 1924 groups, illustrate this change from the normal flora. All animals were killed and the digestive tract examined as soon as it could be removed from the body.

*No. 156.*—Holstein calf, born Nov. 6, 2.30 p.m. At 7.30 a.m., next day, the calf was found by attendant unable to stand and diarrhea starting. Killed at 10.30 a.m.; *i.e.*, when 27 hours old. Before death, liquid, yellowish feces passing from rectum. Fourth stomach more or less distended with colostrum milk. Mucosa covered with a delicate lacework of mucus. Contents contain *B. acidophilus* and enterococci, also cocci in chains. Contents of small intestine yellowish. In the lowest segments a distinct odor of decomposition noticed. In the upper third, the villi are slightly club-shaped and the central lymph sinus distended with fat. The epithelium of the mucosa from the duodenum down is hydropic. In the upper segments, the fluid is in small drops 2 $\mu$  in diameter. These grow larger downwards and in the lowest third they vary from 8 to 20 $\mu$ . Bacilli of the type of *B. coli* increase from above downward and are present in large masses in the lowest third (Fig. 1).

*No. 1084.*—Born Dec. 11, 3 a.m., killed Dec. 13, 9.25 a.m., hence 2 $\frac{1}{2}$  days old when killed. The calf began to scour when about 31 hours old. It became weak and unable to stand within 4 hours. Stools continued fluid until the calf was killed.

The autopsy showed that the umbilical cord had been broken off close to the body, leaving a moist scab over the wound. The fourth stomach contains about 800 cc. of milky fluid in which are curdy masses stained pinkish. The mucosa is covered with a thick, viscid, closely adherent layer, having the consistency and character of mucus. Many petechial hemorrhages in the mucosa under this layer. The small intestine is congested irregularly in spots and the congestion tends to become more pronounced downwards. In the large intestine the congestion is in longitudinal lines, occupying the summits of small folds or ridges. The rectal mucosa is uniformly congested. Nothing noteworthy concerning the gross appearance of the remaining viscera.

There were examined films from contents of the fourth stomach, from six different levels of the small intestine, and from the cecum. In the stomach contents are Gram-positive rods and cocci and a few Gram-negative rods. In the duodenum are many Gram-positive rods and cocci and a few Gram-negative rods. From the 2nd to the 5th segments, the Gram-negative rods gradually increase in numbers while the others are present in small numbers. In the ileum all three kinds are increased, especially the Gram-negative forms. In the cecum all three types are numerous. Plate cultures show a large increase of *B. coli* from the 2nd segment to and including the ileum.

*No. 1097.*—Small Guernsey bull calf, weighing 48½ pounds. Killed when 2½ days old. Calf was with dam about 5 hours. It was in normal condition until 44 hours old. It had probably begun to scour during the night and was found very weak at this time. The feces were fluid, yellowish. Eyes sunken. Temperature 38.9°C. As it was evidently going to die during the coming night, it was killed and autopsied.

The fourth stomach contains only a little milky fluid and some curds. The small intestine is congested in lines, bands, and patches throughout. The large intestine is similarly congested. The central one-half of the liver lobule contains much orange pigment in fine granules and the entire lobule more or less fat. The medulla of kidneys deeply congested. The acidified and boiled urine from the bladder contains about 1/20 column of coagulable protein (0.05 per cent Esbach).

Bacteria scarce in the upper segments of the small intestine. Below the middle *B. coli* very abundant and forming a film of rods over the villi. In the ileum approximately 4 billion bacteria per cc. in a turbid suspension of contents in bouillon.

In the following case, there is evidence from the protein output in the urine that the calf obtained a large amount of colostrum without resisting *Bacillus coli* invasion of the small intestine.

