BACTERIAL POPULATION CHANGES IN THE CECA OF YOUNG CHICKENS INFECTED WITH EIMERIA TENELLA[1,2]

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Eimeria tenella, a protozoan parasite belonging to the class *Sporozoa*, is the etiological agent of cecal coccidiosis of chickens. This organism goes through a typical sporozoan life cycle and, with the exception of sporulation of the oocyst, all stages occur within the host's intestinal tract. Invasion of the intestinal epithelium by sporozoites and merozoites results in considerable tissue destruction and, in acute cases, severe hemorrhaging by the fifth day of infection, at which time mortality among the hosts reaches a peak.

It has been known for some time that under certain poorly defined conditions intestinal bacteria of the chicken may invade the tissues and set up local infections in the liver, spleen, and other organs. Morse (1908) frequently found "*Bacterium aerogenes*," "*B. coli*," "*B. proteus*," and "*Bacillus pyocyaneus*" in various organs of chickens which had succumbed to "white diarrhea." Hadley (1909) found bacterial invasion of the viscera in chickens infected with *Coccidium cuniculi*. This bacterial invasion was studied further by Fantham (1910), who employed coccidia-infected wild grouse. He explained that the sporozoites and merozoites act "as inoculating needles," permitting injurious bacteria to pass into the tissue of the gut, "whence, by way of the blood and lymph, they can reach other organs." Many other workers have reported similar results, which are well reviewed and discussed by Ott (1937). The latter isolated *Escherichia coli*, *Staphylococcus albus*, *Salmonella pullorum*, and an unidentified coccus from the livers of 36 out of 46 one-year-old White Leghorn hens infected with *Eimeria tenella*. Ott found little evidence of bacterial invasion in normal, *E. tenella*-free chickens. By feeding broth cultures of *E. coli* to infected hens, he was able to increase the percentage of livers infected with this bacterium. Ott concluded that there is some relationship between cecal bacteria and cecal coccidiosis.

Recently, Mann (1947) reported considerable success in stamping out cecal coccidiosis by increasing the carbohydrate and reducing the protein and roughage components in the ration. He believed that roughage "caused sufficient damage to the mucosa to pave the way for coccidial invasion." He also thought that the presence of roughage stimulated the activity of certain intestinal bacteria, which assisted coccidial invasion. Mann (1945*a*,*b*) previously found that roughage and protein stimulated proliferation of anaerobic intestinal bacteria,

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particularly those resembling *Clostridium perfringens*. Therefore, Mann concluded that a particular type of cecal bacterial flora seems to be necessary for the development of *E. tenella* in chickens. Also, as pointed out by Mann (1947), the successful use of sulfonamides in alleviating the course of coccidiosis in chickens might indicate action of the drug upon associated bacteria as well as upon coccidia.

Although many workers have shown that cecal bacteria may play an active part in promoting the incidence and severity of cecal coccidiosis in chickens, no extensive study has been made of the kinds and numbers of microorganisms found in the cecum during infection with *E. tenella*. It was thought that such a study might contribute to our knowledge of the etiology of cecal coccidiosis.

TABLE 1

Composition of synthetic diet fed chicks infected with cecal coccidiosis (Cravens, 1947)

Sucrose	64%
Casein (alcohol extracted)	18
Gelatin	5
Salts IV	5
Soybean oil	5
Fish oil (2000 A, 400 D)	1
Thiamine HCl	4 mg/Kg
Riboflavin	6
Nicotinic acid	50
Ca-pantothenate	15
Pyridoxine HCl	4
Alpha tocopherol	3
Biotin	0.02
2-Methyl-1,4-naphthoquinone	0.2
Choline	0.15%
Liver L	2

METHODS

Animals. A pure line strain of *Eimeria tenella* (Railliet and Lucet), isolated by Professor C. A. Herrick of the Zoology and Veterinary Science Departments of this university, was used in infecting the chicks. The method of infection used was that of Edgar and Herrick (1944). Two-week-old hybrid Single Comb White Leghorn (female) × New Hampshire (male) chicks were employed in this investigation. They were usually off feed for from 1 to 2 hours before infection with approximately 10,000 sporulated oocysts. Wisconsin no. 45 mash (Halpin *et al.*, 1944) was fed the chicks during the pre-experimental, 2-week period. After infection the chicks were divided into two groups: one was continued on the same grain mash, and the other was placed on a nutritionally adequate synthetic ration (table 1).

