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# Oregon Agricultural College Experiment Station

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## Immunity or Resistance of the Chicken to Coccidial Infection

By W. T. JOHNSON



CORVALLIS, OREGON

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## INTRODUCTION

Immunity or resistance to coccidial infection is a frequent development with commercial methods of handling fowls. It may also be consistently produced by experimental inoculation. The factors involved in this phase of coccidiosis are closely related to interpretation of methods of control.

**Immunity and resistance defined.** The term immunity is used here to signify absolute insusceptibility to infection, and resistance to designate varying degrees of susceptibility. These definitions, while not strictly in accordance with all textbooks, agree with some writers and appear to be more applicable in connection with this paper.

**Previous knowledge.** The literature includes many observations of the fact that older fowls are less affected with coccidiosis than the young. These statements are unaccompanied by any attempt to explain the reason. That fowls develop resistance to this infection was reported in a previous paper.<sup>1</sup> Resistance was at that time thought to be due to age, but it was later observed and reported<sup>2</sup> that age was not necessarily the determining factor.

Beach and Corl reported<sup>3</sup> an experiment which involved inoculating two groups of fowls with *Eimeria avium*.<sup>\*</sup> One group supposedly had not had access to large numbers of the parasite and the other had previously been inoculated in connection with determining the value of various treatments, and had survived the infection. All of the previously inoculated fowls survived, but thirty-four per cent of the group not previously experimentally inoculated died. In connection with this work the authors make the following statement: "These results make it appear probable that the previously inoculated chicks had acquired resistance against further coccidial infection." Although it would seem probable that at least part of these chicks designated as resistant became so as a result of experimental inoculation, as they imply, the data do not justify the statement that resistance followed such inoculation. It is possible that the supposedly resistant chicks represented those that were so by inheritance. Some young chicks which have not shown evidence of coccidial infection develop no indications of it following inoculation with large numbers of oocysts.

**Relationship of resistance and number of sporulated oocysts ingested.** The fact that the number<sup>4</sup> of sporulated oocysts ingested determines the severity of coccidiosis and that fowls inoculated with small numbers show slight or no symptoms provides a basis for the consistent develop-

<sup>\*</sup>Tyzzar<sup>4</sup> is of the opinion and offers definite information to substantiate it that chickens are hosts for two species of *Eimeria*—*avium* and *tenella*. Tyzzar's determination would evidently establish the oocysts given by Beach and Corl as being at least predominantly *Eimeria tenella*.

ment of resistance to this parasite, providing resistance is due to previous coccidial infection. Unless this was true, development of coccidial resistance would be largely a chance occurrence. It would be irregularly manifested and consistent production would be unobtainable, assuming that coccidial infection is the only means by which resistance to coccidia is produced. In the face of the facts regarding the wide-spread distribution of the parasite this would afford a discouraging situation for the practical poultryman.

**Wide-spread distribution of the parasite and its relationship to immunity.** It appears that avian coccidiosis is universally distributed among commercial flocks over the coast area of the Pacific Northwest. This is substantiated by field observations, reports of poultrymen, post mortem examination of fowls brought to this laboratory, and unpublished data.<sup>6</sup> The infection also occurs naturally in some experimental fowls raised in cages with wire bottoms, so that the feces readily pass through. Wire bottom cage conditions are conducive to coccidial elimination and cannot be approximated in economic commercial poultry production. This writer has therefore come to consider avian coccidiosis as a disease encountered with all commercial flocks in this region, at some time or other. It would seem reasonable to assume that this situation also exists in other poultry districts, even those with different climatic conditions.

With the parasite so widely distributed it would follow that if resistance was the result of previous infection commercially reared fowls would probably show a high degree of resistance. The present writer has repeatedly fed commercially reared fowls sporulated oocysts. In these instances variable but distinct resistance to the parasite was noted. In order to determine further regarding this problem additional inoculations were carried out on a larger scale than previously. These are reported in the following pages.

## MATERIALS AND METHODS

**Fowls used and environment.** Unless otherwise specified, S. C. White Leghorns were used. They were incubator-hatched and reared from the day-old stage in cages, with the exception of those included in Tables I and II, designated as commercially reared. The cages were equipped with hardware cloth bottoms of  $\frac{3}{4}$ -inch or  $\frac{1}{2}$ -inch mesh, with outside feed and drinking equipment, and were kept indoors; the fowls were not exposed to direct sunshine. High mortality not due to infection was to be expected with such conditions. This was caused, to a considerable extent, by trampling and cannibalism. Cannibalism was particularly prominent because of rearing both sexes together, and because of the changing of quarters necessitated when making individual feces examinations.

**Preparation and source of the suspensions.** The oocyst-containing material for the cultures was obtained by using feces and contents of the various parts of the intestinal tract. The oocysts were sporulated in 2½-percent potassium bichromate in distilled water and kept in petri dishes or shell vials at room temperature for varying periods. This culture

material was washed off with tap water, or 2½-percent potassium bichromate in distilled water, and the suspensions kept in shell vials at a temperature of about 7° C., except for the periods May 18 to June 15 and August 13 to September 1, 1926, when they were held at approximately room temperature.\* This method of sporulation and keeping the suspensions permitted of maintaining pathogenicity for extended periods, particularly when washed off with potassium bichromate solution. Six suspensions were used in connection with the work reported in the following pages. All were washed off with tap water except S E and S F.

Throughout the text the coccidial suspensions are referred to as cecal or small-intestine type. It is to be understood that this signifies that the suspensions are not necessarily pure types but are predominantly so.

*Suspension S A†* was prepared April 16, 1926 by washing off shell vial cultures. These cultures had been prepared with small-intestine scrapings and cecal contents, March 18, 20 and 26. They had been kept at room temperature up to April 16. The fowls from which the culture material was taken were incubator-hatched July 29, 1925, and kept indoors from the day-old stage. They were experimentally inoculated March 12, 1926, for the first time. The source of the culture material used March 12 was cecal content of two fowls from a commercial flock. One fowl was six months of age; the age of the other was not recorded. Eight fowls were inoculated March 12, four being July 29, 1925 hatch White Leghorns and four February 10, 1926 hatch Rhode Island Reds. Seven of the eight died of coccidiosis from this inoculation. All seven showed severe small-intestine infection at autopsy as evidenced by hemorrhage and necrotic mucosa. Microscopic examination revealed numerous large-type schizonts and merozoites in one fowl. Four showed slight cecal infection and three apparently none as determined by gross examination.

*Suspension S B* was prepared April 23, 1926, from cultures of cecal content of a year-old commercially reared pullet. Microscopic examination of the small intestine had revealed many oocysts.

*Suspension S C* was prepared May 12, 1926, with a culture made May 1 of cecal feces from cage-reared fowl B 1702. B 1702 had been inoculated October 23, 1925, with a suspension of the cecal type and April 23, 1926, with suspension S B. These were the only times that this fowl had been experimentally inoculated. Suspension S C originated from the small intestine and produced this type predominantly when used to inoculate other fowls. Predilection of the parasite in this suspension was determined by autopsy of B 1719 and B 1727 (Group 3) which were inoculated June 27, 1926.

*Suspension S D* was prepared July 23, 1926. Cultures made July 14, 15, 16, and 17, of cecal feces and contents of fowls under forty-five days of age,

\*Due to refrigeration difficulty.

†If Tyzzer's interpretation is accepted this suspension contained the two species, *Eimeria avium* and *Eimeria tenella*. Development of *Eimeria tenella* took precedence over that of *Eimeria avium* except for one fowl inoculated November 26, 1926. The predominance of *Eimeria avium* in this fowl might be explained on the basis of greater viability of *Eimeria avium* under the conditions of preservation and storage of the suspension.

The other suspensions used in connection with the experiments reported in this bulletin contained *Eimeria tenella* predominantly or exclusively.

were used. They had all been inoculated with suspension S A at various times in the past. While suspension S A was predominantly small-intestine type, suspension S D was similarly cecal. S D also consistently developed some small-intestine infection.