*No. 1069.*—Guernsey bull, born Oct. 14. Developed scours next day. On the 2nd day, it was brought to the Department nearly dead and killed. The rumen and fourth stomach contained a yellowish milk-like fluid and some partly digested curds. Mucosa of small intestine congested throughout. A yellowish material resembling yolk of eggs contained in loops at different levels. Bacteria resembling *B. coli* very abundant in the lower four-sixths. They form thin films or skins over the villi. In the lower half there is also much "dropsical" epithelium. The large intestine is deeply congested throughout. The congested areas partly hemorrhagic. Medulla of kidneys deeply congested. Urine taken from bladder (45 to 60 minutes after killing the animal) becomes solid when acidified and boiled. After diluting with three volumes normal salt solution, the Esbach precipitate still registers 0.85 per cent protein. Cultures containing

bits of kidney and spleen remain sterile. A similar culture of the liver develops several colonies of *B. coli*.

*No. 1071.*—Calf born Oct. 23, killed Oct. 25, between 2 and 2½ days old. Evidences of diarrhœa on the 2nd day of life. Temperature 39.2°C. Weight 77 pounds. The fourth stomach contained about 500 cc. of a yellowish, milky fluid plus rather soft, yellow curds, 2 to 3 cm. in diameter. The mucosa covered with a thin layer of mucus. Minute faded petechiæ beneath it. In the upper two-fifths of the small intestine a little yellowish fluid. Mucosa stippled whitish. Lacteals under serosa appear as white lines. The large intestine contains much opaque fluid, stained yellow. Mucosa normal, except for a congestion near anus. The filtered fluid obtained from contents of colon contains coagulable protein (about 25 per cent of column after acidifying, filtering, and boiling). Moderate congestion of medulla of kidneys. Urine taken from bladder contains 0.18 per cent protein (Esbach). Bits of spleen and kidney in cultures remain sterile. An agar slant plus a bit of liver tissue develops three colonies of *B. coli*.

Microscopic and bacteriological examinations of the small intestine and cecum show a steady increase of *B. coli* from the duodenum down. From the middle of the small intestine on, *B. coli* appears in large numbers in films, covering villi with a continuous layer of rods. *B. coli* colonies are very numerous on the plates; the Gram-positive forms, most abundant in the fourth stomach, diminish downwards. In the cecum, *B. coli* predominates.

*No. 1072.*—Holstein bull calf, born Oct. 27. Killed Oct. 29, 44 hours old. This case resembles No. 1071. Scours began the day before it was killed. The stomachs are well filled with milk and curds, both white and yellowish. The mucosa covered with a thick layer of translucent mucus. Slight and increasing congestion of mucosa of small intestine from above downward. Medulla of kidneys dark red. Urine taken from bladder heavily loaded with coagulable protein (Esbach 0.2 per cent). In this case, *B. coli* was abundant in the ileum and less so in the segment above. Numerous colonies of *B. coli* developed from plates of cecal contents.

*No. 1073.*—Between 2 and 2½ days old when killed. Scouring began during the 1st day and the attendant gave formaldehyde by the mouth. On the 2nd day the animal was very weak, unable to stand. It was then brought to the Department and killed. The fourth stomach contains no colostral or milk rests. The mucosa is covered with a thick layer of mucus. The small intestine contains no food. Congestion of mucosa slight. The medulla of kidneys deeply congested. Urine taken from the bladder heavily loaded with coagulable protein. Urine diluted with two volumes of normal salt solution gives an Esbach precipitate of 0.4 per cent.

In this case *B. coli* was abundant throughout the small intestine but especially so in the ileum. Two types of colonies were present, an opaque and a thinner, partly translucent type. The former greatly predominated.

Although the autopsy indicated that the calf had never suckled, the sterility of spleen, liver, and kidneys, and the proteinuria are opposed to this inference.



*No. 1074.*—This calf was just 4 days old when killed. Scouring began when the calf was 2 days old. When 4 days old it was still very sick and not supposed to survive. The fourth stomach shows superficial erosions following the early hemorrhages. The small intestine is moderately congested, similarly the kidneys. Urine from the bladder contains considerable protein (Esbach 0.2 per cent). Cultures and films from the fourth stomach and different levels of the small intestine show a fair number of Gram-positive rods in the fourth stomach and duodenum, while the segment below the latter is practically sterile. The three remaining segments contain, besides a small number of Gram-positive rods and cocci, an increasing number of *B. coli*. The spleen, liver, and kidneys were sterile.