Microbiological determinations. Two to four animals were picked at random just prior to infection and killed so that normal cecal samples could be obtained. The samples were prepared for bacterial study in the manner previously described for laying hens (Johansson *et al.*, 1948). In the first experiment, only four types

of cultures were prepared: carrot-liver extract ("CL") agar shake tubes (Garey *et al.*, 1941), aerobic agar plates (Johansson *et al.*, 1948), Difco eosin methylene blue agar plates ("EMB"), and Hajna and Perry's (1943) "SF" medium (dilution counts). In the second experiment the following determinations were made: CL agar shake tube counts, aerobic agar plate counts on Baltimore Biological Laboratory's "fluid thioglycolate" medium with 1.3 per cent agar added, plate counts in the latter medium incubated anaerobically in an atmosphere of hydrogen, EMB agar plates, and total spore counts. Spore counts were made by heat-shocking the initial 1:100 dilution (after inoculations had been made in the other media) at 80 C for from 10 to 11 minutes and inoculating tap water dilutions of this material into the following media: typtone yeast extract glucose agar (Johansson *et al.*, 1948) plates; "thioglycolate medium" (Baltimore Biological Laboratory) plates with 1.3 per cent added agar; and shake tubes of the latter medium. Thioglycolate agar plates were incubated anaerobically in hydrogen, and the other plates and tubes were incubated aerobically. All cultures were incubated at 37 C except SF dilution counts, which were incubated at 45 C. Spore plates and tubes were incubated only 24 hours because longer incubation resulted in spreading growth of some of the aerobic sporeformers, which obscured many of the smaller colonies. CL shake cultures and SF dilution tubes were held 72 hours in the incubator, and all other cultures were incubated for 48 hours before making counts. Shake and plate cultures were run in duplicate and 5 tubes per dilution were used in making SF dilution counts. Most probable numbers of the latter were calculated from the table in *Standard Methods for the Examination of Water and Sewage* (1946).

In the first experiment on infected chicks each of the three trials was followed through to the sixth day of infection. Four chicks at a time were taken from each ration just before infection, and on the second, fourth, and sixth day of infection, for bacterial counts on the cecal pouch contents. In the second experiment cecal samples were taken from each of three or four chicks at the time of coccidial infection, and again on the third and fifth day after infection. However, each trial initiated during the second experiment was not followed through because of scheduling difficulties, i.e., the first trial might be represented by samples at 0, 3, and 5 days, while samples from the next trial might have been taken only on the fifth day of infection. Occasional samples were taken from uninfected chicks between the ages of 16 and 20 days that were maintained on both grain and synthetic rations in order to detect any changes that might occur naturally with advance in age of the chicks. All counts are expressed as the number of bacteria per gram of cecal contents; usually the samples were too small to permit dry weight determinations.

Isolation and characterization of enterococci and anaerobes were attempted during the second experiment. Enterococci were isolated from SF dilution tubes by plating out in tryptone yeast extract glucose agar containing 6.5 per cent NaCl and incubated at 37 C for 3 days. Colonies were picked from the salt agar plates and kept on slants of the agar (without NaCl) for further study. The action of these isolates on blood was determined in Difco blood agar base

containing 5 per cent bovine blood. Their ability to liquefy gelatin was determined by stab inoculation into 10 per cent Difco gelatin and incubation at 37 C.