*Suspension S E* was prepared August 31, 1926, of cecal content and cecal feces. A number of typical cecal cores of hemorrhagic origin were used for some of the cultures. Nine of the cultures came from fowls which developed predominant cecal infection. This was determined by autopsy of four fowls inoculated at the same time as those from which this culture material was taken. Five of the cultures came directly from fowls inoculated with suspension S A, which had proved predominantly small-intestine type. Suspension S E was, therefore, mixed from the standpoint of predilection of the parasite. Autopsy and feces examinations proved it to be predominantly cecal type, as judged by the gross appearance of infected intestines and the feces.

*Suspension S F* was prepared December 4, 1926, with cultures from various sources. This suspension consistently produced predominant cecal and slight small-intestine infection in cage-reared fowls.

**Numerical determinations of sporulated oocysts.** In all instances counts were made as to the number of sporulated oocysts in the suspensions used. Determinations consisted in counting the number of sporulated oocysts in 1/100 c.c. of a dilution of the original suspension. Sometimes more than one count was made. These counts are not accurate determinations of the number of viable sporulated oocysts because different counts of the same suspension at the same time vary considerably, and past experience has shown normal-appearing sporulated oocysts to be non-pathogenic, if not dead. Counts were nevertheless considered to be of value.

**Method of Inoculation.** Inoculations were made by the mouth with medicine droppers. The suspension was placed directly into the first portion of the esophagus, or into the pharynx. In all instances where inoculations were made it is understood that sporulated oocysts were given.

**Ration.** With the exceptions noted later, the feed consisted of a modified Wisconsin ration.<sup>7</sup> The Wisconsin ration was mixed by weight, as follows:

Ground yellow corn.....	80 parts
Middlings .....	20 parts
Sodium chloride .....	1 part
Limestone grit .....	5 parts
Bone-meal .....	5 parts
Buttermilk (water substituted at time of inoculation) ad libitum.	

Beginning May 1, 1926, 2½ percent dried sweet skim milk was added to the above to take the place of the liquid buttermilk, and this was continued until June 8, when the dried sweet skim-milk feeding was discontinued and 2½ percent dried buttermilk added. At this time 1 percent cod-liver oil and 7½ percent meat meal were also added to the ration. It was thought that the milk given was sufficient to permit of reasonable

growth, and that this amount would be less than that used by the average commercial poultryman. The ration was so planned as to eliminate, as much as practicable, the factor of milk influencing the infection. When liquid milk was given, this was removed at inoculation, and returned in about two weeks. After dry milk was added, no change in ration accompanied inoculation.

**Technic of smear examinations.** Examination of the feces, intestinal mucosa, and contents was made by the smear method, unless otherwise specified. So far as possible the smears were of moderate depth and of such amount as to insure ready observation. Only the more significant smear examinations are recorded in the tables. Smears were made for the detection of red blood cells when doubt existed as to whether or not feces contained blood.

**Egg records.** Incomplete egg records were kept in some instances, signifying the minimum number laid during such time.

**Fowls used but not reported.** In addition to the fowls included in the tables and the controls, one hundred and eighty-two others were used. The data obtained with these fowls did not add anything other than that brought out by the groups included in the tables; consequently, no further reference will be made to them.

**Fowl groupings and description.** *Group 1* (Table I) consisted of fourteen (eleven of which are included in the table) fowls, reared on a commercial poultry farm and seven (four also in *Group 3*) cage-reared fowls, used as controls. The former were obtained for inoculation purposes from a commercial poultry farm March 26, 1926, placed in cages with one-half inch mesh hardware cloth bottoms, and kept there continuously.

These fowls had been reared under conditions much superior, from the sanitation standpoint, to the average in the Pacific Northwest. They were brooded on a concrete yard to which they were confined when out-of-doors. After brooding they were moved to range houses on new range. As they became older part of the fowls, at least, ranged occasionally over ground used several years before.

Mild coccidial infection was diagnosed in one of the fowls from this flock when they were about seven months old. Only a few oocysts were found.

*Group 2* (Table II) consisted of seventeen mature fowls that reacted to the agglutination test for *Salmonella pullora* infection. They were obtained from nine commercial poultry farms, were placed in wire-bottom cages as soon as received and kept there continuously.

*Group 3* (Table III) consisted of two Rhode Island Reds, inoculated March 26, 1926 and eight Leghorns, not experimentally inoculated previous to this date. The suspension used March 26, was prepared from cultures made by mixing cecal content of a six months pullet with 2½ percent potassium bichromate. There was no definite check on the nature of this infection as to predilection of the parasite. It is probable that the suspension was small-intestine type.

Both Rhode Island Reds passed moderate amounts of pure blood feces, following the March 26 inoculation. They did not show any droopiness.

*Group 4* (Table IV) consisted of eighty-five fowls, eighty hatched May 1, 1926, forty of which were kept separate to serve as checks. The other forty were placed together in a pen, all of which were inoculated except Nos. 35 to 40 inclusive, which served as checks within this pen. These chicks represented the more rapidly developing fowls, as the attempt was made to select cockerels.

As the fowls became older the number was diminished to provide more room. They were then used for other purposes. Removals other than by death from the inoculated pen were met by removals from the check pen.

Five of the fowls in this group were hatched July 25, 1925. These were kept separate. They are not included in the tables.

*Groups 5* (Table V) and *6* (Table VI) were hatched June 7, 1926. Each group occupied a cage, and consisted of seventeen chicks.

An attempt was made to select cockerels for the check fowls. Since this was done when they were under two weeks of age, the consequence was to select the more rapidly developing fowls for this purpose. It was anticipated that inoculation would result in greater mortality to the check fowls, thus disposing of the cockerels, which were the less desirable.

## DATA: NON-TABULAR

*Group 1* (Table I). Three of this group are not included in the table, since one died the day after inoculation and the other two added no data not sufficiently provided by others.

Nos. 864-25, 552-25, 28-25, 440-25, and 74-25 were autopsied May 8, June 18, June 22, June 26, and July 2 respectively. Smears were made of cecal content or scrapings of the small intestines of all of these fowls. No coccidial forms were found in any of them.

May 10, 1927, Nos. 564-25, and 580-25 were the only commercially reared fowls left of *Group 1*. They were killed on this date to determine the presence of coccidial forms. The cecal contents of each fowl were centrifuged in a concentrated sugar solution, and smears made from the surface. No coccidia were found.

*Group 2* (Table II). No microscopic examinations were made of the feces from these fowls. The effect of the inoculations was determined by observing the symptoms and feces and necropsy of the two that died.

*Group 3* (Table III). Examination of feces, May 23, from B 1718, B 1725, and B 1726 revealed moderate numbers of oocysts. A few red blood cells were seen in the smear from B 1726. The feces from B 1722 contained numerous oocysts, May 24. In spite of infection being present in all four of the above-mentioned fowls, no symptoms were shown.

Inoculation on June 5 caused B 1722 to pass a fair amount of pure blood in the feces, beginning June 11. Enough was passed to raise the question of his ability to survive the attack. He continued to pass pure blood in fair amounts June 12 and 13, after which the feces were normal

or only slightly mixed with blood. B 1721 passed distinctly more blood than B 1722. B 1722 was active June 11, inactive June 12 and 13, and then became active again. B 1725 passed a small amount of pure blood and blood-tinged feces, June 11 and June 13, but no more after those dates. B 1725 remained active throughout this attack, ate well, and was decidedly less affected than B 1722.

The June 15 and 20 inoculations were not followed by bloody feces. A smear made with material from a cecal discharge passed by B 1718, June 22, showed two oocysts in three times across the smear with the high dry lens. These oocysts may have been from an inoculation previous to June 15. Further examinations might have revealed more evidence of infection. Numerous oocysts were seen in a smear from cecal feces of B 1726, on this date, probably the result of the June 15 inoculation. Numerous oocysts were being passed by B 1726 and B 1718 June 27. In all probability these oocysts were due to the June 20 inoculations. B 1718 was noted to have laid June 21 and 24. These two fowls had shown no symptoms from the infection, appearing normal at all times.