*No. 1083.*—Calf killed when nearly 4 days old. Early history not definitely known. The attendant stated that scouring began on the 3rd day. The stomachs contained in all about 1,350 cc. of milky fluid and curds, both yellow and white. The mucous membrane of the fourth stomach covered with a layer of tenacious mucus. The small and large intestines congested in streaks. The contents are fluid and yellowish in color. Kidneys slightly congested. Urine from the bladder contains considerable coagulable protein (Esbach 0.15 per cent).

In the small intestine, *B. coli* appears in considerable numbers in the 3rd segment and grows more abundant in the 4th and 5th segments (ileum). Similarly the contents of cecum contain many *B. coli*. The tube containing a bit of liver tissue contains *B. coli*. Spleen and kidney cultures sterile.

*No. 1089.*—A large Holstein bull calf began to scour when a day old. The attendant treated it with formaldehyde. It was still with the dam when 3 days old. Temperature 40.1°C. Taken to the Department and killed at this time. Attached to the caudal margin of the left lobe of liver is a multilocular cyst with very thin, transparent walls. Each element of the cyst is about hen's egg in size and distended with a thin, blood-tinted fluid, which in all amounted to about 500 cc. There is a slight, patchy and streaked congestion of the lower half of the small intestine and rectum. Both stomachs well filled with milk and curds, about 2 liters in all.

In this case a few *B. coli* were in the 3rd segment of the small intestine. They were more numerous in the 4th and 5th and very abundant in the 6th (ileum).

Calves killed in the course of scours after the 3rd day present the same intestinal flora as those killed when 1 or 2 days old.

*No. 1078.*—Bull calf, weighing 90 pounds, killed when 5 days old. It was kept with the dam for about 12 hours and then transferred to a Department unit and fed ordinary milk. Up to 4 days of age, the calf appeared normal as to its digestive functions, then its temperature rose slightly above normal and it partly refused food. On the 5th day, feces became fluid. The calf became very weak and was killed. The autopsy shows congestion of the mucosa of stomach, small and large intestines, especially the last named. Urine taken from the blad-

der contains only a trace of protein. *B. coli* invasion was marked in the ileum and adjoining (4th) segment. Otherwise Gram-positive rods abundant throughout. Cultures from spleen, liver, and kidneys sterile.

*No. 1075.*—Twin calf killed when 8 days old. Both twins began to scour on the 2nd day. One died when 7 days old. In this animal there was irregular, increasing congestion of the small intestine downward. The large intestine was similarly congested. The urine taken from bladder contained a small amount of coagulable protein. Gram-positive rods and cocci are present in relatively large numbers in the upper portion of the small intestine and diminish downwards. *B. coli* type, present in small numbers in the duodenal level, increase steadily towards the cecum, where they are quite numerous. The spleen, liver, and kidney cultures remained sterile.

*Group d.*—Calves dying of scours, when promptly chilled and refrigerated, if the organs cannot be removed immediately, show the same conditions as in those killed. The small intestines are flooded with *Bacillus coli*, in part only the result of postmortem multiplication, as it had been noted from observations on calves killed moribund or examined immediately after natural death that the multiplication probably goes on at a very rapid pace in the hours before death.

*No. 1094.*—Holstein heifer calf, weighing 80 pounds. Born Feb. 20, at noon, died Feb. 24, 9.40 a.m. This calf remained with the dam, taking colostrum for 4 hours. Thereafter it was segregated and fed freshly drawn cow's milk, at first from the bottle. A sample of urine collected 18 hours after birth contained about 0.15 per cent protein (Esbach). Diarrhea set in when the calf was 24 hours old. Signs of weakness came on 20 hours later. The feces were fluid and yellowish to greenish in color. On the 3rd day the calf was somewhat stronger, able to stand up and move about in the stall. The feces were soft, yellowish. Temperature 39.6°C. Respirations very rapid. On the 4th day early the calf was very weak and refused to take milk. 20 minutes later it was found dead. It was immediately cooled off with a stream of water and placed in a refrigerator slightly above 0°C.