Potential anaerobes were isolated from anaerobic thioglycolate agar plates (both spore and nonspore plates), and sometimes from CL agar shakes that evidenced excessive gassing. Isolation was accomplished in freshly steamed "thioglycolate medium" containing excess $CaCO_3$; isolates were then incubated at 37 C until adequate growth occurred. Each isolate was subsequently plated out by loop dilution in thioglycolate agar and incubated anaerobically for from 24 to 48 hours. A single, well-isolated colony was then picked into the modified Brewer's thioglycolate medium, incubated at 37 C until growth reached a maximum, and put away in the icebox for future study. Some of these cultures were anaerobic or microaerophilic, gram-positive cocci or rods which produced little or no gas in Brewer's medium. These cultures were studied further by cultivation on tryptone liver extract glucose agar slants incubated both aerobically and anaerobically; their action on litmus milk and their ability to ferment glucose, sucrose, lactose, and maltose (fermentation basal: 0.5 per cent yeast extract, 0.3 per cent tryptone, and 0.1 per cent agar, with bromcresol purple indicator added) were observed.

Nearly one-fifth (39) of the "anaerobe" isolates produced stormy fermentations in glucose- or lactose-containing media. Gram stains showed these organisms to be large, gram-positive rods; they were nonmotile and obligately anaerobic. An attempt was then made to identify these bacteria. The motility of 8- to 16-hour cultures grown in Brewer's medium was determined by microscopic examination of wet mounts. Gram stains (Kopeloff and Beerman, *Manual of Methods*, 1945) were made of 18-hour Brewer's cultures. Sporulation was induced by cultivation in Reed and Orr's (1941) proteose-peptone plating agar (adjusted to pH 8.0), incubated anaerobically in hydrogen for 48 hours. Schaeffer and Fulton's (*Manual of Methods*, 1945) spore stains were made to determine the shape and location of spores. The ability of these isolates to ferment lactose, glucose, sucrose, maltose, and sorbitol, produce H_2S, reduce nitrate, produce indole, and liquefy gelatin was determined according to the recommendations of Reed and Orr (1941). Their hemolytic action on bovine blood was tested on Difco blood agar base containing 5 per cent bovine blood. All cultures were streaked out on the egg yolk medium of McClung and Toabe (1947) and incubated anaerobically for 24 to 36 hours to observe production of lecithinase (Nagler's reaction, 1939).

Statistical analysis. A statistical analysis was made of the data from the first experiment. First, an analysis of variance between trials was made for each of the four stages of infection (0, 2, 4, and 6 days) over all cecal counts (expressed as logarithms to the base 10 carried out two places). The between-trials variation, less variation due to media, ration, and interaction of ration and media, was used to resolve significant changes in the flora with advance in infection. Then, Bartlett's chi-square test of homogeneity of variance (Snedecor, 1946) was made on the real between-trials variation of cecal counts in order to determine whether or not it was of the same magnitude at the four stages of infection.

The weighted mean of the between-trials variation was used as the variance in determining the between-days variation for each medium by another analysis of variance. Least significant differences (*LSD*) were then computed between different infection periods for those media showing significant *F* values (either at the 5 or 1 per cent level of significance). A few times missing values were estimated according to the method of Yates (Snedecor, 1946), and used in calculating the statistical average.