All four of the fowls inoculated June 27 and which had been previously inoculated, eliminated oocysts. B 1718, B 1726, and B 1722 passed large numbers and B 1725 somewhat less, as determined by smear examinations. Red blood cells were noted in the feces of B 1718 and B 1722, but none in those of B 1725 and B 1726. B 1722 was the only one which passed pure blood detected by gross examination. This consisted of small specks discharged July 4. The other three resistant fowls passed feces which had gross appearance of being slightly blood-tinged on July 4, but there was no pure blood. All four continued to be active. B 1718 laid July 3 and 5.

Severe coccidial infection was found at autopsy in all six checks. The small intestines manifested sloughing of the mucosa, severe hemorrhage, and dilation. B 1719 and B 1727 presented evidence of little or no cecal infection. B 1728 and B 1721 showed typical hemorrhagic areas in the cecal walls and hemorrhagic content.

The fowls in Group 3 passed distinct amounts of pure blood following the September 12, 20, and October 9 inoculations. They were kept together, preventing individual feces examinations. The inoculation suspension used was cecal type. June 27 they had all been given a very large number of oocysts of the small-intestine type.

**Group 4 (Table IV).** This group had not been inoculated up to May 21.

Autopsy of No. 34 in inoculated pen, which died May 28, revealed a few oocysts in duodenal scrapings and contents of both ceca. No gross evidence of coccidiosis was manifest. Autopsy of two chicks from the check pen, one on June 21, another on June 24, revealed no coccidial forms in a cecal smear from each.

May 28, ten oocysts were seen in three smears made from cecal feces of the inoculated pen of this group. Three smears from the check pen were negative. A smear made from six cecal feces from the inoculated pen June 30 showed a few oocysts and a moderate number of merozoites. No coccidial forms were found in a similarly prepared smear from the check pen.

Since there was not cage room to accommodate all the chicks, some were taken out occasionally to make room for the remainder. These removals are not designated in the tables. They were taken out as follows: Nos. 28, 29, and 30, June 16; Nos. 24, 25, 26, and 27, June 20; Nos. 22 and 23, June 27; and Nos. 18, 19, and 20, July 6.

June 7, three chicks from the check pen were inoculated at the same time as Nos. 31, 32, and 33, with material from the same suspension (SA). One of the check chicks died June 13, the second June 14, the third on June 15. All the chicks inoculated June 7 developed severe coccidial infection of the small intestine, accompanied by blood and possibly some infection of the ceca.

Autopsy of No. 21, which died of coccidiosis, demonstrated considerable hemorrhage from the small intestine and possibly from the ceca. No coccidial forms were seen in four smears obtained from the small intestine. A smear made from the cecal content showed a moderate number of oocysts and merozoites.

At the same time that Nos. 13 and 14 were inoculated, two check fowls were also inoculated. The same suspension was used for all four. In addition to these four fowls, five hatched July 29, 1925, were inoculated with this suspension. These were B 1706, B 1710, B 1704, B 1702, and B 1709. B 1706 had been inoculated October 23, 1925, with a suspension producing cecal infection, and with a possibility of some small-intestine infection. It had barely survived the attack as judged by the amount of blood passed and emaciation following. It had also been inoculated with a suspension producing infection of the small intestine on April 23, 1926. B 1702 had been inoculated October 23, 1925, and again on April 23, 1926, with the same suspension used on these dates for B 1706. A moderate number of oocysts were given to B 1702 and B 1704 on October 23, and as a result these fowls developed a mild cecal infection, with a possibility of some small-intestine infection. B 1710 and B 1709 had been inoculated only once previously. This was on March 26, 1926. The type of infection was not definitely established, but was probably small-intestine type and possibly some cecal type. B 1704 was inoculated again March 26, 1926, with the same material as B 1710 and B 1709.

The five July 29, 1925, fowls, except B 1706, passed considerable pure blood, following the inoculation August 17, 1926. B 1709 died August 29, probably as a result of this inoculation as the ceca were filled with caseous cores. The four fowls—viz., Nos. 13 and 14 and the two checks—all from the same hatch, showed considerable pure blood in their feces following the August 17 inoculation. All appeared to pass about an equal amount of blood. No. 13 and one check fowl died of coccidiosis and showed hemorrhagic cecal cores, typical of severe cecal coccidiosis. The other check fowl and No. 14 did not show gross lesions. This was no doubt due to the prolonged period between inoculation and death.

On November 18, 19, and 20, two check fowls, Nos. 37 and 38, inoculated November 12, passed considerable pure blood. No blood was passed by Nos. 6, 7, 8, and 9. Smears made November 19 and 22 from feces of the four previously inoculated fowls revealed four oocysts. The two check fowls apparently just survived the attack.

The inoculations of Nos. 1, 2, 3, 4, 5, 35, and 36 (Table IV), made November 26, were followed by a small amount of pure blood in the feces of the five previously inoculated fowls, and considerable quantities from the check fowls Nos. 35 and 36. The five fowls showed no loss of appetite. Both of the check fowls were droopy and did not eat. Autopsy of No. 35 showed the ceca bulging with pure blood and from all appearances No. 36 barely survived the attack.

**Group 5 (Table V.)** A smear made June 24, 1926 with material from six cecal feces revealed moderate numbers of oocysts and a few merozoites. Pure blood feces were being passed by this group, June 26. A smear made from one of the feces showed a small number of typical large-type merozoites. No. 97, the only one of the group of seventeen, was droopy and died. On autopsy a moderate amount of pure blood was found in the ceca. There was no gross evidence of coccidiosis in the small intestine, but microscopic evidence was found. The cecal walls had no hemorrhages. Nos. 92 to 96, inclusive, were separated June 26 to permit observing their feces. All continued to be active and no blood was passed. Nos. 81 to 91, inclusive, were separated on June 26, and no bloody feces were passed. On July 7 (all sixteen fowls together) a small amount of pure blood was seen in the feces; a smear made revealed numerous merozoites and red blood cells. The only droopy fowl on this date was No. 87. On July 13, No. 91 died, possibly due to coccidiosis, but probably due to having one toe completely pecked off earlier in the day. Autopsy revealed moderate infection of the small intestine and possibly some of the ceca. It is not impossible that cannibalism in this case occurred after the fowl had developed weakness from coccidiosis. No. 90, which died July 14, had developed severe infection of the small intestine and slight or none of the ceca, as they presented a normal appearance. The small intestine was dilated. Blood is commonly found in connection with severe acute infection of this part of the intestinal tract, but there was no blood in any part of the intestine of this chick. All other chicks were active on this date.

The inoculations made August 27 resulted in very severe coccidiosis in the check fowls Nos. 82 and 85, less in 88 and 89 and least in 93 and 94, as judged by the amount of blood in the feces. Cecal coccidiosis developed in No. 85, evidenced by hemorrhagic cecal cores. There was no definite gross evidence of coccidiosis of the small intestine, but moderate infection was observed upon making a microscopic examination. This fowl had shown marked symptoms upon completion of the incubation period of the parasite and continued so until death. No gross evidence of coccidiosis was shown by the small intestine of No. 89, but the feces contained a moderate amount of cecal content, which was mostly pure blood. Coccidial infection of the small intestine and ceca was found by microscopic examination.

There was pure blood in the feces of Nos. 86 and 87 following the inoculation of September 2. They were kept together; consequently, the feces were not examined individually. Nos. 88 and 89 were showing symptoms from the August 27 inoculation and were not inoculated at this time.

No cause for death could be determined at autopsy of No. 96. Microscopic examinations did not reveal coccidial infection.

Pure blood was passed in moderate amounts by the group consisting of Nos. 86, 87, and 88 on September 18, 19, and 20. These three were kept together, so that individual feces examination was not possible.

Separate fecal examinations were made of Nos. 83 and 84 on October 14. Considerable pure blood was passed and autopsy revealed the ceca of both fowls filled with blood. The small intestines of these two fowls did not contain any pure blood. No. 83 evidenced no gross lesions of coccidiosis in the small intestine. The only gross lesions in that of No. 84 were yellowish or whitish patches in the small-intestine mucosa, which is typical of a percentage of coccidial cases.

On October 17 and 18, No. 81 was distinctly weak and the comb was very pale; No. 82 was distinctly active and the comb apparently normal in color. It should be noted here that No. 82 was inoculated before on August 27 and with a cecal-type suspension.

