INFLUENCE OF A VITAMIN K ACTIVE SUBSTANCE ON INFECTIONS OF *EIMERIA TENELLA* IN CHICKENS

by

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TABLE OF CONTENTS

I. INTRODUCTION AND STATEMENT OF PROBLEM

Eimeria tenella (Railliet and Lucet, 1891), the causative agent of cecal coccidiosis is undoubtedly the most thoroughly studied of the eight known species of Eimeria of chickens; in fact, of all the species of the order Coccidia.

Its complete life cycle was delineated in the superlatively executed studies of Tyzzer (1929). Numerous authors have subsequently contributed to knowledge concerning circumstances associated with its life history, and the conditions associated with or caused by it in the chicken. Cytological, histochemical, and physiological studies have been made on the various stages of its life cycle. Histopathology, immunology, and environmental control of the infection have also been investigated.

Recently, a tremendous amount of research has been directed toward preventive and curative therapeutics. These studies have involved the role of diet, use of sulfa drugs, and many other chemical compounds and antibiotics. In addition, age of host, vaccines, genetic factors, sub-clinical infections, and attenuated oocysts have been studied in connection with the development of immunity in birds.

The length of time between inoculation and death from cecal coccidiosis was determined by Rosenberg and Herrick (1951). They showed that if the time of inoculation was changed from 11 A.M. to 11 P.M. the time required for death

to occur, or mortality rate to reach its peak was shifted from 121 hours to 127 hours.

Several investigators have studied the blood volume, blood plasma volume, erythrocyte number, hemoglobin, and blood sugar during cecal coccidiosis infections in chickens. Their findings will be discussed later.

Couch (1954) stated that additional vitamin K was needed by chickens during periods of stress, such as that produced by light cecal coccidiosis infections. He suggested that extra amounts of vitamin K be given to chickens during this time.

Frost and Spruth (1955) made the following statement: "Treatment for coccidiosis may actually accentuate the tendency to hemorrhage by increasing the need for vitamin K." They also noted that an increased need for vitamin K was present during periods of stress.

Harms and Tugwell (1956) stated that no mortality occurred in chickens from cecal coccidiosis infections if the birds were fed a vitamin K active material (menadione) at one gram per ton of feed beginning at one day of age. If, however, they received no additional vitamin K until they were three weeks of age, then 20 grams of menadione per ton of diet started at time of inoculation had no effect on mortality rate from cecal coccidiosis.

Baldwin, Wiswell, and Jankiewicz (1941) postulated that additional vitamin K, given to chickens under certain

conditions, might be beneficial in building-up their prothrombin level. They were subsequently able to show that birds inoculated with E. tenella oocysts and then given an excessive amount of vitamin K in the form of a vitamin K active material suffered a much lower mortality rate than did inoculated birds not receiving additional vitamin K in their diet.

The purpose of the present work was to study the effect of administration of oral doses of a vitamin K active substance on the cecal hemorrhage occurring in chickens inoculated with E. tenella, and to re-evaluate and supplement the reports of Baldwin, Wiswell, and Jankiewicz (1941) and Harms and Tugwell (1956).

That the hemorrhagic phase of E. tenella infections has been neglected is evident by the want of literature on this aspect of the cecal coccidiosis syndrome. While this study was not intended to be a comprehensive study of all the various facets associated with the hemorrhagic condition in fowls, it was hoped that the results would increase knowledge in this area.

II. REVIEW OF LITERATURE

A. General Review of Cause of Hemorrhage

Tyzzer (1929) was apparently the first to recognize and prove that a single and distinct parasitic species of the genus Eimeria caused cecal coccidiosis of chickens. He described the pathology associated with the infection, time involved for different stages in the life cycle to develop, immune processes (natural, cross, and active types), and cause of mortality. Mayhew (1937) confirmed the work of Tyzzer and presented a detailed microscopic and macroscopic study of the ceca in infected birds.

One of the causes of mortality in tenella infections is assumed to be the excessive loss of blood as the second generation schizonts approach maturity. Tyzzer (1929) gave an excellent description of this process. He felt that the hemorrhage resulted from a widespread leakage of blood vessels rather than from local extravasation. The hemorrhage caused sloughing of the mucosa which reached a peak by the fifth day after infection and corresponded with liberation of the merozoites. He also reported eosinophilic infiltration in the area of the glands invaded by the first generation merozoites. If large areas of the mucosa were damaged, a pronounced inflammatory change would occur, including extensive infiltration of the tissue primarily with lymphoid and plasma cells, and an increase in connective tissue.

The leakage developing in the blood vessels is thought to result from pinching or rupture of the capillary walls from pressures produced by the enlargement of the epithelial cells in which the second-generation schizonts, which invade the lamina propria, have been maturing. It is also possible that heparin, or a heparin-like material, is released from the invaded and adjoining cells as a result of injury. Goodman and Gilman (1955) suggested the possibility of heparin being present in the mast cells of Ehrlich in connective tissue surrounding capillaries and small blood vessels. Best and Taylor (1955) stated that heparin is found in the intestinal wall in relatively large amounts. If sufficient heparin is present, it could be considered a factor in producing conditions conducive to hemorrhage, or in prolonging bleeding by preventing the formation of thrombin.

Assuming that hemorrhage is the main factor contributing to mortality in cecal coccidiosis, it follows that alleviation of the hemorrhage should reduce both morbidity and mortality. Research on cecal coccidiosis, however, has been concerned primarily with the prevention of infection, and the arresting of an infection once it has occurred. Preventive measures are those of sanitation, immunization, and diet. Sanitation includes rotation of the range, spraying the brooder houses, and correct management of litter. Immunization procedures, still in the research stage, cover natural immunity through development of resistant strains of chickens,

and active immunity resulting from infections produced by small numbers of oocysts, "attenuated" oocysts, or by use of "vaccines". The diet is important in keeping the chicks healthy and possibly less susceptible to infection. Coccidiostatic and coccidicidal drugs are sometimes included in the feed or drinking water. In this way infections may be prevented, or if not prevented, held to low levels of morbidity.

Control of infections is, of course, concerned with preventing mortality and morbidity. Many drugs, to be discussed later, have been tested for their coccidiostatic and coccidicidal activities against E. tenella. None is utilized primarily for the prevention of hemorrhage, but several are important in slowing-down, or interrupting some stage of the life cycle.

B. Diet

The administration of drugs with feed or water will be considered under therapy. For a review of the available literature on the effect of diet on cecal coccidiosis, reference should be made to Becker (1952) and a publication by Merck and Co. (1953). Two papers concerned with diet are included here, as they appear to be related to this research problem.

Allen (1932) used two diets in her work. One was high in vitamins and proteins; the other, low in these respects.

She found decreased oocyst production for the first five days after appearance of oocysts in the feces, then an increase on the sixth and seventh day, in chicks on the diet containing a high vitamin and protein content. A five percent mortality was reported for these birds, as compared to a 23 percent mortality for the birds on the low vitamin and protein diet. After 18 days, the weight of the birds fed the diet high in vitamins and proteins was, however, only 79 percent that of the surviving birds on the low vitamin and protein diet.

Edgar and Herrick (1944) recorded the following from a dietary study: Chickens having access to feed at all times were more resistant to infection than those fed only after 7 A.M. The presence of feed in the digestive tract of chickens, when they were being inoculated, made them more resistant to infection than when the digestive tract was empty.

C. Sanitation

In the dissemination of oocysts, the importance of litter and soil on which chickens range has been fully recognized in the past.

Koutz (1953) found oocysts of E. acervulina, E. maxima, and E. tenella viable in built-up litter for a period of at least one year after contamination.

Berg, Hamilton, and Bearse (1956a) demonstrated immunity

in chicks raised on old built-up litter containing coccidia oocysts. No outbreaks of coccidiosis occurred in these birds. This was probably due to development of immunity from a low-grade infection before a serious outbreak could occur. Outbreaks of coccidiosis did occur, however, in chicks raised on new litter containing oocysts.

Horton-Smith, Taylor, and Turtle (1940) suggested the use of gaseous ammonia as a spray at the rate of three ounces for each ten cubic feet of poultry house as a control measure. Andrews (1933) used Toxite, identified only as a coal tar acid, to spray the litter. It was effective in controlling coccidiosis for four, but not five weeks. Weekly spraying controlled E. tenella, E. maxima, E. acervulina, and E. necatrix.

Patterson (1933) found oocysts viable under varying conditions of pH, temperature, soil texture, and humidity for periods up to 21 weeks. In direct sunlight the oocysts survived slightly longer than nine hours.

Gorrie (1950) stated that rigid sanitary conditions would not prevent an outbreak of coccidiosis in some cases, and that over-heating of the brooder house might be a factor favoring these outbreaks.

Warner (1933) was able to infect chickens with sporulated oocysts that had been in the soil up to 197 days. His results agree generally with those of Patterson as reported above.

Delaplane and Stuart (1935), using mixed species of coccidia, found oocysts surviving in soil from four to nine months after removal of chickens. In wooded areas oocysts were still viable after 15 to 18 months. Oocysts recovered from sod plots were non-infective after six months.

D. Immunity

Studies on the development of immunity to the etiological agent of cecal coccidiosis in chickens have been pursued along several lines. These include immunity developed as the result of infections with attenuated, sub-clinical, and clinical dosages of sporulated oocysts. Natural immunity or resistance, active immunization using a "vaccine", and the age of the host have also been covered in these studies.

Tyzzer (1929) found no natural immunity to E. tenella in chickens. He did, however, report immunity following recovery from infections.

Kendall and McCullough (1952) reported that resistance to re-infection with E. tenella was developed by the 96th hour following the initial inoculation. A partial immunity was demonstrated by Gordeuk, Bressler, and Glantz (1951) following infection in chicks inoculated when one day of age.

Mayhew (1932) stated that the age of the chicken was a factor in severity of infection, and that chicks 12 to 13 weeks of age became as severely, or even more severely infected than did seven-week-old chicks.

Herrick, Ott and Holmes (1936) suggested that chickens could be divided into two groups according to age at inoculation, and their degree of resistance to infection. One group consisted of those infected when less than two-months of age, in which high mortality occurred. The second group included those above two-months of age when inoculated, in which there was practically no mortality. All chickens up to and including those 15 months of age, however, were susceptible to coccidiosis, and showed signs of acute coccidiosis infection following inoculation.

Gardiner (1955) stated that chicks one to four weeks of age suffered the greatest mortality among chicks from one to six weeks of age when fed 50,000 to 200,000 oocysts. The chickens were, however, most resistant to infection at two-weeks of age. After four-weeks of age the number of oocysts fed tended to have less importance than it had in younger birds.

Mayhew (1932) suggested the possibility of developing resistant as well as susceptible strains of chickens.

Edgar, King and Johnson (1951) found an increased resistance to infection demonstrable by selection of resistant birds through various generations. They also found New Hampshire birds were most resistant, while White Leghorns were least resistant. Champion (1954) reported the inheritance of resistance to E. tenella infections in birds.

Rosenberg, Alicata, and Palefox (1954) demonstrated

development of 89.2 percent resistance in the F4 descendents of birds in which the F1 generation was only 37.9 percent resistant.

Immunity has been produced by feeding attenuated oocysts, or sub-clinical doses of sporulated oocysts. Jankiewicz and Scofield (1934) demonstrated development of various degrees of immunity following feeding of heated and non-heated sporulated and unsporulated oocysts. Little or no immunity developed in birds fed oocysts that had been heated prior to sporulation. Complete immunity was found in birds that had been inoculated with oocysts heated after sporulation.

Albanese and Smetana (1937) attenuated sporulated and non-sporulated oocysts of E. tenella with X-irradiation. The minimum prophylactic dose was 13,500 r at 75 r per minute. Irradiation of oocysts with 9000 r at 75 r per minute resulted in less severe infections. Irradiation of 2,250 to 4,500 r units at 75 r per minute (sub-lethal level) resulted in an increased production of organisms during the latent period, suggesting a stimulating effect from low-level X-ray irradiation.

Farr (1943) obtained a higher degree of immunity in chickens by feeding light doses of oocysts daily over a 15-day period, then by feeding a single heavier dose. He was unable to demonstrate an effective immunity in chickens following recovery from a single heavy dose. His sub-clinical doses ranged from 1,000 to 7,500 oocysts daily for 15

days.