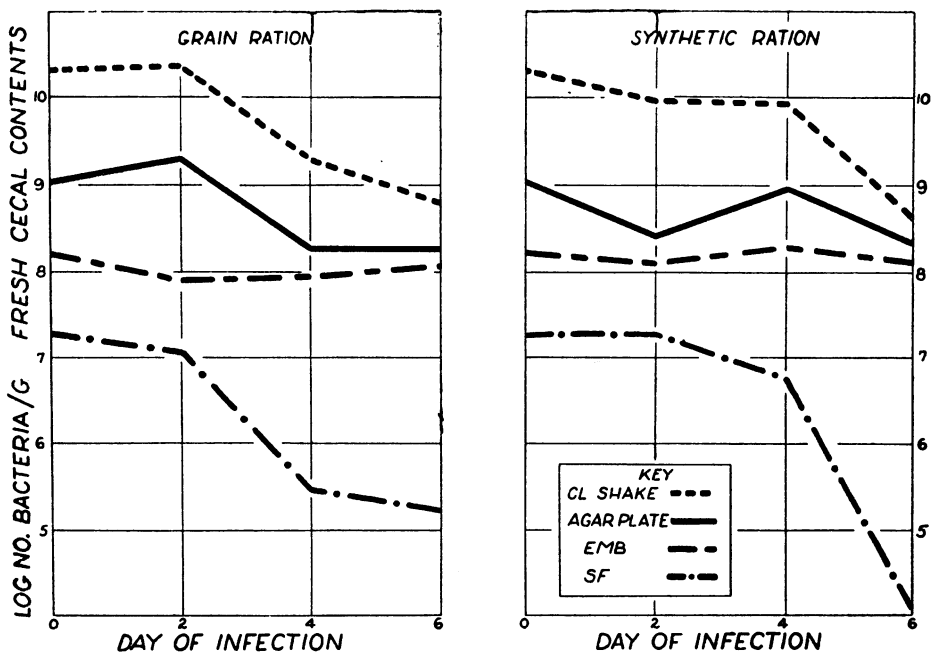

Figure 1. Changes in the cecal flora of chicks infected with *Eimeria tenella*—first experiment.

RESULTS

Experiment 1. The bacteriological results are summarized in table 2 and figure 1. The most striking changes in the cecal flora during infection with *E. tenella* appeared to exist among those groups of bacteria represented by CL and SF counts, most of which were lactic acid bacteria. After the second or fourth day of infection, very few organisms morphologically like lactobacilli could be found in CL shakes. During the latter stages of coccidial infection, CL shake tubes contained primarily coliform organisms. It is interesting to note that coliforms remained unchanged in numbers during the course of infection. Agar plate counts on cecal contents appeared to drop a little in birds on grain, while a slight change occurred in cecal plate counts of chicks on the synthetic ration. The over-all change in the cecal flora was practically the same with either ration. Lactic acid bacteria from chicks on the synthetic diet did not decrease appreciably in numbers until after the fourth day of infection, whereas in birds on the

grain mash, cecal lactics decreased after the second but before the fourth day of infection. The coliforms, as in the intestinal flora of pullets (Johansson *et al.*, 1948), appeared to be predominately *Escherichia* types.

Statistical treatment of these data revealed the difference between trials to be highly significant before coccidial infection, and on the fourth and sixth day after infection; on the second day the between-trials variation was not significant. One thing revealed by analysis of variance between trials is that the within-trials variation (the variance) gradually increased during the advance of infection (from 0.1362 to 0.6747). This indicates that variation between cecal counts increased during the course of cecal coccidiosis. Bartlett's chi-square test of the homogeneity of between-trials mean squares resulted in a slightly over 5 per cent level of probability, indicating that these mean squares were not

TABLE 2

Cecal counts of chicks infected with Eimeria tenella—Experiment 1

MEDIUM	DAYS INFECTED						
	0	2		4		6	
	Grain	Grain	Synthetic	Grain	Synthetic	Grain	Synthetic
CL*	19,884.0	21,822.5	9,108.0	1,920.6	8,509.3	647.7	426.0
	(9)	(4)	(6)	(6)	(6)	(6)	(6)
Agar plate*	1,076.9	2,031.3	254.7	187.8	922.3	187.5	216.2
	(10)	(4)	(6)	(6)	(6)	(6)	(6)
EMB*	130.7	79.8	125.5	90.6	212.3	111.2	134.7
	(10)	(4)	(6)	(6)	(6)	(6)	(6)
SF†	1,953.8	1,236.5	1,965.0	30.7	613.2	16.5	1.0
	(10)	(4)	(6)	(6)	(6)	(6)	(6)

Numbers in parentheses indicate the number of samples from which the average was obtained.

* Counts expressed $\times 10^6$.