Nos. 86, 87, 88, 92, 93, and 94 were kept together, and therefore individual feces could not be identified. Three feces containing a small amount of pure blood were noted October 16. None were passed subsequently.

Feces examinations following the October 20 inoculation showed no blood on October 25, 26, 27, or 28, after which time no bloody feces would be expected to be passed from this inoculation. Eight check fowls, four of which were hatched the same date as the resistant fowls and four a week later, inoculated at the same time with the same suspension but only one-half as much, passed considerable amounts of pure blood. Three died of coccidiosis. Autopsy of five check fowls showed cecal infection to be distinctly predominant.

The inoculations made November 3, 1926, with suspension S A did not result in severe coccidiosis. The fowls continued active and no bloody feces were passed. Infection was produced in at least one of the two fowls Nos. 81 and 82, which was determined by smear examinations of feces. Further evidence that this suspension contained oocysts which had lost their original pathogenicity was obtained later. Two fowls hatched June 15 were each inoculated November 26 with 225,000 oocysts from suspension S A. No distinctive symptoms developed. Autopsy of one of these two, December 4, showed typical yellowish patches of small-intestine mucosa due to coccidial infection. Microscopic examination of small intestine smears revealed numerous small type merozoites but none of the large type.

Suspension S A was highly pathogenic August 30, as it proved fatal to two June-7 hatched fowls following inoculation on this date. Each was given 225,000 oocysts. Very severe small-intestine infection was produced.

**Group 6 (Table VI).** Examination of a smear containing material from four cecal feces revealed two oocysts on June 22. No other coccidial forms were found. Seven and twelve oocysts were found in two smears made of material from four and three cecal feces on June 24. A moderate number of merozoites also were found in the second smear.

It was questionable whether or not No. 114 died from coccidiosis or cannibalism. It was probably not due to coccidiosis. In either event

it was found at autopsy that there was distinct infection of the small intestine. A smear made from the small-intestine mucosa revealed large and small type merozoites.

On July 14, No. 107 of this group was droopy. All others appeared active or only very slightly droopy. A moderately bloody fecal discharge was passed July 16. A smear of this showed numerous merozoites and red blood cells. No. 106 was inactive July 16 and No. 108 very droopy. No. 104 was slightly droopy July 17 and No. 107 was very droopy.

Autopsy of No. 108 showed that this fowl died of very severe small-intestine infection. The small intestine was filled with pure blood. Distinct coccidial hemorrhages were noted on the serous surface and dilation of this organ had occurred. Distinct sloughing of the mucosa, characteristic of very severe small-intestine infection was noted. The ceca contained a moderate amount of pure blood and normal cecal content. The blood may have come from the small intestine. There were no visible hemorrhages in the cecal walls. A different picture was presented by No. 107 at autopsy. The small intestine contained no pure blood, but the ceca were filled with it. Small intestine scrapings showed numerous merozoites.

The fowls left on July 18 were apparently well. No bloody feces were being expelled.

Observations following the July 19 inoculations revealed no blood on July 25, 26, and 27, and the chicks continued to be active and showed normal appetite during this period.

August 23 the fowls in this group inoculated August 17 were separated into three groups according to the inoculations which they had received in the past. Nos. 104 and 106, which were in one group, were noted to have passed one considerable discharge of pure blood. Nos. 104 and 106 were separated to study feces August 23. These two were very active and had passed no blood after being separated up to 6 p.m. on this date. On August 24 they both passed blood in moderate amounts. Both were active and continued so.

August 23, Nos. 110 and 112 passed a slight amount of distinctly bloody feces. The feces on August 24 contained very little blood. They were being kept together and their feces were not observed individually. Both were active at this time and had been so.

No. 100 passed a moderate amount of pure blood feces August 23. The second inoculation of this fowl, made September 2, was not followed by bloody feces.

The ceca of No. 102 were bulging with pure blood, but there was none in the small intestine. Before autopsy, No. 101 became considerably decomposed and accurate determinations were not possible. This fowl had discharged considerable pure blood feces following the August 17 inoculation.

Nos. 103 to 106, inclusive, were segregated and kept in a pen together following the September 2 inoculation. Bloody feces in slight amounts were noted from these on September 9.

On October 26, the three groups included in Group 6 were separated so as to study their feces, according to inoculations previously received. No individual feces examinations were made. No bloody feces were passed by Nos. 103 to 106 inclusive, and 109 to 113 inclusive. October 26, 27, and 28 the group 98, 99, and 100 passed considerable pure blood feces. These were, no doubt, passed by Nos. 98 and 99, since 100 had shown distinct resistance to the inoculation of September 2.

## DATA : TABULAR

## Explanation of abbreviations used in tables.

- O=no inoculation.
- D=died—cause not definitely determined.
- DC=died of coccidiosis.
- DC?=death possibly due to coccidiosis.
- DCa=death due to cannibalism.
- Neg.=no coccidial forms noted.
- 1 O., 2 O., etc.=number of oocysts.
- F. O.=few oocysts.
- M. O.=moderate number of oocysts.
- N. O.=numerous oocysts.
- 1 Me., 2 Me., etc.=number of merozoites.
- F. Me.=few merozoites.
- M. Me.=moderate number of merozoites.
- C. F.=commercial flock fowl.
- C. R.=cage-reared fowl.
- N. B.=no blood in feces.
- T. B.=trace of blood in feces.
- S. B.=slight blood in feces.
- M. B.=moderate blood in feces.
- C. B.=considerable blood in feces.

TABLE I. DATA ON GROUP 1

Fowl	Source	Hatch	Inoculated Date	Oocysts	Feces Examinations	Remarks
552-25	C. F.	Feb., 1925	4/16/26	<sup>1</sup> 450,000	<sup>2</sup> 4/22-2 O. 4/26-28-28 <sup>3</sup> -29 Neg. 5/14-14 Neg.	No symptoms. No bloody feces.
10-25	C. F.	Feb., 1925	4/16/26	<sup>1</sup> 450,000	<sup>4</sup> 4/22 N. O. 4/27-27-28 <sup>4</sup> -29 Neg. 5/15-15 Neg.	Became emaciated, probably due to coccidiosis. Regained weight later. Small amount of bloody feces.
864-25	C. F.	Feb., 1925	4/16/26	<sup>1</sup> 450,000	<sup>5</sup> 4/22-23 Neg. 4/23-2 O. 4/26-26-26 Neg. 4/27-2 O. 4/29-1 O.	No symptoms. No blood in feces.
400	C. R.	Apr. 3, 1926	4/16/26	<sup>1</sup> 185,000		Died of coccidiosis, April 21. Severe hemor- rhage from duodenum and free portion.
37-25	C. F.	Feb., 1925	4/23/26	<sup>6</sup> 1,000,000	<sup>4</sup> 4/29 N. O. 5/18-18-20 Neg. 5/20-2 O.	No symptoms or gross evidence in feces.
			10/ 9/26	<sup>7</sup> 200,000		No symptoms or gross evidence in feces. Au- topsy Oct. 20 showed cecal coccidiosis but none of small intestine.
564-25	C. F.	Feb., 1925	4/23/26	<sup>6</sup> 1,000,000	<sup>5</sup> 5/1 F. O. 5/19-19-21 Neg.	No symptoms or gross evidence in feces.
			10/ 9/26	<sup>7</sup> 300,000		No symptoms or gross evidence in feces.
			4/28/27	<sup>8</sup> 290,000		No symptoms or gross evidence in feces.
B 1723	C. R.	July 29, 1925	4/23/26	<sup>6</sup> 1,000,000		Died April 30, possibly due to coccidiosis. Numerous oocysts and merozoites in small intestine.
474-25	C. F.	Feb., 1925	5/18/26	<sup>1</sup> 1,100,000	<sup>5</sup> 5/24-24 O. 6/1 Neg.	No symptoms. No bloody feces. Laid May 25, 27, 28, June 2 and 4. No symptoms. No bloody feces.
			10/ 9/26	<sup>7</sup> 200,000		