The autopsy was made after 24 hours. The fourth stomach contains about 500 cc. of a thick, milk-soup-like fluid with a few small lumps of casein. Mucosa overlaid with a translucent, mucoid film, easily rubbed off. Beneath, the mucosa is congested. On the pyloric valve an ulcer 8 by 3 mm. in diameter. Small intestine congested throughout in streaks and patches. Peyer's patches, slightly swollen, dark red. Mucosa covered with a thin, creamy, pinkish film of desquamated epithelium. Microscopic examination of scrapings of mucosa at different levels indicates a fatty degeneration of epithelium, usually in patches, increasing in extent downwards. The contained fat from fine granulations to spheres 10 $\mu$ . in diameter. This condition most prominent in ileum. Mucosa

of large intestine congested in longitudinal streaks to rectum where the congestion is continuous.

The liver cells in the central zone of the lobules contain large amounts of orange pigment. Very little fat present. The medulla of kidneys congested to the tips of papillæ. Urine taken from the bladder contains about 0.06 per cent protein (Esbach).

Microscopic examination and cultures of the contents of the small intestine show, besides the usual Gram-positive rods and cocci, large numbers of *B. coli* from duodenum down, most abundant in the ileum. Cultures of bits of liver, spleen, kidneys, as large as large peas, remain sterile.

*Group e. Older Normal Calves.*—Calves which had maintained normal conditions of the intestines were examined from time to time with reference to the intestinal flora. In all cases, *Bacillus coli* was either absent from the plates or else present in very small numbers.

*No. 988.*—Fed cow serum at birth in place of colostrum. The calf maintained a normal condition until 12 days old when it was slaughtered and organs found normal. Films from the mucosa of the lower jejunum and upper ileum were found free from bacteria. One agar slant inoculated directly with a loop of contents of the ileum contained but two colonies after 6 days. The same was true of tubes inoculated with 3 and 6 drops, respectively, of a turbid bouillon suspension of ileum scrapings. Among the colonies were those of *B. coli*.

*No. 1101.*—Born Apr. 1, killed Apr. 29. Holstein bull calf, weighing at birth 92 pounds. This calf remained with the dam for 2 days. It was then placed in a stall by itself and fed fresh cow's milk three times a day. Up to the day of slaughter there had been no digestive disturbances. Its weight, Apr. 26, was 112 pounds. The autopsy brought out no abnormalities. In the contents of the fourth stomach were many Gram-positive rods and some cocci. In the duodenum and following segment were small numbers of the same type. In the 3rd segment bacteria were very scarce. In the 4th and 5th, the Gram-positive rods and cocci were as in the duodenum. In the ileum there was an increase. Plate cultures from this level showed large numbers of colonies of *B. acidophilus* and a small number of *B. coli*. In the cecum, plate cultures of material containing large numbers of bacteria brought out relatively few *B. coli* and many *B. acidophilus* colonies. The type of *B. coli* was identical with that found in very young normal and diseased calves.

*No. 1065.*—Guernsey bull calf born Sept. 6 and kept until Nov. 20, when it was slaughtered. During this period the calf remained normal. At the autopsy no abnormalities were found. The bacteriological examination of the fourth stomach, six different, equally spaced levels of the small intestine, and of the contents of the cecum showed Gram-positive rods and cocci in films and cultures. *B. coli* was absent or else present in such small numbers that it failed to appear.

No. 1067.—Large Holstein bull calf, born Sept. 24, and killed Oct. 17. During this period there were no digestive disturbances. The autopsy showed no abnormalities. Bacteriological examination brought out the same results as in No. 1065. *B. coli* did not appear on any plates, but by incubating a loopful of intestinal contents from the 5th segment in bouillon overnight, *B. coli* was isolated.

Sections of fixed and hardened tissue of the unopened small intestine from five to six different levels of a number of calves, killed when 3 weeks to 2 months of age, have also been examined. These calves were suffering from pneumonia involving over one-half of the lung tissue. In none of the sections were bacteria demonstrable in the lower segments.