† Counts expressed $\times 10^4$.

homogeneous. Actually, one mean square (at 2 days after infection) was out of line and, since a better variance was not available, the weighted mean of the between-trials mean squares was employed in determining the variation between days (Torrie, 1948). Perusal of table 3 indicates the following: (1) CL and agar plate counts changed significantly between days of infection in the ceca of chicks on grain, but not in chicks on the synthetic ration; (2) no significant change in coliform counts occurred in cecal coliforms of chicks on either ration; and (3) the decrease in enterococcus counts during infection was highly significant for both rations. Least significant difference calculations revealed that the drop in CL counts of chicks ingesting grain was not significant until the fourth day of infection ($LSD_{.05}$ log 1.37). Similarily, cecal agar plate counts of grain-fed chicks did not change significantly until the sixth day of infection ($LSD_{.05}$ log 1.34). A highly significant drop in numbers of cecal enterococci was found to occur on the fourth day of infection in chicks ingesting grain, and on the sixth day of infection in chicks on the purified diet ($LSD_{.01}$ log 1.79).

Experiment 2. The results of this study are compiled in table 4 and figure 2. As in the first experiment, the CL shake counts decreased more in the ceca of coccidia-infected chicks on grain than in those on the synthetic diet. Aerobic and anaerobic thioglycolate agar plate counts were surprisingly similar and changed most in birds on grain; in chicks on the synthetic ration these two plate counts appeared to remain fairly constant during infection. Since it was discovered that coliforms made up the majority of the colonies growing on these plates, it is not surprising that anaerobic and aerobic plate counts were similar

TABLE 3

Analysis of variance for between-days' differences in cecal counts

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUMS OF SQUARES	MEAN SQUARE	F VALUE
1. Carrot-liver agar shakes				
Between days, grain	3	17.2741	5.7580	3.405*
Between days, synthetic	3	6.9801	2.3267	1.376
Between trials	52	87.9361	1.6911	
2. Agar plates				
Between days, grain	3	14.2101	4.7367	2.801*
Between days, synthetic	3	3.9831	1.3277	0.785
Between trials	52	87.9361	1.6911	
3. Coliform counts				
Between days, grain	3	6.8276	2.2759	1.346
Between days, synthetic	3	0.6098	0.2033	0.120
Between trials	52	87.9361	1.6911	
4. Enterococcus counts				
Between days, grain	3	54.0650	18.0217	10.657†
Between days, synthetic	3	61.6060	20.5353	12.143†
Between trials	52	87.9361	1.6911	

* *F* significant at the 5 per cent point.

† *F* significant at the 1 per cent point.

in magnitude. Especially in chicks on the synthetic diet, it may be noted that coliforms made up by far the majority of the cecal flora after 5 days' infection with *E. tenella*. It is believed that both CL shake and plate counts were merely coliform counts at this late stage of infection. Lactobacilli were rarely encountered in CL shakes after the third day of infection. Anaerobic spore counts[4] decreased during infection, while aerobic spore counts of cecal contents remained essentially unchanged. Thioglycolate agar shake tube counts of spores markedly

[4] It was found that many of the "aerobic" sporeformers grew well in an atmosphere of 100 per cent hydrogen; hence the anaerobic spore count includes a fair number of aerobes which obscure the true decrease in numbers of anaerobic spores.

TABLE 4

Cecal counts of chicks infected with Eimeria tenella—Experiment 2

MEDIUM	DAYS INFECTED				
	0	3		5	
	Grain	Grain	Synthetic	Grain	Synthetic
CL*	6,942.0	11,076.0	4,429.4	745.3	930.2
	(10)	(8)	(8)	(8)	(8)
Thioglycolate plates—anaerobic*	1,826.8	1,335.4	3,836.3	169.1	789.6
	(10)	(8)	(8)	(8)	(8)
Thioglycolate plates—aerobic*	1,889.3	1,804.1	3,115.2	140.1	739.1
	(10)	(8)	(8)	(8)	(8)
EMB*	359.6	42.4	2,630.8	73.3	760.1
	(10)	(8)	(8)	(8)	(8)
Spore shakes†	171.0	515.0	553.0	79.4	25.9
	(7)	(8)	(6)	(5)	(6)
Spore plates—anaerobic†	1,484.0	215.6	529.0	19.0	230.7
	(10)	(8)	(8)	(6)	(6)
Spore plates—aerobic†	405.0	627.4	210.8	717.1	948.5
	(10)	(8)	(8)	(6)	(6)

Numbers in parentheses indicate the number of samples from which the average was obtained.