580-25	C. F.	Feb., 1925	5/18/26	1,330,000 <sup>1</sup>	5/24 Neg. <sup>5</sup>	No symptoms. No bloody feces. Laid May 27, 28, June 2 and 4.
			10/ 9/26	200,000 <sup>7</sup>	6/4 Neg.	No symptoms. No bloody feces.
			4/28/27	290,000 <sup>8</sup>		No symptoms. No bloody feces.
401	C. R.	Apr. 5, 1926	4/28/26	450,000 <sup>1</sup>		Died of coccidiosis May 24. Severe small-intestine infection with marked hemorrhage into lumen.
405	C. R.	Apr. 5, 1926	4/28/26	225,000 <sup>1</sup>		Died of coccidiosis May 24. Severe hemorrhage into lumen of small intestine.
74-25	C. F.	Feb., 1925	6/ 5/26	550,000 <sup>1, 9</sup>	6/11 M. O. F., Me. 6/13-8 O. <sup>5</sup>	No symptoms. No bloody feces. Killed by other fowls July 2.
440-25	C. F.	Feb., 1925	6/ 5/26	550,000 <sup>1</sup>	6/12-2 Me. 6/13-1 O., 3 Me. <sup>5</sup>	No symptoms. No bloody feces. Killed by other fowls June 26.
1054-25	C. F.	Feb., 1925	6/ 5/26	550,000 <sup>1</sup>	6/11 F. O., M. Me. 6/13-2 O. <sup>5</sup>	No symptoms. No bloody feces.
			10/ 9/26	200,000 <sup>7</sup>		No symptoms. No bloody feces.
28-25	C. F.	Feb., 1925	6/ 5/26	550,000 <sup>1</sup>	6/11 N. O. 6/13-1 O., 4 Me. <sup>5, 10</sup>	No symptoms. No bloody feces. Killed by other fowls June 22.

<sup>1</sup>SA suspension—small-intestine type.<sup>2</sup>Smears were made from cecal feces unless otherwise specified.<sup>3</sup>More than one smear examined the same day.<sup>4</sup>Mixed feces.<sup>5</sup>Small-intestine feces.<sup>6</sup>SB suspension—small-intestine type.<sup>7</sup>SE suspension—cecal type.<sup>8</sup>SF suspension—cecal type.<sup>9</sup>See B1721 and B1728, Table III, for June 5, control fowl inoculations.<sup>10</sup>Small number of red blood cells.

TABLE II. DATA ON GROUP 2

Fowl	Breed	Source	Inoculation		Remarks
			Date	No. oocysts <sup>1</sup>	
943	S. C. White Leg.	Flock A	4/28/27	700,000	N.B. up to May 7. No symptoms.
753	S. C. White Leg.	Flock A	4/28/27	700,000	N.B. up to May 7. No symptoms.
874	R. I. Red	Flock B	4/28/27	700,000	N.B. up to May 7. No symptoms.
1533	R. I. Red	Flock B	4/28/27	700,000	S.B. up to May 7. No symptoms.
232	S. C. White Leg.	Flock C	4/28/27	700,000	N.B. up to May 7. No symptoms.
225	S. C. White Leg.	Flock C	4/28/27	700,000	T.B. up to May 7. No symptoms.
402	S. C. White Leg.	Flock D	4/28/27	700,000	T.B. up to May 7. No symptoms.
A4866	S. C. White Leg.	Flock D	4/28/27	700,000	C.B. up to May 7. Inactive.
			5/ 7/27	850,000	N.B. up to May 16. No symptoms.
A4832	S. C. White Leg.	Flock D	4/28/27	700,000	S.B. up to May 7. No symptoms.
A4860	S. C. White Leg.	Flock E	4/28/27	700,000	C.B. Died of cecal coccidiosis May 4.
A4840	S. C. White Leg.	Flock E	4/28/27	700,000	M.B. up to May 7. No symptoms.
			5/ 7/27	850,000	N.B. up to May 16. No symptoms.
A3066	R. I. Red	Flock F	4/28/27	700,000	C.B. Died of cecal coccidiosis May 5.
A4645	R. I. Red	Flock G	4/28/27	700,000	C.B. up to May 7. Inactive.
			5/ 7/27	850,000	T.B. in one dropping only—May 14.
A4062	B. Rock	Flock H	4/28/27	700,000	N.B. up to May 7. No symptoms.
			5/ 7/27	850,000	N.B. up to May 16. No symptoms.
A4315	B. Rock	Flock H	4/28/27	700,000	T.B. up to May 7. No symptoms.
			5/ 7/27	850,000	N.B. up to May 16. No symptoms.
A4374	B. Rock	Flock I	4/28/27	700,000	N.B. up to May 7. No symptoms.
			5/ 7/27	850,000	N.B. up to May 16. No symptoms.
A4379	B. Rock	Flock I	4/28/27	700,000	N.B. up to May 7. No symptoms.
			5/ 7/27	850,000	N.B. up to May 16. No symptoms.

<sup>1</sup>SF suspension—cecal type.

TABLE III. DATA ON INOCULATIONS AND MORTALITY OF GROUP 3

Fowl	Sex	Hatch	5/12/26	5/13-18	5/19	5/20	5/21	5/22-29	5/30-31	6-1	6/2-4	6/5 <sup>a</sup>	6/6-14	6/15	6/16-19	6/20	6/21-26
403	F	4/ 5/26	675,000 <sup>2</sup>	DC-5/17 <sup>2,4</sup>	--	----- <sup>2</sup>	----- <sup>2</sup>	----- <sup>2,4</sup>	--	----- <sup>2</sup>	----- <sup>2,4</sup>	----- <sup>2</sup>	-----	-----	-----	-----	--
B1722	M	7/29/25	550 <sup>2</sup>	550 <sup>2,4</sup>	0	1,100 <sup>2</sup>	1,650 <sup>2</sup>	1,375 <sup>2,4</sup>	0	1,650 <sup>2</sup>	2,750 <sup>2,4</sup>	550,000 <sup>2</sup>	0	0 <sup>2</sup>	0	0 <sup>2</sup>	0
B1718	F	7/29/25	550	550	0	1,100	1,650	1,375	0	1,650	2,750	0	0	75,000	0	200,000	0
B1719	F	7/29/25	0	0	0	0	0	0	0	0	0	0 <sup>2</sup>	0	0	0	0	0
B1721	M	7/29/25	0 <sup>3</sup>	0	0	0	0	0	0	0	0	550,000	DC-6/11	-----	--	-----	--
404 <sub>1</sub>	M	4/ 5/26	460,000 <sup>3</sup>	DC-5/16 <sup>3,4</sup>	--	----- <sup>3</sup>	----- <sup>8</sup>	----- <sup>8,4</sup>	--	----- <sup>3</sup>	----- <sup>8,4</sup>	----- <sup>2</sup>	-----	-----	-----	-----	--
B1725 <sub>1</sub>	F	2/10/26	3,800 <sup>3</sup>	3,800 <sup>3,4</sup>	0	7,600 <sup>3</sup>	11,400 <sup>3</sup>	9,500 <sup>8,4</sup>	0	11,400 <sup>3</sup>	19,000 <sup>3,4</sup>	550,000	0	0 <sup>2</sup>	0	0 <sup>2</sup>	0
B1726 <sub>1</sub>	F	2/10/26	3,800	3,800	0	7,600	11,400	9,500	0	11,400	19,000	0	0	75,000	0	200,000	0
B1727 <sub>1</sub>	F	2/10/26	0	0	0	0	0	0	0	0	0 <sup>2</sup>	0	0	0	0	0	0
B1728	F	2/10/26	0	0	0	0	0	0	0	0	0	550,000	DC-6/11	-----	--	-----	--

TABLE III. DATA ON INOCULATIONS AND MORTALITY OF GROUP 3 (CONTINUED).