Dickinson, Babcock, and Osebold (1951) established immunity in chicks by feeding sub-clinical dosages of coccidia. They postulated that the degree of immunity would increase with each subsequent chance infection. They suggested feeding small doses of coccidia to young brooder chickens as a method of developing immunity. In contrast to the above findings, Gordeuk and co-workers (1951) were able to produce a higher protective immunity in surviving birds by feeding larger initial doses of sporulated oocysts. The larger dose also, unfortunately, caused a higher mortality than that produced by feeding smaller initial doses of oocysts. The feeding of oocysts in mash resulted in a higher mortality rate than when similar numbers of oocysts were administered orally. They decided that the persistence of immunity depended upon the age at re-exposure, and magnitude of re-exposure.

Johnston (1927) reported a higher resistance or immunity in commercially-reared than in cage-reared fowls. A high degree of resistance developed within ten days after inoculation and was still present six and one-half-months after date of infection.

Babcock and Dickinson (1954) could demonstrate immunity within three to six days, using a standard inoculum of 600 oocysts on the first day followed by 1,000 oocysts on the second day. The same authors were also successful in

developing a low grade immunity in birds fed oocysts over 350 days old. They gave each bird 1,050 to 2,125 oocysts.

Edgar (1956a-1956b) advocated a "vaccine" for immunizing chicks to coccidiosis. The "vaccination" procedure entailed the feeding of a controlled number of oocysts during the first three-weeks of life. A sulfa drug was administered on the 13th and 14th day after inoculation to help control the coccidial infection. The chickens treated in this manner appeared to have developed sufficient immunity to prevent subsequent outbreaks of coccidiosis.

E. Therapy

The importance of E. tenella as a pathogenic parasite can be calculated by the enormity of the literature available on attempts to find good coccidiostatic or coccidicidal drugs. That a number of potentially effective drugs have been found can be ascertained from the literature. An excellent review of the literature from 1933 to 1953 can be found in a publication by Merck and Co. (1953). The therapeutic reference table listed 51 agents that had been tried in treatment of E. tenella. Only one paper on vitamin K appears in this list.

The sulfa compounds appear to be most commonly used in earlier experimental work, with the main emphasis on sulfaguanidine, sulfamerazine, sulfamethazine, and sulfaquinoxaline.

A review of the early development of sulfa therapy appears in a publication by Becker (1952).

Herrick and Holmes (1936), investigating the use of flowers of sulfur as a possible therapeutic measure in coccidiosis, were able to reduce mortality to zero in treated as compared to 70 percent mortality in non-treated infected birds. A five to ten percent level of sulfur caused cloacal irritation in the birds. A ten percent concentration of sulfur was necessary, however, to reduce the mortality, and a 20 percent level necessary to completely prevent losses from coccidiosis. No curative effects from sulfur could be demonstrated once the infection had become established in the birds.

Levine (1943) reported that sulfanilamide suppressed the production of oocysts of six species of Eimeria of chickens, but was ineffective in controlling infections of E. tenella and E. necatrix.

In 1941 Levine stated that sulfanilamide was too toxic for use against coccidiosis in chickens. Sulfapyridine was non-toxic at therapeutic levels, and sulfaguanidine in 0.5 percent concentration prevented infection with intestinal coccidia. The severity of cecal coccidiosis was reduced when sulfaguanidine was fed at the one percent concentration. Farr and Allen (1942) gave two percent sulfaguanidine three-days prior to and until nine-days after the day of inoculation. They reported no symptoms, lesions, or coccidial

forms following this treatment. Birds fed a one percent concentration developed light infection, with no sign of severe lesions. Birds which were treated, however, after the appearance of blood in the droppings received no benefits from sulfaguanidine.

Horton-Smith and Taylor (1942) were able to protect infected chickens by beginning treatment with small daily doses of sulfamethazine and sulfadiazine initiated up to 96 hours after inoculation. Farr and Wehr (1945) observed materially reduced mortality from cecal coccidiosis by feeding one percent sulfamerazine in mash. It was too toxic for continuous use. The 0.25 percent concentration was satisfactory, however, as a prophylactic agent when fed within two days after inoculation. It was less toxic at this level, but some liver and spleen toxicity was present. Hemorrhage was not prevented. The one percent concentration started one-day before to one-to-two-days after inoculation, prevented blood loss during the course of the treatment.

Swales (1946) produced complete protection from mortality by feeding sulfa compounds at one gram per pound in dry mash during the first six days after exposure.

Delaplane, Batchelder, and Higgins (1947), using varying levels of sulfaquinoxaline, were able to reduce mortality to zero or 37 percent depending on the concentration of the drug used. This compared to a mortality of 80 percent in the controls. They found that the drug did not prevent

the formation of immunity to subsequent infections in the surviving birds. Delaplane and Milliff (1948) described the lesions produced in chickens as a result of sulfaquinoxaline poisoning when the drug was fed in mash at concentrations of 0.05 percent. This level is less than that previously reported necessary to protect completely the chickens infected with cecal coccidiosis, when the treatment was started 48 hours after inoculation.

Ripsom and Herrick (1945) found that a single dose of sulfadiazine (0.5 gram tablet), or a one percent concentration in feed, was effective in treating coccidiosis, especially if given on the third day. They report an 80 percent cure. Other drugs which were of doubtful or no value were: sulfathiazole, sulfaguanidine, sulfanilamide, succinyl-sulfonilamide, lime-sulfur, tetraethylthiuram monosulphide, and barium antimonyl tartrate.

Hawkins and Kline (1949) reported that sulfamethazine interfered with the normal weight gain of uninfected birds, and that blood levels of five to six mg percent by the fifth day after infection were necessary to prevent loss in infected birds.

An interesting concept was advanced by Hawkins (1950). He stated that under average conditions, an infected flock would usually recover as rapidly without the administration of sulfamethazine and sulfamerazine as they would with the administration of these drugs. The drugs could be used,

however, in warding off an incipient outbreak if started immediately after the first evidence of cecal coccidiosis, and before the explosive outbreak in the entire flock. These drugs are toxic so they cannot be used continuously and should not be fed longer than three to five days at therapeutic levels. Sulfaquinoxaline would, however, control coccidiosis in a flock when administered continuously at a 0.0125 percent level. He considered sulfaquinoxaline as the best sulfonamide for the prevention of coccidiosis in poultry.

Kendall (1951) found that 0.2 percent sodium sulfamezathine in water or 0.4 percent sulfamezathine in food were concentrations most likely to produce satisfactory therapeutic effects. He stated that these drugs inhibited development of the second generation schizont, thus terminating the life cycle and preventing hemorrhage.

Wilson (1951) recommended sulfaquinoxaline and sulfamezathine in treatment and control of cecal coccidiosis.

Tugwell (1955) found that "Parabis 90" (2-2'-methylene-bis 4- chlorophenol), a mixture of sulfaquinoxaline and micronized sulfur, micronized sulfur, and nitrofurazone were all highly effective in reducing mortality and depressing weight loss in infected birds. Nitrophenide and sulfaquinoxaline were only fairly effective.

Brackett and Bliznick (1949) compared the effectiveness of nitrophenide and sulfaquinoxaline on infections resulting

from small numbers of oocysts. Both drugs were effective in reducing oocyst production. Sulfaquinoxaline had to be used at a higher level than that recommended for field control (0.01 to 0.0125 percent). Nitrophenide proved to be at least twice as effective as the sulfa compounds in suppressing oocyst production.

Many other papers can be found with equally important and interesting studies on sulfonamides as coccidiostatic or coccidicidal agents. It would seem, however, to be beyond the scope of this research problem to include all of these papers.

Gardiner, Farr, and Wehr (1952) studied the effects of nitrophenide as a coccidiostatic agent. They found that nitrophenide in concentrations of 0.075 percent in mash definitely affected development of the second generation schizonts, but the period of hemorrhage was prolonged. Treatment initiated 96 hours after inoculation was ineffective. Treatment was effective, however, if begun by the 48th hour after inoculation.

Nitrofurazone was found to be effective by Harwood and Stunz (1949) at concentrations of one part in 9,000 parts of feed if administered not later than 56 hours after infection. Nitrofurazone was not effective if dissolved in water and given in a metal trough. A mortality rate of 2.5 percent in treated as compared to 76 percent for untreated controls was observed.

Gardiner and Farr (1954) began treatment with 0.0108 percent to 0.0216 percent nitrofurazone, zero to three days after inoculation. A marked decrease in mortality resulted. No reduction in hemorrhage occurred except in those birds receiving treatment beginning zero to two days after date of inoculation.

Harwood and Stunz (1954) reported a two-thirds reduction in mortality by using a concentration of 0.0055 percent furazolidone. If treatment was started within 48 hours after inoculation a 0.011 percent concentration prevented mortality.

Other therapeutic agents used and reported as effective include 4 bromophenylarsonic acid, 2 chlorophenylarsonic acid, 4 chlorophenylarsonic acid, and the sodium salts of the chlorophenylarsonic acids (Morehouse 1946).

Gresev and Koptev (1953) suggested the use of trypan blue as a coccidiostat. The recommended dosages were 1:1,000 to 1.2:1,000 parts in drinking water for seven days in young chicks. A 2:1,000 dilution was effective for chickens two months of age or older.

One of the newer and more effective coccidiostatic drugs was reported by Cuckler et al, (1955). This drug is a carbanilide complex of 4,4'-dinitrocarbanilide and 2-hydroxy-4,6-dimethyl pyrimide. It was found to be effective in preventing E. tenella infections. They proposed the name nicarbazin for this complex.

Cuckler and Malanga (1956) reported that a 0.0125 percent concentration of nicarbazin in feed prevented mortality in three-week-old chicks inoculated with 50,000 sporulated oocysts of E. tenella. Nicarbazin suppressed oocyst production and development of the second generation schizonts and their merozoites. Hemorrhage was prevented or greatly reduced. The birds did, however, develop an immunity against E. tenella. They were resistant to re-infection with challenging doses of E. tenella oocysts and showed normal weight gain during this time.

Cuckler, Malanga, and Ott (1956) found the LD50 of nicarbazin in two-week-old Leghorns as 2,400 mg per kg of body weight. A 0.06 percent concentration in feed depressed growth, 0.007 percent to 0.03 percent levels were well tolerated. Nicarbazin could be fed continuously for eleven weeks in concentrations of 0.005 to 0.02 percent without adverse effects. A feed concentration of 0.010 percent protected chicks against infection with E. tenella in the laboratory. A concentration of 0.01 to 0.02 percent protected chicks under simulated field conditions when exposed to E. tenella. A 0.012 percent level of nicarbazin suppressed development of oocysts.

Ott and co-workers (1956), after an extensive study on the use of nicarbazin for infected and non-infected birds, suggested 0.01 to 0.02 percent concentration as the effective protective level if given in feed. The above

concentrations would protect chickens from cecal coccidiosis if given as late as 24 hours after exposure. It was not effective if started 48 hours after inoculation, and was, therefore, not recommended for therapeutic use. Nicarbazin could be given indefinitely at 0.01 or 0.02 percent concentrations without apparent harm to chickens.

Rubin and co-workers (1956) compared nicarbazin with nitrofurazone and sulfaquinoxaline for coccidiostatic activity. Nicarbazin at 0.0125 percent was more efficient than either nitrofurazone at 0.0055 percent, or sulfaquinoxaline at 0.0125 percent. Birds given 0.1 percent nicarbazin in feed showed pronounced emaciation. If fed at a level above that recommended a decreased food consumption occurred that eventually led to a point of actual starvation.

White-Stephen, Zeibel, and Smith (1955) found that noninfected birds fed nicarbazin or nitrofurazone plus aureomycin did not show an increased rate of growth. Birds fed sulfaquinoxaline, "Polystat", nitrophenide, or "Coccivac" plus aureomycin did show an increased rate of growth.

Walton et al. (1955-1956) could find no significant interference by nicarbazin on penicillin or chlortetracycline, when both were administered to chickens in the feed.

Berg, Hamilton, and Bearse (1956b), using 0.02 and 0.012 percent nicarbazin in feed reported a depression of growth at the higher, but not at the lower level. The lower level was effective in preventing lesions in cecal

coccidiosis infections.

Sherwood, Milby, and Higgins (1956) demonstrated a reduced hatchability of eggs from hens receiving from 0.002 percent to 0.0125 percent concentrations of nicarbazin. They stated that nicarbazin could be used safely as a coccidiostatic drug during the starting and growing period, but should be discontinued one week prior to onset of egg production.

Cuckler and Malanga (1956-1957) checked the effectiveness of 12 antibiotics and reported that two of them, chlortetracycline and "Spiramycin", were effective against coccidiosis. Both were found to have good activity. Spiramycin was the most effective, but both had the same order of potency as sulfaquinoxaline.