* Counts expressed × 10^6.

† Counts expressed × 10^3.

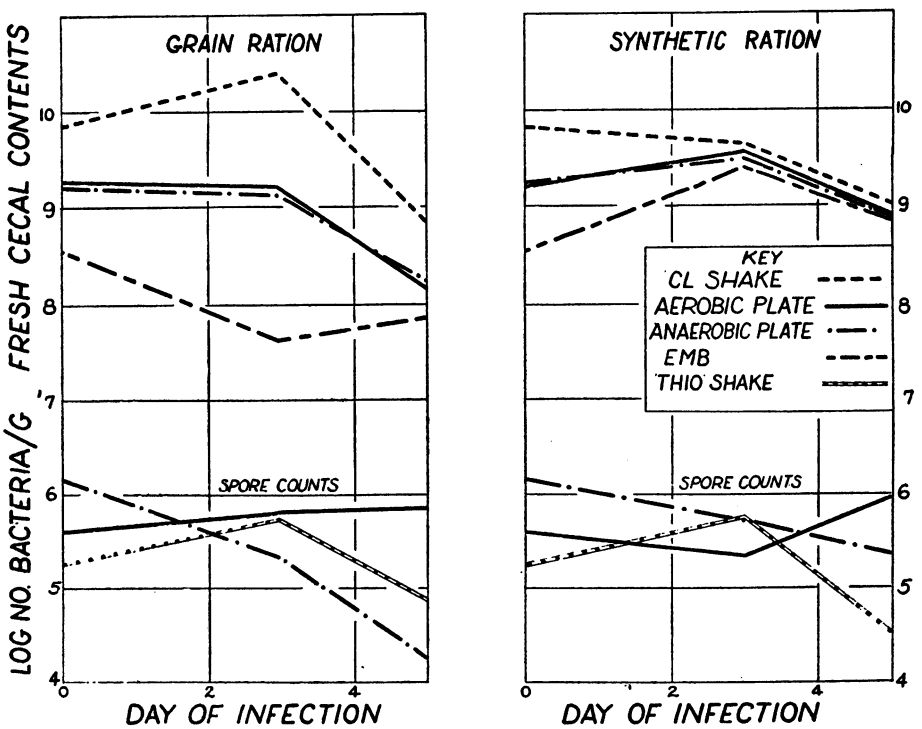

Figure 2. Changes in the cecal flora of chicks infected with *Eimeria tenella*—second experiment.

decreased after the third day. There appeared to be little difference in cecal flora changes between birds on the two rations, but chicks on the synthetic diet possessed a slightly richer cecal coliform flora throughout coccidial infection.

The cecal flora of normal, uninfected chicks of the same age as those that were infected was found to remain the same; hence these changes in the flora which were found to occur in both experiments must not be due to age but to some other factor, presumably, the coccidial infection.

Large, cream-colored lenticular colonies, which are typical of *Clostridium perfringens*, usually were noticed in spore plate counts made before infection on cecal contents diluted as high as 10^{-4} or 10^{-5}; nonspore anaerobic plates of cecal contents rarely produced such colonies beyond 10^{-5} dilution prior to infection. However, on the fifth day of infection, *C. perfringens*-like colonies often were found in abundance on anaerobic plates of the unheated material up to the 10^{-8} dilution, but were rarely seen in the lowest dilution (10^{-2}) of the anaerobic spore plates. These colonies were easily recognized, and when transferred to litmus milk containing reduced iron, always gave a stormy fermentation. Their presence in thioglycolate agar shake tubes was unmistakable because they blew the agar to bits and pushed the cotton plugs completely out of the tubes within 18 hours at 37 C. Thus, it seems that changes in the ceca during infection with coccidia resulted in favorable conditions for growth of the gas-gangrene organism as evidenced by germination of *C. perfringens* spores and their extensive proliferation.