Fowl	6/27 <sup>a</sup>	6/28-7/3	7/4-9/11	9/12 <sup>a</sup>	9/13-19	9/20 <sup>b</sup>	9/21-10/8	10/9 <sup>a</sup>	10/10-26
403.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
B1722.....	2,100,000	0	0	50,000	0	100,00	0	200,000	D <sup>a</sup>
B1718.....	2,100,000	0	0	50,000	0	100,00	0	200,000	D <sup>a</sup>
B1719.....	2,100,000	DC-7/3	--	-----	--	-----	--	-----	-----
B1721.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
404.....	-----	-----	-----	-----	-----	-----	-----	-----	-----
B1725.....	2,100,000	0	0	50,000	0	100,00	0	200,000	0
B1726.....	2,100,000	0	0	50,000	D <sup>†</sup>	-----	--	-----	-----
B1727.....	2,100,000	DC-7/3	--	-----	--	-----	--	-----	-----
B1728.....	-----	-----	-----	-----	-----	-----	-----	-----	-----

<sup>1</sup>Rhode Island Reds.<sup>2</sup>SA suspension—small-intestine type.<sup>3</sup>SC suspension—small-intestine type.<sup>4</sup>Daily inoculations.<sup>5</sup>Inoculations on this date made from same suspension as June 5 inoculations of Group 1.<sup>6</sup>SE suspension cecal type.<sup>7, 8, 9</sup> B1726, B1722, and B1718 died September 18, October 11, and 26, respectively.



TABLE IV. DATA ON INOCULATIONS AND MORTALITY OF GROUP 4 (CONTINUED)

Fowl	8/17	8/18-21	8/22-9/28- 9/1 10/23	10/24	10/25-30	10/31	11/1-6	11/7	11/8-10	11/11	11/12	11/13	11/14	11/15-18	11/19-25	11/26	11/27-12/3	12/4-31
1...	2,000	2,000	0	2,000	0	2,000	0	2,000	0	2,000	2,000	2,000	0	2,000	0	400,000	0	0
2...	2,000	2,000	0	2,000	0	2,000	0	2,000	0	2,000	2,000	2,000	0	2,000	0	400,000	0	0
3...	2,000	2,000	0	2,000	0	2,000	0	2,000	0	2,000	2,000	2,000	0	2,000	0	400,000	0	0
4...	2,000	2,000	0	2,000	0	2,000	0	2,000	0	2,000	2,000	2,000	0	2,000	0	400,000	0	0
5...	2,000	2,000	0	2,000	0	2,000	0	2,000	0	2,000	2,000	2,000	0	2,000	0	400,000	0	0
6...	2,000	2,000	0	2,000	0	2,000	0	2,000	0	2,000	200,000	0	0	0	0	0	0	0
7...	2,000	2,000	0	2,000	0	2,000	0	2,000	0	2,000	200,000	0	0	0	0	0	0	D <sup>12</sup>
8...	2,000	2,000	0	2,000	0	2,000	0	2,000	0	2,000	400,000	0	0	0	0	0	0	0
9...	2,000	2,000	0	2,000	0	2,000	0	2,000	0	2,000	400,000	0	0	0	0	0	0	0
10...	2,000	2,000	0	2,000-D <sup>8</sup>	--	-----	--	-----	--	-----	-----	-----	--	-----	--	-----	--	-----
11...	2,000	2,000	0	2,000-D <sup>10</sup>	--	-----	--	-----	--	-----	-----	-----	--	-----	--	-----	--	-----
12...	2,000	D <sup>7</sup>	--	-----	--	-----	--	-----	--	-----	-----	-----	--	-----	--	-----	--	-----
13...	200,000 <sup>6</sup>	0	0	0-DC-9/5	--	-----	--	-----	--	-----	-----	-----	--	-----	--	-----	--	-----
14...	200,000 <sup>6</sup>	0	0	0-DC-9/11	--	-----	--	-----	--	-----	-----	-----	--	-----	--	-----	--	-----
15...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	400,000	0	DC-12/4
16...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	400,000	0	0
17...	0	0	0	0	0	0	0	0	0	0	400,000	0	0	0	0	0	0	0
18...	0	0	0	0	0	0	0	0	0	0	400,000	0	0	0	0	0	0	D <sup>14</sup>
19...	0	0	0	0-D <sup>11</sup>	--	-----	--	-----	--	-----	-----	-----	--	-----	--	-----	--	-----
20...	0	0	0	0-D <sup>12</sup>	--	-----	--	-----	--	-----	-----	-----	--	-----	--	-----	--	-----

<sup>1</sup>Number of sporulated oocysts given daily of SA suspension—small-intestine type—May 21 to August 21 inclusive, with exception noted (<sup>6</sup>).<sup>2</sup>Fowl 34 died May 28.<sup>3, 4</sup>Fowls 15, 16, and 17 died August 14, 10 and July 19, respectively.<sup>6</sup>SD suspension—cecal type.<sup>7</sup>Fowl 12 died August 21.<sup>8</sup>SE suspension—cecal type—September 2 to November 26, inclusive.<sup>9, 10, 11, 12</sup>Fowls 10, 11, 39 and 40 died October 7, September 5, October 7 and 17, respectively.<sup>13, 14</sup>Fowls 7 and 38 died December 27 and 31, respectively.

TABLE V. DATA ON INOCULATIONS AND MORTALITY OF GROUP 5

Fowl	6/7-16	6/17*	6/18-20	6/21	6/22-25	6/26	6/27-30	7/1	7/2-4	7/5	7/6-9	7/10	7/11-18	7/19	7/20-8/16
81.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
83.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
84.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85.....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
86.....	0	1,500	0	4,000	0	8,000	0	16,000	0	40,000	0	120,000	0	200,000	0
87.....	0	1,500	0	4,000	0	8,000	0	16,000	0	40,000	0	120,000	0	200,000	0
88.....	0	1,500	0	4,000	0	8,000	0	16,000	0	40,000	0	120,000	0	200,000	0
89.....	0	1,500	0	4,000	0	8,000	0	16,000	0	40,000	0	120,000	0	200,000	0
90.....	0	1,500	0	4,000	0	8,000	0	16,000	0	40,000	0	120,000	0	200,000	0
91.....	0	1,500	0	4,000	0	8,000	0	16,000	0	40,000	0	120,000	0-DC-7/14 0-DCa-7/13	-----	-----
92.....	0	1,500	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
93.....	0	1,500	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
94.....	0	1,500	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
95.....	0	1,500	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
96.....	0	1,500	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
97.....	0	1,500	2,000	2,000	2,000	DC	-----	-----	-----	-----	-----	-----	-----	-----	-----

TABLE V. DATA ON INOCULATION AND MORTALITY OF GROUP 5 (CONTINUED)

Fowl	8/17-26	8/27†	8/28-9/1	9/2‡	9/3-11	9/12	9/13-19	9/20	9/21-10/8	10/9	10/10-19	10/20	10/21-11/2	11/3*
81.....	0	0	0	0	0	0	0	0	0	200,000	0	400,000	0	200,000
82.....	0	200,000	0	0	0	0	0	0	0	200,000	0	400,000	0	200,000
83.....	0	0	0	0	0	0	0	0	0	200,000	0-DC-10/15	-----	-----	-----
84.....	0	0	0	0	0	0	0	0	0	200,000	0-DC-10/15	-----	-----	-----
85.....	0	200,000	0	0	DC-9/8	-----	-----	-----	-----	-----	-----	-----	-----	-----
86.....	0	0	0	50,000	0	100,000	0	100,000	0	200,000	0	400,000	0	200,000
87.....	0	0	0	50,000	0	100,000	0	100,000	0	200,000	0	400,000	0	200,000
88.....	0	200,000	0	0	0	100,000	0	100,000	0	200,000	0	400,000	0	200,000
89.....	0	200,000	0	0	DC-9/5	-----	-----	-----	-----	-----	-----	-----	-----	-----
90.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
91.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
92.....	0	0	0	2,000	2,000	2,000	2,000	2,000	2,000	200,000	2,000	400,000	0	200,000
93.....	0	200,000	0	2,000	2,000	2,000	2,000	2,000	2,000	200,000	2,000	400,000	0	200,000
94.....	0	200,000	0	2,000	2,000	2,000	2,000	2,000	2,000	200,000	2,000	400,000	0	200,000
95.....	0	0	0	2,000	2,000	2,000	2,000	2,000	2,000-DC-9/25	-----	-----	-----	-----	-----
96.....	0	0	0	D	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
97.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

\*SA suspension—small-intestine type—for all inoculations up to August 27 and on November 3.

†SD suspension—cecal type.

‡SE suspension—cecal type—for all inoculations after August 27, except November 3.