Brumpt (1942-1943) recommended the use of quinacrine in treatment of coccidiosis. This drug was not as effective in poultry coccidiosis as was "Stoversal" (Acetarsone).

Zimmermann (1952) tested a number of drugs previously used in antimalarial therapy research. He found that the following drugs were the most effective in reducing oocyst production: cupric dimethyldithio carbamate, dimethylthithioper carbamide acid, O-nitrophenyl ester, and a-(diamylaminomethyl-1,2,3,4-tetrahydro-9-phenanthrene-methanol) hydrochloride.

Lux (1954) was unable to demonstrate a correlation between compounds showing anticoccidal activity and compounds

known to possess significant antibacterial activity. He found anticoccidial activity in 2,4-diamino 5 aryl-6-alkyl-pyrimidines and 2,4 diamino 5 aryl-6,6-dialkyl-5,6-dihydro-1,3,5-trigines. He also reported synergisms between these drugs and the sulfonamides.

Kendall and Joynes (1956) found synergistic action between pyrimethamine and sulfadimidine or sulfaquinoxaline in E. tenella infections. When treatment was stopped an acute infection followed.

The probability of the development of drug resistant strains of organisms is one of the many aspects of therapy that the research workers must consider. Until recently, emphasis in treatment of coccidiosis was apparently placed on the sulfa drugs. These drugs were available in commercial feeds, consequently one might expect some sulfa resistant strains of coccidia to develop.

Harwood and Stunz (1953) were unable to demonstrate resistance to nitrofurazone or sulfaquinoxaline in three reportedly resistant strains of E. tenella studied.

Waletzky, Neal, and Hable (1954) reported field strains of E. tenella that were resistant to sulfaquinoxaline and sulfamethazine, but found no increased resistance to nitrophenide, nitrofurazone, or arsenosobenzene. These strains were 40 times more resistant to sulfaquinoxaline and five times more resistant to sulfamethazine than were non-resistant strains.

Cuckler and Malanga (1955) studied 40 strains of coccidia that were reportedly drug resistant. Of these, 13 strains were resistant to both nitrophenide and nitrofurazone; 18 strains were resistant to sulfaquinoxaline; and nine strains were resistant to all three drugs. Nicarbazin was effective in inhibiting all strains of coccidia, whether sensitive or resistant to the other agents.

F. Hemorrhage

The occurrence of lethal hemorrhage with E. tenella infections presents a problem that fortunately is not encountered in many diseases of chickens. Internal and subcutaneous hemorrhages are, however, found in cases of inadequate diet and with some sulfa therapeutic practices. The following material is a review of literature on hemorrhage produced by inadaquate diets, and as a side effect of sulfa therapy.

Dam and Schønheyder (1934) observed subcutaneous hemorrhage in chicks reared on an artificial diet. Sixty to 70 percent of the chicks developed hemorrhage within 15 to 20 days after being placed on the diet. If the birds were fed cereal and seeds the hemorrhage did not appear, or would disappear in those birds in which it had already occurred.

McFarlane, Graham, and Richardson (1931) found hemorrhage in birds fed a basal diet to which fish meal oil

extracted with ether had been added. The birds would bleed for 12 to 14 hours following injury. No hemorrhage occurred in birds fed the basal diet to which normal fish meal oil had been added.

Goldhaft and Wernicoff (1951) reported subcutaneous hemorrhage occurring in poultry in the United States. They presented the following as possible causes: (1). Antibiotics given in feed might kill intestinal bacteria and thus prevent the production and utilization of vitamin K. (2). Unheated soybean oil contains an anticoagulant which significantly delays the blood clotting time in chickens fed such a diet. Menadione bisulfite added to the drinking water at a level of 10 mg per gallon and given continuously for a period of 48 to 72 hours prevented or terminated the hemorrhages. Four grams of menadione bisulfite per ton of feed did not prevent hemorrhage.

Griminger, Morrison and Scott (1956) were, however, unable to demonstrate an anticoagulant in unheated soybean meal. They found no differences in prothrombin time in chicks fed raw soybean and chicks not fed raw soybean meal.

Bornstein and Samberg (1954) noted subcutaneous hemorrhage in a flock of chickens in Israel. The diet contained no alfalfa meal and was apparently deficient in vitamin K. Intramuscular injections of menadione sodium bisulfite returned blood clotting time to normal and prevented further hemorrhages.

Hare and co-workers (1953) found that spontaneous hemorrhage was produced in birds fed a simplified ration. They concluded that the oil in the diet was related to the incidence of hemorrhage and mortality in birds. Menadione at 0.18 mg per pound of ration prevented both hemorrhage and mortality.

Griminger et al. (1953a) likewise felt that coccidiostatic drugs and/or antibiotics fed in the diet adversely influenced the normal intestinal flora's synthesis of vitamin K, which supplied a part of the chicken's normal requirement.

Davies (1954) found sulfonamide poisoning in chickens following use of sulfa drugs. This condition was characterized by multiple hemorrhages in many of the body tissues. Necrotic lesions were found in the spleen. No beneficial effects were produced from the administration of vitamin K with sulfa drugs.

Frost and Spruth (1955) found that chicks on a low vitamin K diet did not show increased clotting time when given 0.05 percent concentration of sulfaquinoxaline or arsonilic acid. A 0.1 percent concentration of sulfaquinoxaline did, however, cause an increased clotting time. Clotting time could be lowered by feeding menadione sodium bisulfite at 0.72 grams per ton of feed. Menadione sodium bisulfite at 90-180 mg per ton of feed overcame the increased clotting time of chicks on the basal diet low in

vitamin K. Field outbreaks of hemorrhagic diseases apparently resulting from over medication with sulfa drugs could be alleviated by giving 100 mg menadione sodium bisulfite per gallon of water. Under normal conditions as little as 0.1 mg of this drug per gallon of water was sufficient to prevent hemorrhage.

Frost, Perdue, and Spruth (1955) found menadione sodium bisulfite effective in treatment of field outbreaks of hemorrhagic syndrome in fowls.

Yacowitz and co-workers (1955) demonstrated a hemorrhagic condition in chickens fed a normal diet plus sulfaquinoxaline. Sulfaquinoxaline concentrations of 0.06 to 0.1 percent in feed were sufficient to cause hemorrhage. Vitamin K protected chicks three-weeks of age, but had no effect on chicks five-weeks of age. Penicillin given with sulfaquinoxaline tended to increase the toxicity of the sulfa drugs.

Yacowitz, Carter, and Ross (1955) showed that chickens fed 0.1 percent sulfaquinoxaline starting at four to five weeks of age developed extensive hemorrhage. A 0.15 percent level of sulfaquinoxaline started in chicks at one-day of age caused depression of growth rate, but no hemorrhages.

G. Hemoglobin and Other Blood Determinations

Though much of the research work in cecal coccidiosis has been directed toward therapeutic methods, some work has been done on the blood picture present during the infection. The literature on this material seems to be predominantly studies of the hemoglobin concentration and blood-clotting time.

Pratt (1938) completed a rather thorough study on blood volume versus body weight, and on hemoglobin before and during cecal coccidiosis infections. He found that the blood volume was greatly reduced during coccidiosis, as was the body weight. The ratio of blood volume to total body weight was slightly decreased by the disease. Before infection, blood was equal to 11.1 percent of the total body weight, as compared to 9.4 percent during the hemorrhagic phase of the infection. He found a decrease of 71 ml of blood between the day of inoculation and the sixth day after inoculation. A weight loss of 24 percent was observed by the fifth day. This was before hemorrhage had started. He also reported a reduced hemoglobin concentration during the acute stage of infection.

Herrick (1939) recorded the following conditions in infected chickens on the fifth day: Reduced erythrocyte count and hemoglobin concentration, loss of blood volume, and reduced metabolic rate. The osmotic pressure of the blood

remained fairly stable.

Natt and Herrick (1955) found a 50 percent decrease in erythrocytes on the fifth and sixth days of the infection. Eight more days were required for the erythrocyte count to return to normal. They suggested using hematocrit as a method for checking severity of cecal coccidiosis infection.

Natt and Herrick (1956) stated that the blood volume loss resulted from the decreased erythrocyte number. There was no decrease in plasma volume during the hemorrhagic phase of the infection.

Rosenberg and Herrick (1950) attempted to use hemoglobin values as an index of resistance to cecal coccidiosis. They found no significant difference in hemoglobin values between those chickens that had survived, and those that had died as a result of E. tenella infection. The normal hemoglobin value for 11-day-old chicks was 8.44 to 8.93 grams per 100 ml of blood. Chickens surviving coccidial infections had hemoglobin levels of 4.37 to 4.81 grams as compared to 3.28 to 4.04 grams for those which had died with cecal coccidiosis. They concluded that hemoglobin values could not serve as an index of resistance to cecal coccidiosis.

Olson (1935) listed several factors necessary for hemoglobin determination of chicken blood, using various methods. He gave an average hemoglobin level for chickens of 8.79

grams per 100 ml of blood. In 1937 Olson reported a hemoglobin level of 9.73 grams for chickens from 14 to 224 days of age for both male and female.

Hayden (1927-1928) reported hemoglobin values as 74 percent with a range of 70 to 80 percent. The grams of hemoglobin per 100 ml of blood were not given. Dukes and Schwarte (1931) gave the hemoglobin range as 9.4 to 10.2 grams in adult females. Cook and Harmon (1933) found a hemoglobin range of 5.3 to 19.0, with 11.9 grams as the average. They suggested that dietary iron, egg production, and splenic reserve factors all influence hemoglobin concentrations in chickens.

Natt (1951) found a reduction in erythrocytes of approximately 50 percent on the fifth and sixth days of cecal coccidiosis infections. A normal erythrocyte count was found after eight days. Hematocrit also decreased 50 percent and likewise required eight days to return to normal. He recommended hematocrit as a fast and easy method of determining the severity of an infection.

Pratt (1941), employing artificial hemorrhage, concluded that the following physiological abnormalities in chickens with cecal coccidiosis were due to cecal hemorrhage:

1. Increased blood sugar level.
2. Lowered muscle glycogen.
3. Reduced blood volume.

Dougherty and Herrick (1952) reported little difference in carbohydrate metabolism capacity in infected and control

birds. They disputed Pratt's thesis that hemorrhage caused the disturbances in infected chickens, since the hemorrhage in infected birds was minute and prolonged instead of rapid and of short duration; as was the artificial hemorrhage produced by Pratt.

Schildt and Herrick (1955), from an *in vitro* study of cecal pouches, indicated that cecal activity ceased at the end of the fourth day after infection and normal function did not return for a period of about three weeks. Crop activity decreased on the third day and stopped on the fifth day. A period of approximately two weeks elapsed before crop adtivity returned to normal.

H. Prothrombin and Clotting Time

A technic for determination of prothrombin time was devised and outlined by Quick (1936). He extracted thromboplastin as an aqueous emulsion from the brain, lungs, and thymus of the rabbit. In this test, only one variable is present. This variable is prothrombin. The concentrations of the other constituents are known, or can be calculated. The time required for the clot to form after combining calcium, thromboplastin, and plasma is known as the prothrombin time. A curve can be constructed of prothrombin times by using from 10 percent to 100 percent plasma concentrations. As the percentage of plasma increases, the time necessary for the clot to form decreases. The prothrombin time can,

therefore, be considered as a measurement of the time necessary for a clot to form, or of the concentration of prothrombin in the plasma. The prothrombin time and the blood clotting times are not directly proportional, because a minimum prothrombin time demands 100 percent concentration of prothrombin, whereas the clotting time would be normal with prothrombin levels of approximately 80 to 100 percent.

Quick (1945) stated that the prothrombin time could increase as much as five times before any effect on coagulation time was evident.

Warner, Brinkhous, and Smith (1936) found that only a low level of prothrombin was needed to form a normal fibrin clot. They also found complete conversion of plasma prothrombin into thrombin when an optimal amount of calcium and an excess of thromboplastin were added.

The same authors (1939) stated that the rate of thrombin formation was directly related to the conversion of prothrombin into thrombin, and not to the amount of thromboplastin present in the blood plasma.

There appear to be few papers published on the prothrombin time of chickens.

Quick (1945) reported prothrombin time in chickens of ten seconds.

Almquist and Klose (1939a) reported prothrombin time of 26.1 seconds for chicks receiving menadione while on a basal diet.