One hundred seventy-six isolations were made from CL agar shake cultures and anaerobic plates (both of heat-shocked and non-heat-shocked preparations). Of this total, 39 were identified as *C. perfringens* and the rest were found to be chiefly coliform bacteria, cocci, and lactobacilli, or unidentified microaerophilic or anaerobic gram-positive rods. All cultures identified as *C. perfringens* fermented glucose, lactose, maltose, and sucrose, produced a stormy fermentation in litmus milk, reduced nitrate to nitrite, did not produce indole, produced H_2S, and gave a positive lecithinase reaction. Only one such culture did not liquefy gelatin, and only four did not hemolyze bovine blood (the others produced a weak to strong alpha hemolysis). Spores of a few of the cultures were observed and found to be oval, excentric to subterminal in location; the sporangia were slightly swollen. Sporulation of the isolates was induced with great difficulty, and only a few spores were seen in any culture. The cultures were nonmotile, gram-positive, and were fairly large regular rods. Thus, the morphological, cultural, and biochemical studies of these sporeforming anaerobes seems to agree with the description of *C. perfringens* given in *Bergey's Manual of Determinative Bacteriology* (1948).

Eight cultures of enterococci were isolated from SF dilution cultures of eight different coccidia-free chicks, varying in age from 7 to 30 days. They were all large, irregularly-shaped, gram-positive cocci, able to grow in a medium containing 6.5 per cent NaCl, to produce either alpha or gamma hemolysis of bovine blood, and unable to liquefy gelatin. From this preliminary study of a few enterococcus isolates from young chickens, it appears that they are *Streptococcus faecalis*.

Seven gram-positive, anaerobic or microaerophilic nonsporeforming rods found among the 176 isolates appeared to be lactobacilli. Six of them fermented sucrose, maltose, lactose, and glucose, while one fermented sucrose, maltose, and glucose with the production of acid but no gas. Growth, if any, on aerobic slants was scant, whereas stab cultures grew well. None strongly coagulated or reduced litmus milk, but all produced acid in this menstruum. No attempt was made to classify these isolates as to species.

A few of the microaerophilic coccus isolates were inoculated into tubes of SF medium and incubated at 45 C; no growth appeared during 1 week of incubation. This indicates that some of the coccus isolates were not enterococci. They were found to ferment sucrose, maltose, lactose, and glucose with the production of acid but no gas. Further study of these coccus isolates was not made.

DISCUSSION

There have long been indications that the etiology of cecal coccidiosis is complex, and that destruction of intestinal epithelium with resultant hemorrhage by the protozoan parasite, *Eimeria tenella*, is but one aspect of the disease. Bacteria belonging to the coliform, coccus, and sporeforming anaerobe groups have been frequently isolated from the liver, spleen, heart, and blood of chickens which have succumbed to coccidiosis infection (Morse, 1908; Hadley, 1909; Fantham, 1910; Ott, 1937). No doubt secondary infection is faciliated as a result of weakened cecal walls and a general emaciation of the animal. Mann (1947) goes further and claims that "coccidiosis in chicks is a secondary complaint, the primary aetiological agents being bacteria which proliferate to excess in the presence of roughage or protein." In addition, it is possible that toxic products of bacteria may penetrate the infected epithelium to confuse the picture even more.