TABLE VI. DATA ON INOCULATIONS AND MORTALITY OF GROUP 6

Fowl	6/7-16	6/17*	6/18-20	6/21	6/22-25	6/26	6/27-30	7/1	7/2-4	7/5	7/6-9	7/10	7/11-16	7/17
98	0	0	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0	0	0	0
103	0	1,500	0	4,000	0	8,000	0	16,000	0	40,000	0	120,000	0	0
104	0	1,500	0	4,000	0	8,000	0	16,000	0	40,000	0	120,000	0	0
105	0	1,500	0	4,000	0	8,000	0	16,000	0	40,000	0	120,000	0	0
106	0	1,500	0	4,000	0	8,000	0	16,000	0	40,000	0	120,000	0	0
107	0	1,500	0	4,000	0	8,000	0	16,000	0	40,000	0	120,000	0	DC
108	0	1,500	0	4,000	0	8,000	0	16,000	0	40,000	0	120,000	0	DC
109	0	1,500	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
110	0	1,500	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
111	0	1,500	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
112	0	1,500	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
113	0	1,500	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
114	0	1,500	2,000	2,000	2,000	2,000	2,000-DC 7-6/28	-----	-----	-----	-----	-----	-----	-----

TABLE VI. DATA ON INOCULATIONS AND MORTALITY OF GROUP 6 (CONTINUED)

Fowl	7/18	7/19	7/20-8/16	8/17†	8/18-9/1	9/2‡	9, 3-11	9/12	9/13-19	9/20	9/21-10/12	10/13-19	10/20
98.....	0	0	0	0	0	0	0	0	0	0	0	0	200,000
99.....	0	0	0	0	0	0	0	0	0	0	0	0	200,000
100.....	0	0	0	200,000	0	550,000	0	0	0	0	0	0	200,000
101.....	0	0	0	200,000	0-DC 8/25	-----	-----	-----	-----	-----	-----	-----	-----
102.....	0	0	0	200,000	0-DC 8/23	-----	-----	-----	-----	-----	-----	-----	-----
103.....	0	200,000	0	0	0	50,000	0	100,000	0	100,000	0	0	200,000
104.....	0	200,000	0	200,000	0	50,000	0	100,000	0	100,000	0	0	200,000
105.....	0	200,000	0	0	0	50,000	0	100,000	0	100,000	0	0	200,000
106.....	0	200,000	0	200,000	0	50,000	0	100,000	0	100,000	0	0	200,000
107.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
108.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
109.....	2,000	2,000	2,000	0	0	2,000	2,000	2,000	2,000	2,000	2,000	0	200,000
110.....	2,000	2,000	2,000	200,000	0	2,000	2,000	2,000	2,000	2,000	2,000	0	200,000
111.....	2,000	2,000	2,000	0	0	2,000	2,000	2,000	2,000	2,000	2,000	0	200,000
112.....	2,000	2,000	2,000	200,000	0	2,000	2,000	2,000	2,000	2,000	2,000	0	200,000
113.....	2,000	2,000	2,000	0	0	2,000	2,000	2,000	2,000	2,000	2,000	0	200,000
114.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

\*SA suspension—small-intestine type—up to and including August 16 inoculations.

†SD suspension—cecal type—7/23.

‡SE suspension—cecal type—9/2 and later.

## INTERPRETATION OF DATA

**Resistance determinations.** Considering the number (as determined by smear examinations) of coccidial forms or amount of blood in the feces as criteria in determining the degree of infection, it is obvious that most of the commercially reared fowls were highly resistant and some possibly immune. Since the smear examinations of the feces may not have been sufficiently exhaustive, it is probable that some fowls from which coccidial forms were not noted were not immune. The difference between a fowl which is immune and one which is highly resistant does not appear to be of much consequence so far as economic poultry production is concerned.

**Relation of age to resistance.** The high degree of resistance shown by the commercially reared fowls could not have been due to age. This was demonstrated when mature fowls No. 10-25 (Table I) and A 4866, A 4860, and A 3066 (Table II) became severely affected. Cage-reared fowls, including mature ones, moreover, when not previously inoculated were regularly susceptible. The fowls in Group 2 (Table II) were inoculated at approximately the same age or older than those in Group 1 (Table I.) Cage-reared fowl B 1725 (Table III) which was inoculated when five months younger than those in Group I, was equally or nearly as resistant. Other experimentally inoculated fowls which were highly resistant or immune at seven months or less of age are: B 1718, B 1722, and B 1726 (Group 3); Nos. 1 to 9, inclusive (Group 4); Nos. 81, 82, 86, 87, 88, 92, 93, 94 (Group 5) and Nos. 98, 99, 100, 103, 104, 105, 106, 109, 110, 111, 112, 113 (Group 6).

Besides A 4866, A 4860, and A 3066, one other in Group 2 passed considerable pure blood and became slightly inactive, but recovered quickly. Six passed small amounts of blood, but otherwise showed no evidence. The remaining seven gave no clinical evidence that infection had taken place. Thus thirteen of the seventeen were highly resistant, of which some were possibly immune. No smears were made of the feces of any in Group 2.

**Cage-rearing and susceptibility.** Fowls reared in cages and not previously experimentally inoculated were almost without exception highly susceptible to both small-intestine and cecal types of infection.

**Effect of two types of suspensions.** Using suspensions which were cecal or small-intestine in type permitted studying each type of infection separately as well as studying the reciprocal relationship of cecal and small-intestine infection.

All in Group 2 were inoculated with a suspension of predominantly cecal type and Group 1 with a suspension of predominantly small-intestine type.

The data indicate that inoculation with small-intestine type does not produce resistance to cecal infection. This was demonstrated by Nos. 13 and 14 (Table IV). These fowls had been inoculated with 112,716 small-intestine type oocysts between May 21 and August 16, receiving a maximum of about 2,000 and a minimum of 44 per day. On August 17

they were inoculated with 200,000 oocysts of the cecal type suspension S E along with two check fowls. There was no significant difference in severity of coccidiosis in Nos. 13 and 14, as compared with the check fowls. All four passed considerable pure blood and died of coccidiosis. Group 3 previously inoculated fowls proved very resistant to the June 27 inoculation of the small-intestine type, but distinctly susceptible to the cecal type following the September 12, 20, and October 9 inoculations. This is in agreement with other data obtained at this laboratory.

**Effect of moderate inoculations.** Cage-reared fowls given less than 2,000 oocysts daily evidenced no ill effects. This was demonstrated by the early inoculations of Group 4. Nos. 21 (Table IV), 97 (Table V), and 114 (Table VI) died upon being given, with a few exceptions, 2,000 oocysts daily. Autopsy showed all three to be infected. Death of Nos. 21 and 97 was due to coccidiosis. It is probable that No. 114 did not die of coccidiosis. No. 21 had been given a total of 25,000, from June 21 to July 5, dying of coccidiosis on the latter date. Death was probably due to the action of less than 20,000 oocysts of the small-intestine type. The fowl had received 3,716 oocysts from this same suspension from May 21 to June 16, not receiving more than 774 nor less than 44 any day. No. 97 died from the effects of possibly less than 10,000 oocysts given during the period June 17 to June 21, inclusive, and No. 114 was inoculated with 19,500 oocysts from June 17 to June 23, inclusive.

It is probable that Nos. 21 and 97 were very susceptible to coccidial infection. This is not unexpected, since occasionally fowls also show what appears to be an inherited resistance.

The inoculations with 2,000 or more oocysts daily resulted in immunity or a high degree of resistance in all surviving fowls when sufficient numbers had been given. Nos. 6, 7, 8, and 9 (Table IV) evidenced immunity or marked resistance to cecal infection. Each had been given not to exceed 2,000 oocysts daily, from September 2 to November 10, of a cecal type suspension (S E) to a total of 134,000. Nos. 1, 2, 3, 4, and 5 (Table IV) were inoculated the same as Nos. 6, 7, 8, and 9 up to November 10 and continued to be inoculated up to November 18, to make a total of 146,000 oocysts. Nos. 1, 2, 3, 4, 5, and two check fowls (35 and 36) were inoculated November 26, with 400,000 oocysts, Nos. 6 and 7 with 200,000, and Nos. 8 and 9 and two check fowls (37 and 38), November 12, with 400,000 oocysts. Those previously inoculated were immune or highly resistant at this time, and the check fowls highly susceptible. Nos. 1 to 9 inclusive had each received 122,716 oocysts of small-intestine type, previous to August 22. This suspension had produced some infection of the ceca. The maximum number of oocysts, including both types, given any fowl (excluding Nos. 13, 14, 31, 32 and 33) of this group up to November 10, was 256,716.