Perdue, Spruth, and Frost (1957) reported an average prothrombin time of 19.0 ± 0.7 seconds and 32 ± 1.3 seconds for birds on a diet containing Klotogen F at concentrations of 360 and 45 mg per ton of feed respectively. They considered 19 seconds as the normal prothrombin time. Birds given vitamin K_1 at 22.5 mg and 360 mg had average prothrombin times of 41 ± 4.2 seconds and 22 ± 0.6 seconds, respectively.

March, Biely, and Touchburn (1955) stated that the prothrombin time of chicks was influenced by the amount of vitamin K fed to their dams. Prothrombin time for day old chicks ranged from 26 seconds to 73 seconds and reflected the vitamin K accumulation by the dams. The dams had similar prothrombin times whether they were or were not fed dehydrated greens. Their prothrombin time was 22 to 30 seconds.

Griminger, Morrison, and Scott (1956) reported prothrombin time averages of 24 to 242 seconds on 38 birds fed a normal diet.

The blood clotting time in chickens has been reported in various papers. Scarborough (1931) lists coagulation times for 20 determinations ranging from 20 seconds to 4 1/2 minutes.

Sebrell and Harris (1954) found a ten minute clotting time in vitamin K deficient chickens. They suggested that this would represent a prothrombin level approximately 20 to 30 percent of normal. They were able to obtain a normal

clotting time within four to six hours after feeding 11 units of vitamin K_1, or 0.3 to 0.64 units of methylnaphthoquinone per chicken.

Kornberg, Daft, and Sebrell (1944), while attempting to produce a vitamin K deficiency in rats, obtained more uniform results by including 0.1 percent sulfonamides in the diet. Hypoprothrombinemia was produced which was reversible by oral administration of 40 micrograms of a vitamin-K active substance three times each week. A prothrombin level below 20 percent usually resulted in spontaneous subcutaneous hemorrhages. No spontaneous hemorrhages occurred when the prothrombin level was 30 percent or greater. Drugs causing a hypoprothrombinemia were sulfapyrazine, sulfadiazine, sulfathiazole, succinyl sulfathiazole, sulfanilamide, and sulfaguanidine.

Endicott, Kornberg, and Daft (1944) also reported that hemorrhagic lesions were produced in rats given sulfa drugs in their diets.

Mushett and Seeler (1947) produced hypoprothrombinemic lesions in rats and dogs within 24 hours after feeding sulfaquinoxaline in the diet. No lesions or other abnormalities were found in histological studies of liver tissue. They attributed the hypoprothrombinemia to the action of sulfa drugs in either decreasing or inhibiting the production of prothrombin in the liver. They found that the prothrombin level in the blood decreased as the blood level of

sulfaquinoxaline increased. Menadione was ineffective in correcting the hypoprothrombinemia. Vitamin K_1 and 2-methyl-1,4-naphthoquinone disulfite were effective in returning the plasma prothrombin level to normal.

Asplin and Boyland (1947) reported a hypoprothrombinemia in chickens fed sulfamezathine. A condition resembling vitamin K deficiency occurred. Birds on sulfamerazine had a prothrombin time as high as 43.3 seconds. The prothrombin time of birds on sulfadiazine, sulfathiazole and sulfapyrazine ranged from 22.9 to 27.3 seconds. The average prothrombin time for control birds was 23.2 seconds.

Frost and Spruth (1955) used a vitamin K active material (menadione sodium bisulfite) in controlling hemorrhagic conditions in chickens on a deficient diet. They found that 10 to 100 mg of menadione per gallon of water resulted in a return to normal clotting time (3 to 7 minutes) within 15 hours. The regimen recommended was 90 to 180 mg per ton in feed, or 10 to 100 mg in drinking water for three to four days. They suggested that an increased hemorrhagic tendency might occur as a result of using sulfaquinoxaline in treatment of coccidiosis. They also suggested feeding an increased amount of vitamin K to chickens to counteract the hemorrhagic tendency.

Black et al. (1942) stated, however, that sulfonamides inhibit the ability of the liver to produce prothrombin.

Mushett and Seeler (1947) disagreed with the above

conclusion. They claimed that vitamin K_1 and 2 methyl-1,4-naphthoquinone bisulfite were effective in overcoming the hemorrhagic tendency found after sulfa therapy.

Ansbacher (1938) was able to obtain normal clotting time within four to six hours after feeding vitamin K_1 to deficient chicks.

Reynolds, Warden, and Luther (1953-1954) found less than three percent incidence of hemorrhage in vitamin K deficient birds. They found the following procedures necessary to produce vitamin K deficiency.

1. Feed must be kept dry.
2. Water containers cleaned daily to prevent bacterial contamination.
3. Coprophagy must be avoided.

Stamler, Tidrick, and Warner (1943) reported that only one to two micrograms of 2 methyl-1,4-naphthoquinone per day were necessary to maintain a normal prothrombin level, and only about one-twentieth of this amount was necessary to maintain sufficient prothrombin in the blood to protect the chickens from hemorrhagic manifestations. These requirements did not increase with age, nor did increased amounts of menadione produce an elevated prothrombin level.

Griminger and co-workers (1953b) found that five percent alfalfa meal in the feed reduced the average blood clotting time from 15 minutes to two minutes. Menadione, five mg per kg in the diet, was as effective as one percent alfalfa meal in reducing clotting time.

A 0.36 mg level of menadione or one to five percent alfalfa meal restored normal clotting time in birds fed 15 ppm of oxytetracyclines or five ppm of 3-nitro-4 hydroxy-phenylarsonic acid, according to Sweet, Romasen, and Combs (1954).

Ware and co-workers (1953) stated that 0.18 mg of menadione per pound of ration prevented hemorrhage and mortality but did not sustain normal blood clotting time in chicks fed a simplified diet.

Anderson et al. (1954) reported a return to normal clotting time and prevention of additional hemorrhages in birds on a simplified, vitamin K deficient diet, after feeding ten mg of menadione in one ml of corn oil. The clotting time dropped from 17.19 minutes to 2.25 minutes within 24 hours. The feeding of fecal droppings to these birds was also helpful in preventing hemorrhage and in maintaining normal blood clotting time.

I. Natural and Synthetic Vitamin K

Dam (1929) was apparently the first to report subcutaneous and internal hemorrhages in chickens on synthetic diets. This condition in chickens could not be cured by feeding citrus juices.

McFarlane, Graham, and Richardson (1931), while maintaining a flock on a diet of ether-extracted fishmeal, noted

bleeding conditions in the birds. Vegetable proteins prevented bleeding, while cod liver oil was ineffective. Holst and Holbrook (1933) noted a similar condition which could be cured by feeding cabbage. They attributed this condition to deficiency of vitamin C.

Dam and Schønheyder (1934) demonstrated the presence of a material in cereals and seeds which would prevent hemorrhage. The following year Dam (1935) proposed the name "Vitamin K" (koagulation-vitamin) for the antihemorrhagic factor. This material was found in hog liver, hemp seeds, and vegetables. It was fat soluble, and was a necessary dietary factor in the prevention of hemorrhage and for the maintenance of normal blood clotting time. Its presence in many green leafy vegetables was demonstrated by Dam and Schønheyder (1936). Alfalfa was shown to be one of the richest sources of natural vitamin K.

Almquist (1936) succeeded in extracting vitamin K_1 from alfalfa meal with hexane.

McKee and co-workers (1939) reported the isolation of vitamin K_1 and K_2 from alfalfa.

Fieser (1939) identified a synthetic compound 2 methyl-3-phytyl-1,4-naphthoquinone as identical to vitamin K . This material was successful in reducing prothrombin time in trial tests.

Jukes (1937) found vitamin K in the fecal droppings of chickens deficient in this vitamin. This apparently

represented poor absorption of the vitamin by the chickens. He also reported the transfer of vitamin K from mother hen to chick via the egg. Almquist, Mecchi, and Klose (1938) agreed that vitamin K could be transferred via the egg. They stated that the vitamin K level of chicks was highest at two weeks of age. At three weeks of age they found a range in clotting time in chicks from 2.9 minutes to more than 60 minutes.

March, Biely, and Touchburn (1955) stated that the amount of vitamin K given the dam was reflected in the prothrombin time of the baby chick.

Considerable interest has been manifested in materials with vitamin K-like activity. The most productive work appears to be that done in connection with materials having a quinone radical in their structure.

Ansbacher and Fernholz (1939) noted hemorrhage in chicks when their prothrombin level fell below one-fifth of normal. The condition could be corrected by using a synthetic material possessing vitamin K-like activity. They were the first to record the marked antihemorrhagic activity of menadione (2 methyl-1,4-naphthoquinone).

Almquist and Klose (1939b) suggested using menadione as a cheap substitute for vitamin K_1. Menadione, according to them, could maintain the prothrombin of chick's blood at a normal level, if adequate amounts were given.

Almquist and Klose (1939c) found antihemorrhagic

activity in a pure synthetic phthiocol (2-methyl-2-hydroxy-1,4-naphthoquinone).

Tishler and Sampson (1939) stated that 2-methylnaphthoquinone possessed antihemorrhagic activity of the same order of magnitude as vitamin K_1.

Thayer and co-workers (1939) reported that the activity of 2-methyl-1,4-naphthoquinone was ten units per mg compared to 660 units for vitamin K_2, and 1,000 units for vitamin K_1. According to Ansbacher and Fernholz (1939) the activity of menadione was almost equal to that of vitamin K_1.

MacCorquodale and co-workers (1939) were able to demonstrate vitamin K activity only in the quinone derivatives of 1,4-naphthoquinone.

Richards and Shapiro (1945), using dogs, mice, and rabbits, found the LD50 of menadione bisulfite at 250 mg per kilo of body weight. Death resulted from respiratory paralysis, preceeded by convulsions, lacrimations, and exophthalmos. The lethal agent in menadione bisulfite ("Hykinone") was found to be the quinone radical.

Evans (1944) reported that chickens were unable to utilize vitamin K produced by bacteria in their intestinal tracts due probably to low absorption ability of the intestinal wall. Chickens kept on a low vitamin K diet had a low plasma prothrombin level. Vitamin K_1 showed the greatest activity in alleviating this condition.

Dam, Schønheyder, and Tage-Hansen (1936) were able to

obtain a precipitate from the plasma of both normal birds and birds suffering from K avitaminosis. In the former case, the precipitate was prothrombin. The precipitate from the birds without vitamin K was found to be inactive as prothrombin. Vitamin K was present in the precipitate of the birds receiving vitamin K in their diet, but was not present in the birds not receiving vitamin K.

III. MATERIALS AND METHODS

A. General Methods

The chickens used in this study were male hybrids obtained from the Hy-Line Hatcheries of Des Moines, Iowa, when one day old. These chicks were kept in brooders until the day of inoculation. Chickens were obtained whenever possible throughout the year. The birds were maintained on a diet free of sulfa drugs and antibiotics. Alfalfa meal, 4.63 percent, was present in the diet to furnish vitamin K. This level represents approximately the normal amount of vitamin K required for chickens. In all experiments the birds were five weeks of age when inoculated. This age is within the range at which they are considered most susceptible to cecal coccidiosis infections. The original E. tenella used were obtained from laboratories at Iowa State College as sporulated oocysts. This strain had previously been isolated, identified, and used for research purposes. In subsequent experiments birds were infected with oocysts recovered from those birds infected with original oocysts obtained from Iowa State College. These oocysts were sporulated in two percent potassium dichromate solution prior to time of inoculation.

The five-week-old birds were weighed, numbered and divided into two groups in matched pairs, according to weight. The birds of Group 1 received menadione sodium bisulfite after inoculation, and were designated as the treated birds.

The birds of Group 2 received no drugs, and were designated as the untreated inoculated controls. In the first three experiments a third group of birds was used. These were the non-inoculated, non-treated control birds, used for weight studies.

B. Hemoglobin

Prior to inoculating the birds with sporulated oocysts of E. tenella, blood was withdrawn from the heart for prothrombin time studies. Blood was also withdrawn from the lateral wing vein of birds in the first three experiments for hemoglobin concentration determinations. These studies had to be discontinued after the third experiment as the photrometer was no longer available.

A Leitz Photrometer was used to determine the percentage concentration of hemoglobin. The grams of hemoglobin per 100 ml of blood was then read from a previously prepared chart. The lateral wing vein was punctured with a sharp 22 gauge needle, and blood was allowed to flow freely from the wound. Twenty-five hundredths ml of blood was drawn into a hemoglobin pipette and mixed with five ml of a 0.15 percent sodium carbonate solution. No correction was needed using this technic.

C. Prothrombin

The prothrombin time studies were performed according to the procedure devised by Quick (1936). A microtechnic was employed which permitted using smaller amounts of chicken blood for each determination. No attempt was made to dilute the plasma for determination of partial prothrombin times.