#### DISCUSSION.

The significance of *Bacillus coli* in the pathology of the intestinal tract may now be briefly formulated. The relation of this species to the normal tract has already been outlined, and it is evident from what has been stated that a comparison of the normal and the pathologic state in terms of *Bacillus coli* involves a quantitative factor. *Bacillus coli* is probably present in all levels of the small intestine in the normal animal, but frequently in such small numbers that only incubation of contents in bouillon overnight brings it to light. In all cases of intestinal disturbance *Bacillus coli* is present in the lower small intestine in larger numbers than in health, and as the disease progresses the numbers of living bacilli not only increase locally but the infection spreads towards the duodenum. In calves killed in an advanced stage the mucosa is carpeted with layers of *Bacillus coli* attached to the top plates of the epithelial cells (Fig. 1). This carpeting may extend from the ileocecal valve to the second fifth level, *i.e.* nearly into the duodenum, before death. Animals have been found still in good condition, usually during the second 24 hours of life, with a heavy infection of the 4th and 5th segments of the small intestine with *Bacillus coli*. These are interpreted as preclinical cases of scours.

The action of *Bacillus coli* in bringing about the local hyperemia and flux and the general intoxication is probably a result of the absorption of toxins produced and set free during multiplication. This phase of the etiological conditions is still under investigation.

Tracing the conditions back we naturally come to the problem as to the forces which hold *Bacillus coli* in check, for it is obvious that we are dealing here with an equilibrium quite delicate during the early days of life between *Bacillus coli* and certain inhibitory agencies residing in the normal functions of the entire tract. Are the multiplication and consequent intoxication due to an inadequate functioning of the mucosa and associated glands, or to an unusual virulence of the invading *Bacillus coli*? In individual cases it is readily conceivable that a failure of the peptic stomach and its secretions in the early hours of life may give *Bacillus coli* the necessary opportunity. It is, however, to be inferred, from the large number of cases which have been studied since 1917, that to explain the persistent epidemics and numerous sporadic cases of scours the genesis of scours as a herd disease is the result of both inadequate digestive functions as well as increasing virulence of *Bacillus coli*. These two factors reciprocally favor each other in the sense that the depression of normal functions favors multiplication and rapid passages from case to case, and consequent rise in virulence, where animals are numerous. Experimental evidence in favor of this conception of scours may be presented in a later publication. At present we are relying more on a comparative study of spontaneous cases in formulating the above hypothesis.

Among the conditions leading to an inhibition of normal protective functions a failure of the fourth stomach as stated above is of importance. Frequently, advanced and moribund cases show a coating of mucus, not present in the normal calf. This suggests a fetal condition of relative inactivity. Attention has been called to a peculiar, dropsical condition of the intestinal epithelium in many cases, seen only during the 3 first days, and also of fetal origin.<sup>5</sup> Its relation to scours is undetermined. Fat stasis has also been mentioned as occurring in the upper half of the small intestine.

In the early days another factor may be of importance. This is the overdistension of the fourth stomach with colostrum milk. Carpenter and Woods<sup>6</sup> have recently referred to this factor. At the autopsy of young calves the great variation in the contents of the fourth stomach has frequently been noticed. In some cases this or-

<sup>5</sup> Carpenter, C. M., and Woods, G., *Cornell Vet.*, 1924, xiv, 218.

gan extended down to the pelvis. Attention has also been called to the large amount of coagulable protein in the contents of the ileum. This passing into the large intestine becomes the white scours. Whether this undigested colostrum is still protective, or whether it acts as a culture medium for *Bacillus coli* and other bacteria, is not known. Large quantities of colostrum have been fed experimentally without manifest injury.<sup>7</sup>

In the apparent overfeeding of colostrum there is concealed another factor, the time after birth when the first food is taken. The overdistension may be due in some cases to a delayed feeding which stimulates the calf to overfeed. This delay may also be significant in permitting *Bacillus coli* to get a foothold in the small intestine in spite of the large, but delayed dose of colostrum. The cases reported were not under observation at birth and hence no information is at hand in evaluating this delay as favoring scours.