Mann (1945*a*, *b*) studied "six-day disease" of chicks, which he found to be caused by invasion by various fecal bacteria, chiefly *Clostridium welchii*, enterococci, and coliforms, through the intestinal wall. His description of the appearance of the cecal pouches of birds afflicted with this infection is similar to that of cecal coccidiosis: occlusion of the ceca by a hard core with hemorrhage in varying degrees. He was able to control this disease by using a ration high in natural carbohydrate, and low in animal protein, roughage, and residual oils capable of destroying vitamin A. McGaughey (1944) reported similar results in a bacteriological study of "six-day disease" of chicks. He was able to reproduce the disease by feeding cultures of *C. welchii* to day-old chicks. McGaughey suggested that the cause of death, which is fulminating, might be a *C. welchii* intoxication. Mann (1947) believes the bacteriology of cecal coccidiosis to be similar to that of "six-day disease," and that it too could be controlled by a ration like the one employed to subdue morbidity due to "six-day disease."

The results of the first experiment show that coliform organisms, primarily *Escherichia* species, do not decrease in numbers in the ceca during the course of coccidiosis, whereas lactic-acid-producing bacteria all but disappear. The second experiment agreed with the first and, in addition, indicated that condi-

tions in the ceca of infected chicks were modified so as to favor proliferation of sporeforming anaerobes, especially *C. perfringens*. Marsh and Tunnicliff (1944) found many coccidia-infected sheep to develop enterotoxemias caused by *C. perfringens* (type D). Therefore, the results obtained in this study strengthen the belief that *C. perfringens* may be a secondary invader in chicks infected with coccidia, and that the activity of *E. tenella* makes their growth possible.

There is considerable evidence in the literature that injury to the intestinal tract of an animal may result in visceral invasion by *C. perfringens*. Williams (1927) pointed out that, when the small bowel of man becomes paralyzed or obstructed, conditions become ideal for growth of *Bacillus welchii*. Stabins and Kennedy (1929) artificially obstructed the jejunum of dogs and found an abrupt increase in numbers of *C. welchii* within the obstructed segment. *C. welchii* was found to invade the blood stream in five human cases in which the primary lesion was in the intestinal tract (Cruickshank and Davidson, 1944). The latter state: "Injury and disease of the alimentary canal, which normally harbours organisms of the gas gangrene type. . . may allow the entry of these organisms into the blood stream." Since cecal coccidiosis of the chicken results in occlusion of the cecum together with injury to the intestinal mucosa and epithelium, a favorable environment is established for the growth of *C. perfringens*. None of the *C. perfringens* isolates were typed in the present study, but Taylor and Gordon (1940) found only type A among the 88 cultures isolated from 12 of 13 chickens examined.

The coliform organisms may also be involved in the etiology of cecal coccidiosis. This study has shown that they are present in large numbers throughout the course of coccidiosis infection. In a previous investigation (Johansson *et al.*, 1948) it was found that the cecal contents of mature hens had a coliform count considerably lower than that found in the ceca of young chickens. This might possibly be a factor related to the reduced severity of cecal coccidiosis which is known to occur with advance in the age of chickens (Herrick *et al.*, 1936).

Although no outstanding difference was noted between the cecal flora of chicks on the two rations employed in this work, Herrick (1947) has found the grain mash to favor an appreciably higher incidence, severity, and mortality in the chicks infected with *E. tenella* than did the purified ration.

SUMMARY AND CONCLUSIONS

The cecal flora of 2-week-old chicks on a grain mash or on a synthetic diet changed during the course of infection with *Eimeria tenella*. Numbers of lactobacilli and enterococci were reduced considerably by the fourth or fifth day of infection. Coliform organisms were found to remain unchanged in numbers in the ceca during cecal coccidiosis. The growth of anaerobes resembling *Clostridium perfringens* was stimulated by coccidial infection of the cecum.

A statistical analysis was made of the enumerative data from one of these experiments and revealed that some of the changes which occurred in the cecal flora of chicks maintained on two different rations during the course of coccidial infection were statistically significant.

It is believed that *C. perfringens* and *Escherichia coli* complicate the etiology of cecal coccidiosis of the domestic chicken.

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