Difficulty was encountered in preventing the blood from clotting in the needle and syringe in trial studies. This was alleviated by rinsing the needle and syringe in sodium oxalate solution before withdrawing the blood. A 21 or 22 gauge needle and a two ml syringe were used for withdrawing blood from the heart. The birds were placed on their right sides with the wings folded back. The puncture was then made between the ribs on the left side and directly into the heart. Blood could be withdrawn easily in this manner. Nine tenths ml of blood was taken and immediately placed into tubes containing 0.1 ml of a 0.1 molar solution of sodium oxalate. These tubes were centrifuged within one-half hour after the blood was obtained. The plasma was removed and placed in a clean test tube. One tenth ml of this plasma was transferred to a small tube containing 0.1 ml of thromboplastin, mixed, and then set in a water bath previously heated and maintained at 37° C. The tube was allowed to remain in the water bath for a few seconds before adding the calcium chloride solution. One tenth ml of a 0.02 molar solution of calcium chloride was forcefully blown into the above mixture. The

tube was twirled constantly after the addition of calcium chloride. The prothrombin time was that time necessary for a fibrin clot to form in the tube after the addition of calcium chloride to the plasma and thromboplastin mixture.

A commercially prepared thromboplastin material was used on trial runs, and in Experiment I. The commercial thromboplastin was then discarded in favor of thromboplastin prepared from the brain of chickens. The thromboplastin was prepared according to the procedure of Quick (1936). All of the brain tissue was used. The membranes around the brain were removed and the brain was washed to remove as much blood as possible. The excess water was removed by blotting on absorbent paper. The brain was weighed, macerated, and sufficient 0.85 percent sodium chloride solution added to make a ten percent suspension. The material was thoroughly mixed and then heated at 60°C. for 15 minutes to remove any trace of prothrombin or thrombin arising from blood remaining in the tissues. The solid materials were removed by centrifugation. The opalescent material (supernatant) was either used directly, or divided into four ml lots and frozen for later use. The prothrombin times for all of the birds in any given experiment were always run with the thromboplastin prepared from a common source.

D. Infection and Treatment

Fecal examinations were made of droppings collected from the birds before they were inoculated, to check for coccidiosis infections. These examinations were negative in all cases.

The sporulated oocysts were prepared for use as follows: Potassium dichromate solution containing the oocysts was centrifuged, the supernatant was removed and replaced with distilled water. The oocysts were washed twice in distilled water, centrifuged, and then resuspended in distilled water. A count of sporulated oocysts was made using a hemocytometer. Additional water was added as needed to obtain a suspension or mixture containing approximately 25,000 sporulated oocysts per ml of material. The birds were inoculated with from 25,000 to 50,000 sporulated oocysts, per os, according to weight.

On the day of inoculation and for three succeeding days birds of Group 1 of each experiment were given one ml of menadione sodium bisulfite solution orally. This material was obtained from Abbott Laboratories. It was distributed under the trade name of "Klotogen F", and was packaged in capsules containing 0.25 grams of menadione sodium bisulfite per capsule. The material was water soluble, and possessed vitamin K-activity. One capsule was dissolved in 80 ml of water, giving a concentration of 3.125 mg per ml. Each bird of Group 1 received one ml of this solution, orally, daily for

four days, or a total of 12.5 mg of menadione sodium bisulfite. The solution was mixed fresh daily immediately before use. The capsules were kept in a brown bottle in a darkened area at all times.

E. Tissue and Other Studies

The birds were placed in clean individual cages after inoculation. They had access to feed and water at all times. The birds were weighed every other day. Blood was taken periodically for the studies mentioned above. In most cases no blood was withdrawn for prothrombin time determinations after the sixth day following inoculation. It was felt that the withdrawal of blood from the heart at this time might tend to have adverse effects on those birds already losing blood from cecal hemorrhages.

All of the birds that died during the course of the experiments or that were sacrificed at the termination of the experiments were necropsied and examined grossly for intestinal and cecal hemorrhages. Microscopic studies were made of slides prepared from the cecum of birds of three experimental groups. When a bird died, or was sacrificed, the ceca were removed and placed in formalin. One cecum from each bird was later removed from formalin and the middle one-third of the enlarged portion of each was fixed, sectioned, and mounted for microscopic studies. Serial sections were made from each block. The slides were stained with Giema's,

Delafield's hematoxylin, or Delafield's hematoxylin and eosin stains.

Several other studies were attempted during the course of these experiments. A decrease in the daily amount of drug given was tried. The mortality rate, however, of the birds receiving the menadione sodium bisulfite equalled that of the infected controls. An anticoagulant (bishydroxy dicoumarin) was used in two experiments. This was administered orally to one group receiving menadione sodium bisulfite, and alone to another group. Dosages of two mg per bird produced no obvious effects. A higher level of seven mg per bird resulted in death to a majority of birds in both groups. This line of research was discontinued in favor of using only menadione sodium bisulfite.

IV. RESULTS

A. Mortality

Nine experiments were completed and tabulated. The procedure in all cases was essentially the same. The birds were weighed and divided into two groups of equal pairs according to weight. The prothrombin time for each bird was determined on the day of inoculation and on the fifth or sixth day thereafter. Hemoglobin concentration determinations were made on day of inoculation and three times during the course of infection.

The mortality rate in the two groups of birds in each experiment showed quite a large range. In the birds receiving menadione sodium bisulfite, the range was from 8.3 percent to 41.7 percent. The inoculated controls had a mortality range of from 25 percent to 90 percent. The overall mortality for the nine experiments was 28 birds or 29.1 percent of Group 1 as compared to 57 birds or 59.3 percent of Group 2. The mortality of the Group 2 birds was slightly more than twice that of the treated birds. These results were analyzed by Chi square test and it was found that the menadione sodium bisulfite treated bird's increased survival rate was significant at the one percent level of probability. The mortality, survival, and percentages of deaths are given in Table 1.

It can be seen from Table 1 that in each experiment a

Table 1. Comparison of mortality rate between infected birds given menadione sodium bisulfite (Group 1), and infected birds given no medication (Group 2)

Exp. no.	Number of birds per group	Group 1 died	1 survived	Percentage mortality	Group 2 died	2 survived	Percentage mortality
I	7	2	5	28.6	6	1	85.7
II	8	3	5	37.5	6	2	75.0
III	7	1	6	14.6	4	3	57.1
IV	16	3	13	18.8	4	12	25.0
V	10	4	6	40.0	9	1	90.0
VI	12	5	7	41.7	10	2	83.7
VII	14	5	9	35.7	8	6	57.1
VIII	12	1	11	8.3	4	8	33.3
IX	10	4	6	40.0	6	4	60.0
Totals	96	28	68		57	39	
Mean percent deaths				29.1			59.3

larger number of non-treated birds than treated birds died. In Experiments IV and IX the number of deaths was only slightly lower in the treated than in the non-treated groups. The data from Experiments I, II, III, V, and VIII showed that the percent of deaths in Group 1 birds was less than one-half the percent of deaths in the Group 2 (non-treated) birds. In Experiment VII there was a 20 percent greater loss in Group 2 birds than in Group 1 birds. It is also interesting to note that in seven of the experiments the non-treated birds suffered a mortality rate of 50 percent or greater compared to a mortality rate of less than 50 percent in the

treated birds. In the other two experiments the non-treated birds suffered a 25 percent and a 33.3 percent mortality, respectively, compared to 18.8 percent and 08.3 percent in the treated birds. In all experiments except IV, the non-treated birds showed 20 percent or greater mortality rate than the treated birds. In Experiment V a 50 percent greater mortality was noted.

A third group of birds was included in Experiments I, II and III. These were non-inoculated controls for weight comparison studies. No exact relationship could be determined from the weight studies, except that Group 1 birds showed a weight gain greater than that of Group 2 birds, and less than that of the non-inoculated control birds. In Experiments IV through IX the weights were recorded every-other-day for the two groups. Again no conclusions could be drawn. The birds of Group 1 showed a slightly greater average daily gain, but it was not significant by Chi square test.

B. Hemoglobin

Hemoglobin studies were made on the birds of the first three experiments. The mean hemoglobin concentrations for the birds prior to and following inoculation are given in Table 2. There was a slight difference in the pre-inoculation mean hemoglobin concentration between the three groups in the three experiments. This difference was, however,

Table 2. Mean hemoglobin concentrations, with standard deviations for the birds of Experiments I, II, and III. Hemoglobin expressed in grams per 100 ml of blood

Exp. no.	Group no.	Determinations with standard deviations			
		First	Second	Third	Fourth
I	1	7.10±0.72	6.78±1.43	5.63±0.64	2.75±0.17
	2	7.38±1.12	7.26±0.97	5.49±1.47	2.80[a]
	3	7.59±0.77	7.46±0.89	6.09±0.41	7.40±0.95
II	1	7.21±0.83	7.79±1.13	6.23±0.72	3.02±0.62
	2	6.59±0.67	7.24±0.89	5.50±0.80	2.70±1.20[b]
	3	6.99±1.01	7.36±0.41	8.30±0.73	9.07±0.95
III	1	6.82±0.49	6.99±0.38	4.09±1.41	3.17±0.76
	2	7.20±0.95	6.86±0.62	4.03±1.96	3.28±1.38[c]
	3	7.21±0.32	7.65±0.76	8.62±0.20	8.99±0.37

[a] Only one bird survived for fourth determination.

[b] Only two birds survived for fourth determination.

[c] Only three birds survived for third and fourth determinations.

only 0.99 grams between the lowest and highest mean. The mean hemoglobin concentration for birds of Group 1 and 2 had changed only slightly by the second determination. There was a noticeable drop by the third determination. The final determination showed a decided drop in the mean concentration as compared to the original level and to the control non-inoculated birds (Group 3).

The hemoglobin concentrations for the individual birds of Experiments I, II, and III are given in Tables 3, 4, and

Table 3. Hemoglobin concentrations for birds of Experiment I. Results expressed in grams per 100 ml of blood

Group no.	Birds no.	Date of determination 8/9[a]	8/11	8/13	8/15
1	1	5.95	4.00	4.80	3.00
	2	7.40	6.80	6.08	2.80
	3	6.80	8.30	5.45	2.65
	4	7.05	8.00	6.80	
	5	6.80	6.80	5.45	2.55
	6	7.40	6.20	5.60	
	7	8.30	7.40	5.25	2.75
	total	49.70	47.50	39.43	13.75
	mean	7.10	6.79	5.63	2.75
2	8	8.00	7.05	5.45	
	9	5.45	6.55	4.80	
	10	8.30	6.80	4.60	
	11	6.20	6.07	5.75	
	12	7.40	7.40	5.95	
	13	8.30	8.00	3.60	
	14	8.00	8.95	8.30	2.80
	total	51.65	50.82	38.45	2.80
	mean	7.38	7.26	5.49	2.80
3	15	7.05	7.40	6.37	8.30
	16	7.05	8.77	5.60	8.00
	17	8.30	7.65	5.95	6.50
	18	6.80	5.75	5.75	6.20
	19	8.60	7.65	6.80	8.00
	20	8.30	7.52	5.95	6.80
	21	7.05	7.52	6.20	8.00
	total	53.15	52.26	42.62	51.80
	mean	7.59	7.46	6.09	7.40

[a] Date birds were inoculated.

5. A fairly wide range was recorded for hemoglobin concentrations.

In Experiment I, (Tables 2 and 3) prior to inoculation, a range of 5.45 grams to 8.60 grams was found in the three groups. Group 1 birds showed a mean of 7.10±0.72 grams hemoglobin. Group 2 birds had a mean hemoglobin reading of 7.38± 1.13, and Group 3 had a mean of 7.59±0.77 grams hemoglobin.

By the fifth day after inoculation the hemoglobin had dropped for all birds in Groups 1 and 2 except number 14. The mean hemoglobin for Group 1 birds had dropped to 5.63± 0.64 grams or a drop of 1.47 grams. This had dropped to a mean of 2.75±0.17 grams by the seventh day. The Group 2 birds had a mean hemoglobin value of 5.49±1.47 grams on the fifth day and 2.80 grams on the seventh day. There was, however, only one bird alive in this Group by the seventh day. The non-inoculated birds had a mean hemoglobin value of 7.59± 0.77, 6.09±0.41 and 7.40±0.95 grams on the first, fifth and seventh days, respectively.