The relation of virulence of *Bacillus coli* to scours is under investigation and only certain data gathered in studying normal and pathological calves can be cited at this time. To measure virulence of *Bacillus coli* is made difficult by a number of facts. The group known as *Bacillus coli* is ubiquitous and the mere presence of *Bacillus coli* in minimal numbers is of no immediate significance. There are in the digestive tract of domestic mammals and birds many varieties of *Bacillus coli* and changes go on under cultivation. Moreover, calves quickly acquire a high degree of resistance to *Bacillus coli* entering the digestive tract after the 1st week. In spite of these difficulties the purely bacteriological studies of isolated races of *Bacillus coli* furnish some evidence of value.

In the early cases studied, beginning in the winter of 1917, the dominant race of *Bacillus coli* was saccharolytic and motile. Later this type was supplanted by one which is non-motile and fails to act upon saccharose. This type has maintained itself up to the present, although other races may be isolated from intestinal contents if special methods are used. In the intestinal tract and organs of a young calf which was sent to this Department from a distant herd recently, a saccharolytic, motile race was the only one isolated. This could be readily distin-

<sup>7</sup>Smith, T., and Little, R. B., *J. Exp. Med.*, 1924, xxxix, 303.

guished from the local type in certain peculiarities of its colonies on agar plates.

In general the races of *Bacillus coli* found in practically pure cultures in the organs of cases of *Bacillus coli* septicemia and in large numbers in the intestinal tract of ordinary scours had in common certain characters, such as a moist, rich growth, forming round, convex colonies which sometimes tend to flow down the agar slope.

If we put all the facts together, the inference seems admissible that scours is associated with the multiplication of special races of *Bacillus coli* and that such races are developed and maintained in large herds. This will account for the different races of *Bacillus coli* which have been charged by other observers as the cause of scours. Each large herd through the presence of calves below par at birth may thus develop and maintain its own type of scours organism which, however, is not virulent enough to make any headway in naturally strong calves properly cared for as regards food and housing.

During the period from 1917 on during which scours was being observed, the investigations were restricted to one large herd of cows. Accessions to this herd from outside were frequently made, however. During the latter half of 1918, four animals came to autopsy from which a paratyphoid bacillus was isolated. Two of these were a trifle over 4 and 5 months old, respectively. The other two were young calves. One died when 9 days old, the other was killed at the same age. It is only this latter pair which comes within the scours period. This infectious agent did not get a foothold in the herd and no cases were encountered after this. Outbreaks due to paratyphoid bacilli should be clearly distinguished from the early scours since the nature of the disease and the lesions observed are different. Such distinction is called for in the interests of a simpler etiological classification and a better understanding of the early disease.

In the course of these studies, it has become evident that *Bacillus coli* is not disseminated from the digestive tract after death to appear in the internal organs. The presence of *Bacillus coli* in the spleen, kidneys, and liver is the result of antemortem dissemination. There may, however, be focal multiplication, post mortem, which increases greatly the number of bacteria originally present, unless the body is promptly chilled and refrigerated. Even under these conditions local

multiplication goes on for a time until the body is chilled throughout. It is thus possible to distinguish between scours with and without bacteremia in calves autopsied some time after natural death.

A large amount of work has already been done on this ubiquitous disease, notably by Jensen, Poels, Titze and Weichel, and others dealing with small groups of cases. This work has been summarized up to 1911 by Jensen.<sup>8</sup> It will be seen from his review that this affection had been ascribed to *B. coli*, *B. proteus*, *B. pyocyaneus*, streptococci, and variants of these species. Jensen's own investigations have shown that forms of *B. coli* are the dominant organisms in this disease. He divides the disease into enteritis with and without bacteremia, thus covering the subsequent classification of the writer and associates into calves which have been inadequately or not at all protected by colostrum and those which have been so protected. Jensen was able to produce a fatal hemorrhagic enteritis in young calves by feeding a small dose of creolin, pyocyanin, or iodine trichloride in the milk. In such animals *B. coli* was found in the internal organs after death. Jensen also called attention to the fact that feeding calves during the first 24 hours of life with boiled milk brought about severe, often bloody diarrhea and death. He infers that normal types of *B. coli* may gain virulence under these conditions. It is not clear whether his hypothesis postulates an immediate increase in virulence in every case showing enteritis, or whether a gradual increase in virulence through repeated passages associated with a certain fixation of these characters is meant. His work has been extended and confirmed by Christiansen.<sup>9-11</sup>