Moderate numbers of oocysts given daily, or nearly so, developed resistance with distinctly less manifestation than when large numbers were given at varying intervals. With the exception of Nos. 21 (Table IV), 97 (Table V), and 114 (Table VI), none of those receiving 2,000 oocysts daily showed any symptoms that were noted.

**Inoculations necessary to produce resistance.** Besides predilection of the parasite, an important determining factor is the number of oocysts

ingested. One or more inoculations do not necessarily produce a marked resistance to a subsequent one. Several inoculations of Nos. 31, 32, and 33 (Table IV) produced no recognizable resistance. They had been given daily inoculations from May 21 to June 5, 44 oocysts being the minimum and 166 the maximum number. A total of 826 oocysts in a suspension of the small-intestine type had been given during this time. The above three fowls were inoculated June 7 with 225,000, 113,000 and 113,000 oocysts, respectively, using the same suspension as when inoculating them previously. Fatal coccidiosis resulted in all three.

This brings out what appears to be a point of difference in comparison with coccidial resistance production in some other animals. Andrews<sup>18</sup> investigations with cats and dogs led him to suggest that one inoculation regularly establishes a high degree of resistance or immunity in these species. One inoculation of the chicken is very unlikely to confer immunity such as Andrews describes.

In instances where the fowls survived ingestion of a large number of oocysts, considerable resistance was shown to subsequent inoculations of the same type of suspension. This was demonstrated by commercially reared fowls A 4866, A 4840, and A 4645 (Table II). The same was true of Nos. 82 (Table V) and 100 (Table VI). This evidence is substantiated by other data at this laboratory.

**Time required for resistance production.** Four of the above five fowls provided data which established that a comparatively short time is necessary for developing a high degree of resistance. The second inoculation of these fowls, in which they demonstrated that they were highly resistant, took place as follows: A 4866, A 4860, and A 4645, nine days after previous inoculation, at which time they were distinctly susceptible, and No. 100, sixteen days after previous inoculation. These inoculations were all of the cecal type.

**Duration of immunity.\*** Nos. 564-25 and 580-25 (Group I) remained highly resistant if not immune in connection with the inoculation of April 28, 1927. The last previous experimental inoculation was October 9, 1926, at which time they showed no gross infection. The interval between these inoculations was six and one-half months. This resistance was probably maintained for a longer period.

**Natural resistance production a chance occurrence.** The production of resistance affords one answer to the question occasionally raised as to why some poultry raisers continue to be successful year after year, when using the same runway, particularly for rearing. Coccidial control under such circumstances has, as a rule, been a matter of chance.

\*Eleven fowls were inoculated October 13, 1927 (since the preparation of this manuscript) with two hundred and ninety thousand oocysts of suspension S F (cecal type). These consisted of the following: B 1725 (Group 3); Group 5 fowls, 87, 92, and 94; Group 6 fowls, 103, 104, 111, 112, and 113 and two cage-reared control fowls hatched May 23, 1927. No. 103, which had previously proved highly resistant, succumbed to cecal coccidiosis. The two control fowls died of cecal coccidiosis. The remaining fowls continued to be highly resistant and some possibly immune. No satisfactory explanation can be offered at present for the susceptibility shown by No. 103 to the October 13, 1927 inoculation. It is possible that the fowl designated above as No. 103 was actually another fowl. Accidental liberation of three pens of fowls about nine months previous to the October 1927 inoculation may have resulted in the identity of No. 103 being lost. The last experimental inoculation of any of the nine was eleven to twelve months prior to that of October, 1927.

Apparent freedom from coccidiosis when using the same yard year after year has led some to regard soil contamination as unimportant in coccidial infection. Personal observation has revealed that statements of this nature must be accepted with reserve, as severe outbreaks of coccidiosis have been found in connection with establishments supposedly continuously successful and free of this disease. When no trouble occurred resistance probably developed following repeated inoculations with small numbers of oocysts. Where serious trouble developed it would signify ingestion of large numbers of oocysts as an initial inoculation, with none or a comparatively small number previously ingested.

**Relation of inheritance to resistance.** The statement is sometimes made that repeated annual occurrences of coccidiosis in connection with breeding establishments will eventually produce a resistant strain. The fact that resistance may be developed by repeated natural inoculations with small numbers of oocysts tends, however, to prevent resistance occurring through breeding. In such instances fowls which are naturally susceptible may acquire resistance but produce susceptible offspring. Large numbers of oocysts given at one time with slight or no previous infection consistently destroy susceptible young fowls. Selecting *young* chicks which survive such inoculations may afford a means of producing resistant strains.

**Mechanism of coccidial resistance.** The mechanism of coccidial resistance was not definitely determined, but the data indicate that this is not a problem of anti-body formation. It would appear that a mechanical resistance is developed. This view is supported by the following: first, repeated inoculations (Group 4) over an extended period were required to develop marked resistance with small numbers of oocysts administered daily; second, survival (Groups 2 and 6) following infection produced by a large number of oocysts at one time resulted in marked resistance in a few days to the same type; third, (Groups 3 and 4) fowls highly resistant to a given suspension continued to develop coccidial forms in small numbers following ingestion of this same suspension; and fourth, fowls (Group 3) highly resistant to small-intestine infection were susceptible to cecal infection. None of the data contradicted the hypothesis that immunity involved infection of all parts of the intestinal tract at some time or other.

Coccidial infection was the only cause studied as a factor in producing resistance. It is not impossible that other agencies may also produce resistance to this infection.

**Effect of coccidial infection as to health.** The present study was conducted primarily to obtain data as to the production of resistance. Incidentally some information was obtained as to the relationship of resistance production to the general health. Since this constitutes a very important problem requiring further study it is planned to continue investigations into this phase. Further consideration is therefore withheld for the future.

**Evaluation of coccidiosis control methods.** Knowing that coccidial infection produces resistance provides a possible standard of evaluating

the success of coccidiosis control methods. Fowls reared under conditions permitting considerable coccidial infection should be highly resistant, while with the reverse true, marked susceptibility would be expected. The value of the highly artificial methods of brooding and rearing being developed and recommended at present for the control of coccidiosis can not be determined without studying their relation to the resistance problem.

## CONCLUSIONS

Commercially reared fowls may develop fatal coccidiosis after maturity, but more often possess a high degree of resistance if not immunity.

Cage-reared fowls may with few exceptions be maintained very susceptible to coccidiosis up to and including maturity.

A high degree of resistance, if not immunity, may be regularly developed by experimental inoculation.

## SUMMARY

1. In the present experiments 346 commercially and cage-reared fowls were included.

2. Some commercially reared mature fowls were found to show considerable susceptibility to coccidial infection, but more showed marked resistance, if not immunity.

3. A high degree of resistance to coccidial infection was regularly produced experimentally in both developing and mature cage-reared fowls. An equal degree of susceptibility was, almost without exception, maintained when desired by proper management.

4. Suspensions were used which regularly produced predominant cecal infection.

5. Suspensions were used which regularly produced predominant small-intestine infection.

6. No reciprocal relationship between small-intestine and cecal infection was indicated.

7. One or more inoculations did not necessarily produce a clinically observable resistance to a later inoculation.

8. Resistance to coccidial infection was dependent upon the degree of infection, as well as predilection of the parasite.

9. A high degree of resistance to cecal infection was produced in very susceptible fowls within fifteen days from the time of previous inoculation.

10. Infection of the ceca and small intestine was simultaneously produced.

11. Daily inoculations with two thousand or less sporulated oocysts resulted in resistance with less manifestation of disease than when larger numbers were given at greater intervals.

12. There was no apparent difference in predilection of the parasite of a given suspension regardless of whether given to fowls of brooder age or older.

13. Two fowls were highly resistant at least six and one-half months after the final inoculation.

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