In Experiment II (Tables 2 and 4), the Group 1 birds had a mean hemoglobin value of 7.21±0.83 grams before inoculation. The Group 2 birds had a value of 6.59±0.67 grams and the non-inoculated Group 3 birds had a mean hemoglobin value of 6.99± 1.00. Little change was found in the hemoglobin values by the third day for the infected birds. The hemoglobin had dropped for all of the birds by the fifth day. The treated birds showed a decrease of 0.98 grams, the non-treated birds

Table 4. Hemoglobin concentrations for birds of Experiment II. Results expressed in grams per 100 ml of blood.

Group no.	Birds no.	Date of determination 2/15[a]	2/17	2/19	2/21
1	92	7.35	7.35	7.35	2.40
	73	6.20	7.10	6.05	2.85
	86	6.65	6.90	6.20	3.05
	72	8.05	8.30	5.95	4.05
	48	8.05	9.95	7.10	2.75
	93	6.65	8.30	5.65	
	54	6.40	7.80	5.15	
	66	8.30	6.65	6.40	
	total	57.65	62.35	49.85	15.10
	mean	7.21	7.79	6.23	3.02
2	59	6.90	7.10	4.20	
	95	7.35	6.20	6.05	3.55
	89	5.80	6.40	5.60	
	78	6.20	6.40	4.80	
	75	7.60	8.30	6.30	
	30	6.40	7.35	5.25	1.85
	77	5.80	7.60	5.45	
	60	6.65	8.55	6.35	
	total	52.70	57.90	44.00	5.40
	mean	6.59	7.24	5.50	2.70
3	50	7.10	7.60	8.55	9.60
	82	5.80	7.60	8.05	8.55
	55	6.20	8.05	8.80	9.05
	85	6.00	7.10	7.60	8.80
	38	6.65	6.90	7.10	8.05
	90	8.05	7.60	8.05	8.55
	96	7.60	7.10	8.55	8.80
	100	8.55	6.90	9.70	11.15
	total	55.95	58.85	66.40	72.55
	mean	6.99	7.36	8.30	9.07

[a] Date birds were inoculated.

had a 1.09 gram decrease in hemoglobin. Birds of both groups had a much lower value and mean hemoglobin on the seventh day. The control birds showed an increase in hemoglobin of 2.08 grams from the first to the last determination.

Table 5 gives the hemoglobin concentrations for the birds of Experiment III. Again a decreasing hemoglobin concentration was found. The third determination was made on the sixth day instead of the fifth day as in the preceeding two experiments. A definite drop in hemoglobin was seen for the third and fourth determinations of Group 1 and 2 birds, whereas the hemoglobin increased for the control birds. It will also be noted that four of the inoculated non-treated birds had died on the sixth day prior to the time blood was taken for hemoglobin determination.

The lowest hemoglobin concentration found for the birds receiving the drug was 2.40 grams. The reading was taken from one bird on the seventh day. This bird survived the infection. A low of 1.85 grams was recorded for one bird in the infected control on the seventh day. This bird also survived the infection. The lowest level recorded for any of the birds that succumbed was 3.60 grams. This was for bird number 13 of Group 2 in Experiment I. The highest recording, on the fifth day for any bird that had died by the seventh day, was 6.35 grams in the non-treated birds and 6.40 grams in the treated birds.

Table 5. Hemoglobin concentrations for birds of Experiment III. Results expressed as grams per 100 ml of blood

Group no.	Birds no.	Date of determination 3/10[a]	3/12	3/15	3/17
1	57	6.20	7.60		
	34	7.35	7.60	3.75	2.85
	83	7.10	6.90	3.20	2.55
	32	7.35	6.90	4.60	2.75
	88	6.20	6.90	3.20	2.85
	76	6.65	6.65	6.90	4.60
	26	6.90	7.35	3.90	3.40
	total	47.75	48.90	24.55	19.00
	mean	6.82	6.99	4.09	3.17
2	80	5.80	5.80	2.25	1.85
	46	7.10	7.35		
	70	8.30	6.65	4.25	4.60
	63	8.55	6.90		
	52	7.10	6.65		
	36	6.90	7.80	5.60	3.40
	41	6.65	6.90		
	total	50.40	48.05	12.10	9.85
	mean	7.20	6.86	4.03	3.28
3	87	6.90	7.10	8.30	8.80
	58	7.10	7.80	8.55	8.80
	68	7.10	6.20	9.05	9.60
	37	6.90	8.30	8.55	8.80
	56	7.35	7.80	8.30	8.55
	79	7.35	8.30	8.80	9.05
	29	7.80	8.05	8.80	9.35
	total	50.50	53.55	60.35	62.95
	mean	7.21	7.65	8.62	8.99

[a] Date birds were inoculated.

C. Prothrombin Time

Prothrombin time determinations were made on all birds in all experiments with the exception of Experiments VIII and IX. Prior to the beginning of the experiments, a group of birds was maintained for the purpose of practicing the technic for prothrombin time determinations. Some difficulty was experienced in the practice group, and with the birds of Experiment I in obtaining satisfactory results. It was felt that these problems were largely overcome before the beginning of Experiment II.

The prothrombin times obtained for birds of Experiment I are not included. These were determined using a commercially prepared thromboplastin. The prothrombin time for most of the birds in this experiment was in excess of five minutes. The prothrombin times for birds in Experiments II through VII were obtained using thromboplastin prepared from the brain of chickens. The blood from birds of Group 1 in Experiment II was difficult to handle, especially after they had received menadione sodium bisulfite. The blood of four of these birds appeared to be hypercoagulable, as it coagulated before it could be emptied from the syringe. The remaining four birds of this Group had a mean of three to six seconds longer prothrombin time than did either the inoculated or non-inoculated controls. This cannot be considered as significant, however, since these data represented only half of the birds in Group 1 (Table 7).

Table 6. Mean prothrombin times with standard deviations for birds of Experiments II, III, IV, V, VI, and VII. Time is expressed in seconds

Exp. no.	Group no.[a]	Birds per group	Prothrombin time with standard deviations	
			First	Second
II	1	8	56.1 ± 8.2	50.5 ± 4.9
	2	8	55.4 ± 9.5	43.0 ± 8.9
	3	8	52.2 ± 9.2	45.8 ± 7.3
III	1	7	51.7 ± 16.7	36.0 ± 8.4
	2	7	39.7 ± 21.4	39.7 ± 9.3
	3	7	37.3 ± 22.5	52.7 ±17.5
IV	1	16	85.9 ± 29.9	56.4 ±13.3
	2	16	65.0 ± 21.3	55.7 ±17.9
V	1	10	76.7 ± 26.3	17.0 ± 2.6
	2	10	81.2 ± 19.5	17.4 ± 3.9
VI	1	12	38.8 ± 17.2	16.2 ± 8.3
	2	12	34.4 ± 11.3	21.5 ± 7.7
VII	1	14	43.6 ± 12.3	41.6 ± 2.0
	2	14	44.6 ± 15.9	48.7 ±11.0

[a] Group 1 - inoculated, treated birds.
Group 2 - inoculated, non-treated birds.
Group 3 - non-inoculated, non-treated birds.

The mean prothrombin time with standard deviations obtained for each group before, and five to six days after, inoculation is given in Table 6. The mean showed quite a large range in time. In all of the Experiments except II and V the treated birds showed a much greater decrease in prothrombin time than did the non-treated inoculated birds. The difference between Group 1 and Group 2 birds was 6.2 seconds in

Table 7. Prothrombin time for birds of Group 1 treated birds, and Group 2 non-treated birds. Prothrombin time expressed in seconds

Experiment number	Group number	Bird no.	Prothrombin time 2/15[a]	Prothrombin time 2/20
II	1	92	58	45
		73	63	50
		86	53	50
		72	45	NC[b]
		48	45	NC[b]
		93	55	NC[b]
		54	65	NC[b]
		66	65	57
		total	449	202
		mean	56.1	50.5
	2	59	60	35
		95	48	34
		89	60	36
		78	75	51
		75	50	40
		30	48	43
		77	55	45
		60	47	60
		total	443	344
		mean	55.4	43.0

[a] Day birds were inoculated.

[b] Blood clotted in syringe.

Experiment II, and 4.1 seconds in Experiment V, with the non-treated birds having the shortest time. The non-treated birds in these two experiments, however, suffered a high mortality, which was 75 percent and 90 percent, respectively. The treated birds of these two experiments had a 37.5 percent and 40 percent mortality rate, which was approximately half

that of the non-treated birds.

Table 8 gives the data on prothrombin times for birds of Experiment III. The birds in Group 1 (treated) showed a fairly similar range in prothrombin times, with the exception of bird 34 which had a prothrombin time of 18 seconds. The second determination showed a shorter time for all of the birds except for bird number 34 which showed a doubling of prothrombin time. The mean time for this group dropped from 51.7 seconds to 36.0 seconds after receiving menadione sodium bisulfite.

The non-treated-infected birds had a wider range in individual prothrombin times before inoculation, ranging from 19 seconds to 75 seconds. The second determination fell within a range from 28 to 52 seconds. The mean time, however, remained the same for both determinations. A drop of 15.7 seconds was recorded for the treated birds, as compared to no decrease in time for the Group 2 non-treated infected birds.

The prothrombin times for birds of Experiment IV were given in Table 9. The initial readings for Group 1 birds were higher than that recorded in the preceeding two experiments. These times did, however, correspond to those of the succeeding experiments. The higher reading was probably due to a different thromboplastin mixture from that used previously. The interesting observation in the experiment was the drop in time for most of the birds, only one bird having

Table 8. Prothrombin time for birds of Group 1 treated birds, and Group 2 non-treated birds. Prothrombin time expressed in seconds

Experiment number	Group number	Bird no.	Prothrombin time 3/10[a]	Prothrombin time 3/14
III	1	57	58	25
		34	18	37
		83	41	40
		32	65	45
		88	60	25
		76	60	45
		26	60	35
		total	362	252
		mean	51.7	36.0
	2	80	20	35
		46	56	45
		70	35	52
		63	19	50
		52	50	37
		36	23	32
		41	75	28
		total	278	279
		mean	39.7	39.7

[a] Day birds were inoculated.

an increased prothrombin time and one remaining the same at the second reading. The mean drop in time was 29.5 seconds for Group 1 birds, while Group 2 birds had a decreased time of ten seconds by the second determination. Individually, four of the non-treated birds showed an increase, none remained unchanged. The data, obtained for the birds of Experiment V, are given in Table 10. These

Table 9. Prothrombin time for birds of Group 1 treated birds, and Group 2 non-treated birds. Prothrombin time expressed in seconds

Experiment number	Group number	Bird no.	Prothrombin time Dates 4/7	4/12
IV	1	181	117	50
		124	45	55
		35	105	40
		102	67	60
		191	65	60
		164	99	50
		143	95	50
		132	63	50
		111	70	70
		94	135	57
		43	67	65
		198	110	85
		140	83	75
		40	53	30
		69	70	48
		127	130	57
		total	1374	902
		mean	85.9	56.4
	2	115	160	110
		61	75	45
		131	54	35
		45	45	40
		147	47	69
		31	61	55
		104	70	65
		165	95	62
		190	120	55
		200	45	55
		53	90	62
		81	63	47
		42	65	55
		51	45	30
		49	45	53
		35	60	53
		total	1045	891
		mean	65.0	55.7

birds had a high initial prothrombin time, but the second reading was extremely low as compared to other results. No explanation can be made for this extreme change in time. The treated birds had a mean decrease of 59.7 seconds, while the non-treated birds had a mean decrease of 63.8 seconds. The second time was 17.0 seconds for Group 1 birds and 17.4 seconds for Group 2 birds.

Table 11 includes the prothrombin data from Experiment VI. A different thromboplastin mixture was again used for Experiments VI and VII.

Three of the treated birds, and five of the non-treated birds had died before blood was taken for the second prothrombin time determination. A decreased prothrombin time was observed for all of the remaining treated birds, while the time for most of the remaining non-treated birds remained fairly constant. The mean decrease in time was 22.6 seconds for Group 1 birds and 14.3 seconds for Group 2 birds.

A mean decrease of two seconds was found on the fifth day for the treated birds, compared with an increase of 4.1 seconds for Group 2 birds of Experiment VII (Table 12).