Titze and Weichel,<sup>12</sup> working chiefly with cultures sent to the laboratory from different outbreaks of scours, found that of 210 strains, 160 belonged to *B. coli*, 24 to *B. enteritidis*, 2 to paratyphoid B, and the rest to miscellaneous species. Stickdorn<sup>13</sup> studied 118 cultures of *B. coli* stated to have been isolated from scours cases. Of these, only 23 fermented saccharose. Further divisions were made when other sugars and alcohols were used. In these investigations it is not clear whether the authors' cultures were isolated from the internal organs or from the small intestine. It may be assumed that they were isolated from the blood and internal organs, since *B. coli* in the digestive tract has not been in general regarded as significant. This significance appears when the quantitative factor, obtained before natural death, is determined. The articles quoted do not take

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<sup>8</sup> Jensen, C. O., in Kolle, W., and von Wassermann, A., *Handbuch der pathogenen Mikroorganismen*, Jena, 2nd edition, 1913, vi, 121.

<sup>9</sup> Christiansen, M., *Maanedsskr. Dyrlæger*, 1915, xxvii.

<sup>10</sup> Christiansen, M., *Centr. Bakt., 1. Abt., Orig.*, 1917, lxxix, 196.

<sup>11</sup> Christiansen, M., *Maanedsskr. Dyrlæger*, 1917, xxix.

<sup>12</sup> Titze, C., and Weichel, A., *Arb. k. Gsundtsamte*, 1909-10, xxxiii, 516.

<sup>13</sup> Stickdorn, W., *Centr. Bakt., 1. Abt., Orig.*, 1915, lxxvi, 245.



this into consideration. It is obvious from the results reported in this and earlier papers that a distinction must be made between *B. coli* invading the small intestine only and the entire body as well.

Küthe<sup>14</sup> examined different sections of the digestive tract of calves obtained from abattoirs. He finds *B. coli*, *B. bifidus*, and *B. acidophilus* as normal flora. Carpenter and Woods<sup>8</sup> studied the bacteriology of the digestive tract of fourteen normal calves from 1 to 98 days of age and of thirteen diseased calves from 2 to 67 days old. The series is too broad with reference to age and there are too few animals of the same scours-susceptible age to permit definite conclusions on the distribution of bacteria in the digestive tract. The inferences drawn by the writers differ from those of Carpenter and Woods, who state that the bacteria "for the first 48 hours seem to have little difficulty." In the opinion of the writers the first 48 hours are the critical period. If the colon group multiplies freely during this period the calf is doomed.

#### CONCLUSIONS.

New-born calves receiving no colostrum, or else receiving it after some delay, may die of *Bacillus coli* septicemia, of which scours is a local manifestation.

Calves receiving an insufficiently protective dose of colostrum may become victims of various bacterial diseases such as arthritis, nephritis, omphalitis, and possibly pneumonia.

Calves receiving a sufficient dose of colostrum may still develop scours of various degrees of severity due to the local multiplication of various types of *Bacillus coli* in the small intestine.

There exists in the young calf a delicate balance between certain strains of *Bacillus coli* and the mucous membrane and digestive ferments, which, upset in favor of *Bacillus coli*, produces scours. The necessary conditions for such attacks are in part inherited defects of the digestive tract, both morphological and functional, and special types of *Bacillus coli*, resident in the herd and environment.