If the prothrombin times were combined for all of the treated birds, and compared to the combined totals for the non-treated birds, a total of 4028 seconds would be obtained for the treated birds and 3615 seconds for the non-treated birds. The mean time would be 60.1 seconds and 53.9 seconds respectively, or a difference of 6.2 seconds shorter

Table 10. Prothrombin time for birds of Group 1 treated birds, and Group 2 non-treated birds. Prothrombin time expressed in seconds

Experiment number	Group number	Bird no.	Prothrombin time Dates 5/26	5/31
V	1	105	60	18
		161	91	20
		196	60	20
		44	92	17
		95	98	15
		171	40	18
		97	108	17
		172	78	19
		91	90	13
		184	50	13
		total	767	170
		mean	76.7	17.0
	2	113	60	13
		156	60	10
		168	100	17
		173	60	20
		123	97	22
		166	65	15
		98	70	18
		84	97	22
		129	100	19
		67	103	18
		total	812	174
		mean	81.2	17.4

prothrombin time for the non-treated birds. The total time for the second recorded determinations would be 2254 seconds and 2531 seconds, or a mean of 37.6 seconds and 40.2 seconds, respectively. The prothrombin time for all birds of Group 1 had dropped from a mean of 60.1 to a mean of 37.6 seconds.

Table 11. Prothrombin time for birds of Group 1 treated birds, and Group 2 non-treated birds. Prothrombin time expressed in seconds

Experiment number	Group number	Bird no.	Prothrombin time Dates 6/21	6/26
VI	1	157	39	
		103	25	15
		152	40	
		167	83	
		119	45	35
		189	55	23
		153	23	12
		110	26	15
		179	35	15
		116	25	09
		148	26	12
		180	43	10
		total	465	146
		mean	38.8	16.2
	2	136	37	
		112	57	33
		176	22	23
		126	26	21
		127	47	
		187	38	22
		186	47	
		108	21	
		183	27	
		160	31	10
		178	25	25
		109	35	17
		total	413	151
		mean	34.4	21.6

Table 12. Prothrombin time for birds of Group 1 treated birds, and Group 2 non-treated birds. Prothrombin time expressed in seconds

Experiment number	Group number	Bird no.	Prothrombin time Dates 8/18	8/23
VII	1	518	60	65
		593	34	33
		537	40	48
		564	42	50
		512	25	18
		530	40	41
		516	53	41
		517	30	40
		561	37	32
		556	60	25
		595	45	43
		540	28	44
		582	67	51
		576	50	51
		total	611	582
		mean	43.6	41.6
	2	558	55	25
		557	70	40
		515	33	50
		525	44	37
		506	33	57
		522	25	50
		591	33	35
		599	42	50
		536	40	60
		571	35	58
		528	82	60
		562	53	45
		600	38	61
		589	41	54
		total	624	682
		mean	44.6	48.7

This would represent a 22.5 second decrease in prothrombin time by the second determination. The time for the non-treated birds had changed from 53.9 to 40.2 seconds, or a mean of 13.7 seconds decrease in time. The treated birds, therefore, showed a mean reduction in prothrombin time of 8.8 seconds below that of the non-treated birds. If the birds of Experiments II and V were not included, the treated birds showed a mean decrease of 11.9 seconds below that of the non-treated birds.

The prothrombin times recorded in these experiments were not presented as the normal mean time for chickens. They did offer, however, a comparison of the time before and after inoculation with E. tenella and also after receiving, or not receiving menadione sodium bisulfite.

D. Histopathology

Histological studies were made from tissue sections prepared from the ceca of birds from Experiments VII, VIII, and from a special experimental Group not included in the previously presented data. This latter group of birds was treated in the same manner as were previous groups, except that the birds were sacrificed on the sixth through the tenth day after inoculation, solely for histological studies. In this experiment birds were sacrificed when necessary so that a bird from both groups was available for tissue studies. This was done so that a comparison of tissue could be made between the

treated and non-treated birds.

Serial sections were cut from the ceca of all birds of the special Experiment, from the birds of Experiment VII that had died, and from paired birds sacrificed after the seventh day in Experiment VIII. These sections were stained with Giemsa's stain for preliminary studies. From this preliminary study a number of slides were selected for further study. Additional serial sections were then prepared from the same blocks and stained with Delafield's hematoxylin, or Delafield's hematoxylin and eosin. The slides stained with hematoxylin were easier to study as the tissues of the ceca and the parasites were more clearly defined.

The description given by Tyzzer (1929) was used as a guide for histopathology associated with cecal coccidiosis. Normal histology as given by Calhoun (1954) was used as a guide for normal tissue.

Examination of serial sections from the ceca of birds of the non-treated group that had died during the infection, normally on either the fifth or sixth day, showed that severe hemorrhage had occurred. Blood and tissue debris filled the lumen of the cecum. The epithelial cells that were still intact were generally occupied by parasites. Portions of the tunica propria and submucosa were displaced by large collections of schizonts. The ceca of some birds showed large areas of sloughed tissue. An estimation of the amount of damage in some of the sections indicated that at least

one-half of the epithelial cells and a large portion of the tunica propria were missing.

Compared to the above observations, the ceca from the birds receiving menadione sodium bisulfite showed generally, much less severe hemorrhage even though the birds had died. In some of the sections it was difficult to distinguish any blood cells or plasma outside the blood vessels. The epithelial cells were as heavily parasitized as were those of the non-treated birds. The degree of sloughing of tissue was less prominent, and in many sections the tissue appeared almost normal, except for the presence of the parasites. The vitamin K active material apparently exerted no direct effect on the parasites, since abnormal forms were not found in the sections studied.

There was a generalized infiltration of lymphoidal cells in all of the sections. In those sections made from birds that were sacrificed, lymphoidal cells and connective tissue infiltration were quite prominent in both groups.

Photographs were made from sections showing the marked difference in hemorrhage and tissue destruction between treated and non-treated birds. These are included below in Figures 1 through 10.

The birds used for tissue studies were also compared as to weight gain or loss, and for prothrombin time. Treated birds that were sacrificed after the seventh day had gained more weight than had the non-treated birds. This might

indicate a less acute cecal coccidiosis, less hemorrhage, or a larger food intake by the birds receiving menadione sodium bisulfite. The weight difference was approximately 28 grams per bird.

Treated birds that died from cecal coccidiosis had a mean prothrombin time prior to inoculation of 62.2 seconds. The first prothrombin time following inoculation was 36.6 seconds, or a mean decrease of 25.6 seconds. The non-treated birds had a pre-inoculation mean prothrombin time of 58.8 seconds and a mean of 47.3 seconds by the second determination. This represents a decreased mean time of 11.5 seconds, which is less than half the decrease obtained for the treated birds. The change in prothrombin time would indicate that while menadione sodium bisulfite was successful in bringing about a decrease in prothrombin time, this alone was not sufficient to prevent death from cecal coccidiosis, or that some factor or factors other than hemorrhage contributed to the mortality of these infected-treated-birds. The following are suggested as possible contributory factors: Intravascular clots resulting from increase of available thromboplastin and prothrombin, or shock resulting from hemorrhage and histamine-like materials released from injured cecal tissue. No research was done to either prove or disprove the above suggestions. Additional work will be necessary to determine the reason for death in those birds that had exhibited such a marked decrease in prothrombin time. Theoretically, a

decreased prothrombin time should also cause a decreased clotting time and a subsequent decrease in severity of hemorrhage. This was apparent from the histological studies. Therefore, no reason can be advanced that would explain this discrepancy.

V. DISCUSSION

This study covered aspects of E. tenella infections that had previously been almost entirely neglected. The papers by Baldwin, Wiswell and Jankiewicz (1941) and Harms and Tugwell (1956), were the only references found to this problem in the literature. Baldwin, Wiswell and Jankiewicz (1941) used one-week-old chicks and infected them with 5,000 to 6,000 oocysts. One group of infected birds was given "Hykinone", a vitamin K active substance, and another group was used as an infected control. Each bird of the treated group received 0.25 ml of Hykinone per day for a period of four days. The authors reported a ten percent mortality in the treated birds, as compared to a 70 percent mortality in the non-treated birds.

Harms and Tugwell (1956) reported 6.8 percent mortality in three-week-old birds receiving 20 grams of menadione per ton of feed. Control birds on a basal diet also experienced 6.8 percent mortality from cecal coccidiosis.

The age at which chickens are most susceptible to cecal coccidiosis is still unsettled, but between four to eight weeks of age appears to be optimal for maximal infection. The birds used in this research were five-weeks-old. This age was chosen as the birds were within the optimal range, were large enough for easy handling, and for the withdrawal of blood by heart puncture. Larger numbers of oocysts were given to these birds than were given by Baldwin, Wiswell, and

Jankiewicz (1941). The chickens also received slightly larger doses of the vitamin K active substance. The birds of Group 1 were given 12.5 mg of menadione sodium bisulfite over the four day period. This was 2.5 mg more of the vitamin K active material than was used by the above authors. It was found, however, that if smaller amounts than 12.5 mg were used it was much less effective, or completely ineffective in preventing a high mortality in infected birds.

Frost and Spruth (1955) stated that menadione sodium bisulfite had at least four times greater potency than had menadione (Klotogen), and that one gram per ton was the minimal effective dose for prevention of hemorrhage in chicks on a vitamin K deficient diet.

Griminger et al. (1953b) found that 20 mg of menadione per kg given to birds simultaneously with coccidiostatic drugs was sufficient to return the blood clotting time to normal in approximately 72 hours.

Statistical analysis of data on weight studies indicated that the inoculated birds receiving menadione sodium bisulfite did not show a significant increase in weight gain over the non-treated inoculated birds. The slight weight gain by the treated birds in these series of experiments might have been due, in part, to the smaller loss of blood from hemorrhage, or to the birds eating more during the hemorrhagic phase of the disease.

The information obtained from the first three

experiments on the study of hemoglobin indicated that the menadione sodium bisulfite apparently exerted little or no direct effect on the hemoglobin concentration of the infected birds. The difference in grams per 100 ml of blood on the final determinations was too small to be significant. The hemoglobin concentration method could be used as a means of determining if hemorrhage was occurring, as in the case of cecal coccidiosis.

The prothrombin time studies indicated that the administration of menadione sodium bisulfite had a tendency to decrease the mean prothrombin time. In all of the experiments the mean prothrombin time for the treated birds decreased. This decrease in time was greater than any found in the infected non-treated birds with the exception of Experiments II and V, which have been previously mentioned. In Experiment V the inoculated controls had a lesser prothrombin time than did those birds receiving the vitamin K active substance. Why this happened is unknown, but an explanation will be attempted.

Shafiroff et al. (1943), working with normal animals, found that a rapid and progressive hemorrhage rendered the blood hypercoagulable and caused a marked reduction in clotting time.

While it is recognized that clotting time and prothrombin time are not directly proportional, it is reasonable to assume that a decrease in prothrombin time would be found in

animals exhibiting a marked decrease in clotting time. Another problem arises as to whether the hemorrhage resulting from E. tenella infection can be termed as rapid and progressive. Dougherty and Herrick (1952) claimed that the hemorrhage was minute and prolonged. In Experiment V of this research study, however, six of the nine birds in Group 2 were lost on the same day, this would indicate that the hemorrhage, apparently could not have been very minute or much prolonged.

Bradford, Herrick, and Wolfe (1947) recovered a lethal factor from the cecal fluid of birds infected with E. tenella. This material was present on the fifth through the thirteenth day after inoculation. When injected in infected or healthy birds, 0.1 ml was sufficient to cause death of the bird. Death was apparently caused by intravascular clots. The authors identified this material as a thromboplastin or thromboplastin-like substance.

This thromboplastin-like material from the ceca of infected birds could conceivably have been picked up by the blood stream in sufficient quantities to lower the clotting time; and the prothrombin time might possibly be lowered slightly. This may have been a contributing factor in Experiments II and V, in which a lower prothrombin time was found in the untreated birds. These birds also experienced a high mortality rate, which could have been caused, in part, by intravascular clotting.

The exact method of action of vitamin K is unknown. It is known, however, that it does not itself enter into the blood coagulation mechanism. This was shown by Dam, Schønheyder, and Tage-Hansen (1936). They demonstrated the presence of vitamin K in the plasma, but were unable to form a fibrin clot using it. They also reported the inability of plasma from K avitaminosis birds to form a fibrin clot. The clot could be formed, however, using plasma from birds receiving vitamin K.

Collentine and Quick (1951) postulated that vitamin K acted as a prosthetic group which combined with an apoenzyme to form the active enzyme which was responsible for synthesis of prothrombin.

Dessau, Lipchuck, and Klein (1954) found that the absence of vitamin K in diets of mice resulted in acute hemorrhage and mortality. When vitamin K and vitamin E were both absent, cardiac lesions and hemorrhage occurred. This might indicate that vitamin K by itself, or in conjunction with vitamin E exerted a homeostatic effect on the tissues and systems of the body.