The immediate indications are a great increase in the number of *Bacillus coli* in the lowest third of the small intestine with a spreading of the invasion towards the duodenum as the disease gains headway. Under these conditions a general intoxication results. The bacilli form layers or films attached to the top plates of the epithelial cells. At this time morphological changes in the cells are not yet recogniza-

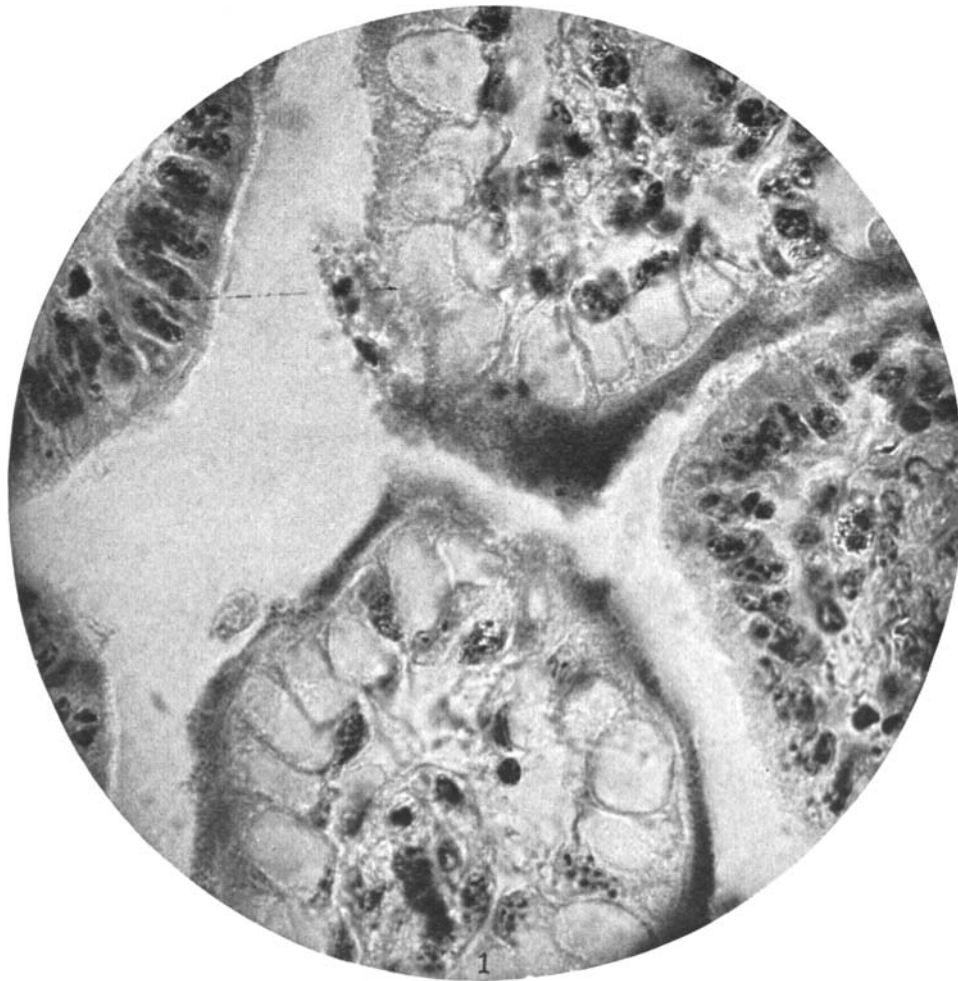
<sup>14</sup> Küthe, H., *Centr. Bakt.*, 1. Abt., Orig., 1915, lxxvi, 409.

ble. The vacuolar or hydropic condition frequently found antedates the bacterial invasion and may be present in the absence of bacteria. In mild or recovered cases, during the 1st week a fatty infiltration of the epithelium is not infrequently present.

In a large number of clinically normal calves, from 1 day to 3 months of age, Gram-negative bacilli were not present in sufficient numbers to be detected in sections, although enriched cultures, when attempted, revealed them.

#### EXPLANATION OF PLATE 8.

FIG. 1. For a brief history of this animal (No. 156) see page 94, and Fig. 7 of the accompanying paper by Smith.<sup>5</sup> Section of the unopened small intestine above ileum fixed in Zenker's fluid and alcohol. The tips of four villi in view, two of which show the hydropic condition of the epithelium.<sup>5</sup> Dense masses of *B. coli* are attached to the top plates of these. They are also present on villi not so changed. Eosin and methylene blue.  $\times 812$ .



(Smith and Orcutt: Bacteriology of intestinal tract of calves.)