The present concept of the importance of vitamin K is that it is necessary for the formation of prothrombin. This material is formed primarily in the liver. It is thought that vitamin K either becomes part of the prothrombin molecule, or is a necessary factor in the construction of prothrombin. An excess of vitamin K in the body does not

produce an excess of prothrombin, but does under normal condition maintain prothrombin at an optimal level.

The entire mechanism in the formation of the blood clot is uncertain, but production of fibrin probably occurs in a series of steps. A number of current theories on blood coagulation will be found in a publication by Albritton (1952). The following is a summary of one of the present theories of blood coagulation:

1. Platelets disintegrate----------thromboplastin
2. Factor V + prothrombin--(thromboplastin + Ca)--thrombin
3. Fibrinogen----------(thrombin)----------fibrin clot.

The clotting process is held in check in the blood by the absence of free thromboplastin. The thromboplastin is thought to be released when the endothelium of blood vessels is traumatized or the platelets are ruptured. All of the above factors are probably necessary for blood coagulation to occur <u>in vivo</u>. The prothrombin is thought to be produced constantly in the liver. The body's store of prothrombin becomes depleted within 24 hours if the liver is incapable of its continued production. Factor V, a globulin, is also thought to be produced by the liver. Its production and concentration parallel those of prothrombin. It is actually utilized in the conversion of prothrombin to thrombin.

The process in the chicken is similar in all respects, except that the source of thromboplastin is different. The thromboplastin is assumed to be released from injured tissue

cells of the body and not from platelets. There are no platelets in chicken blood according to Sturkie (1954) and Bainbridge and Menzies (1919).

The vitamin K active materials given the birds in these experiments would not result in an excess of prothrombin, but would be available for conversion to or utilization in the formation of prothrombin if needed during the period of hemorrhage from cecal coccidiosis. It is conceivable that in the non-treated birds the coagulation process which should occur during the hemorrhage phase of cecal coccidiosis would utilize most if not all of the normally available prothrombin. This would cause a depression of the blood's clotting ability in these birds. This apparently occurred during the earlier portion of the hemorrhage phase, as the chickens had a depressed blood clotting time then.

Couch (1954) stated that chickens under stress, such as when infected with cecal coccidiosis, probably needed additional vitamin K in their diet to help them recover from this period of stress. Menadione sodium bisulfite is not as active as vitamin K_1 in returning prothrombin times to normal in deficient chicks, or in birds under stress, but it is three to four times as effective as menadione according to Frost, Perdue, and Spruth (1956).

The data obtained from this study did not in all cases show a markedly decreased prothrombin time in infected birds receiving the vitamin K active material (Tables 9-12). They

do, however, indicate a tendency toward a more rapid formation of the fibrin clot. Prothrombin time would not decrease below a certain level regardless of the amount of vitamin K or prothrombin available. It was suggested that the additional vitamin K active material available to the birds resulted in the production of enough prothrombin to allow them to withstand better the stress of hemorrhage, and possibly reduce the total amount of blood lost. That it had some effect was obvious from the difference in the mortality rate between Group 1 and Group 2 birds. The mortality rate was 28.1 percent in Group 1 and 59.3 percent in Group 2 birds.

Studies of the tissue sections showed that the main and apparently only major difference existing between infected birds receiving menadione sodium bisulfite, and those not receiving this drug was the degree of hemorrhage that had occurred. This decreased severity in hemorrhage resulted in less marked destruction of tissue from the hemorrhage and less sloughing of the tunica propria and epithelial cells in the treated birds. The lesser degree of sloughing of tissue helped prevent additional hemorrhage.

Histological studies were made on birds from the fifth through the tenth day. The treated birds that had died showed less evidence of hemorrhage having occurred than did the non-treated birds. Birds surviving the infection showed comparable states of repair of tissue. A marked infiltration of lymphoid cells and connective tissue was observed. The

additional vitamin K-active material received by the treated birds was apparently utilized by the chickens in producing the extra prothrombin needed.

The additional prothrombin present during this period of hemorrhage, would permit the coagulation process to continue and less hemorrhage would occur. If prothrombin was low, then the coagulation process would be depressed and hemorrhage could continue until the bird died.

There was no apparent increase in rate of tissue repair in the treated birds. There was, however, less tissue destruction so a lesser amount of tissue repair was necessary in the surviving treated birds.

VI. SUMMARY

A 50 percent decrease in mortality rate was found in five-week-old chickens, inoculated with cecal coccidiosis, following the administration of menadione sodium bisulfite.

Menadione sodium bisulfite, a material possessing vitamin K activity, was given orally for four successive days: starting on the day of inoculation. The daily dose was 3.125 mg dissolved in one ml of water and given orally with a catheter.

Results obtained from hemoglobin studies indicated that vitamin K was ineffective in maintaining a normal hemoglobin level during the infection.

The prothrombin time was slightly reduced in most of the infected birds by the second determination, following the last administration of menadione sodium bisulfite.

Tissue sections showed a reduced severity in cecal hemorrhage among the treated birds regardless of whether they had died or had survived the infection. There was also less tissue destruction observed in these birds.

Comparison of the prothrombin time of infected birds that had received vitamin K active material, and still died, and non-treated birds that had died would indicate that some additional factor or factors other than hemorrhage contributed to the death of the birds with cecal coccidiosis.

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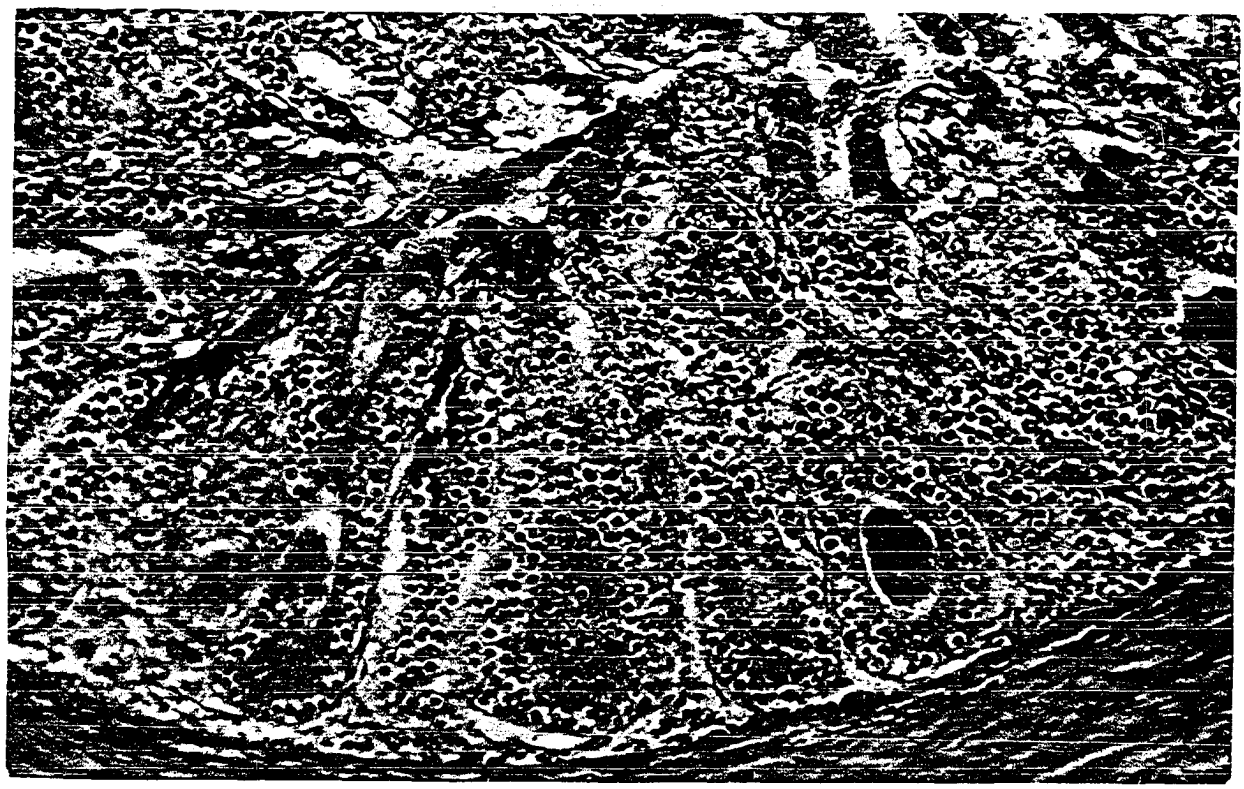
IX. APPENDIX

Figure 1. Photomicrograph. Treated infected bird number 521. This bird died on the sixth day following inoculation. The epithelial cells lining the lumen of the cecum were highly parasitized. Little tissue damage or hemorrhage was evident in the sections. The lumen of the cecum was relatively free of blood and debris. X100.

Figure 2. Photomicrograph. Non-treated infected bird number 572. This bird also died on the sixth day. The tunica propria was markedly disorganized. The lamina muscularis and a portion of the submucosa were intact as can be seen in the lower left portion of the photomicrograph. Severe hemorrhage and tissue destruction had apparently occurred in this portion of the cecum. No clear line of demarcation could be determined between the core in the lumen and the tissue of the cecum. Parasitic forms, apparently oocysts, were scattered throughout the submucosa. No normal epithelial cells could be distinguished. X100.



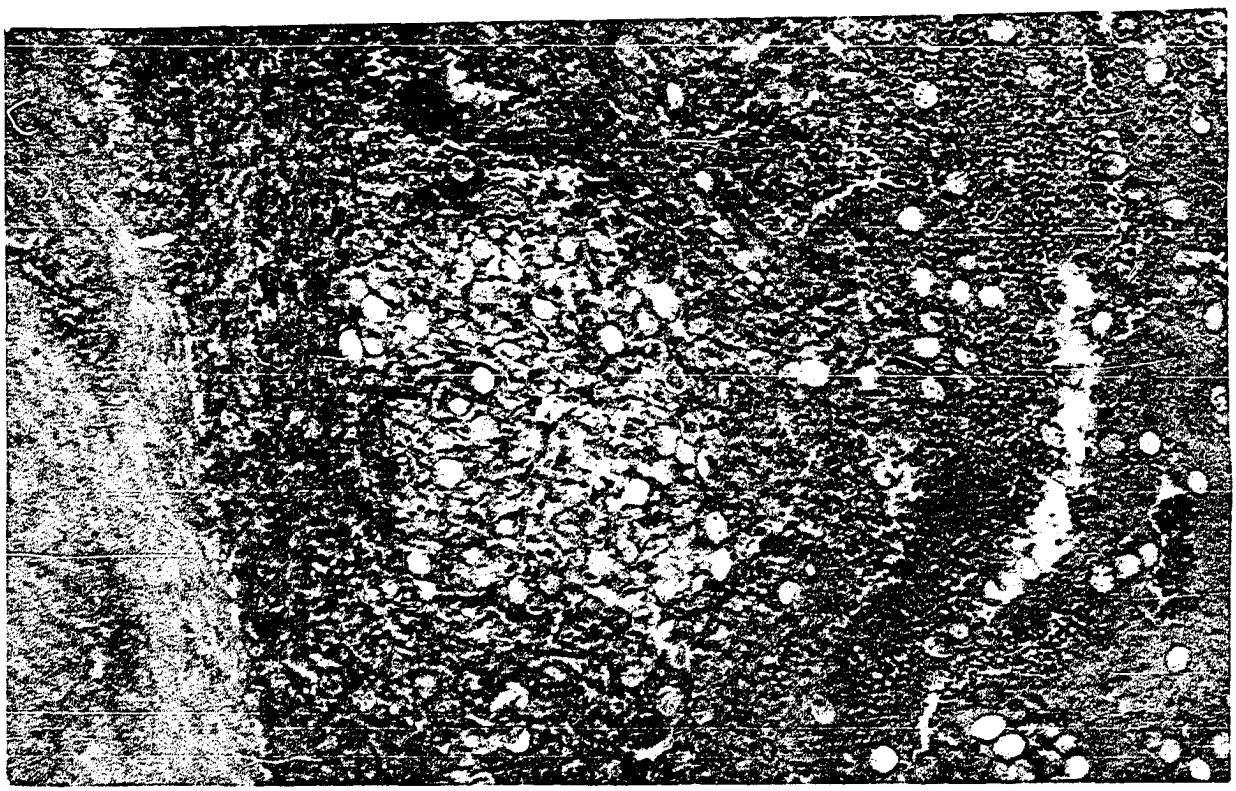
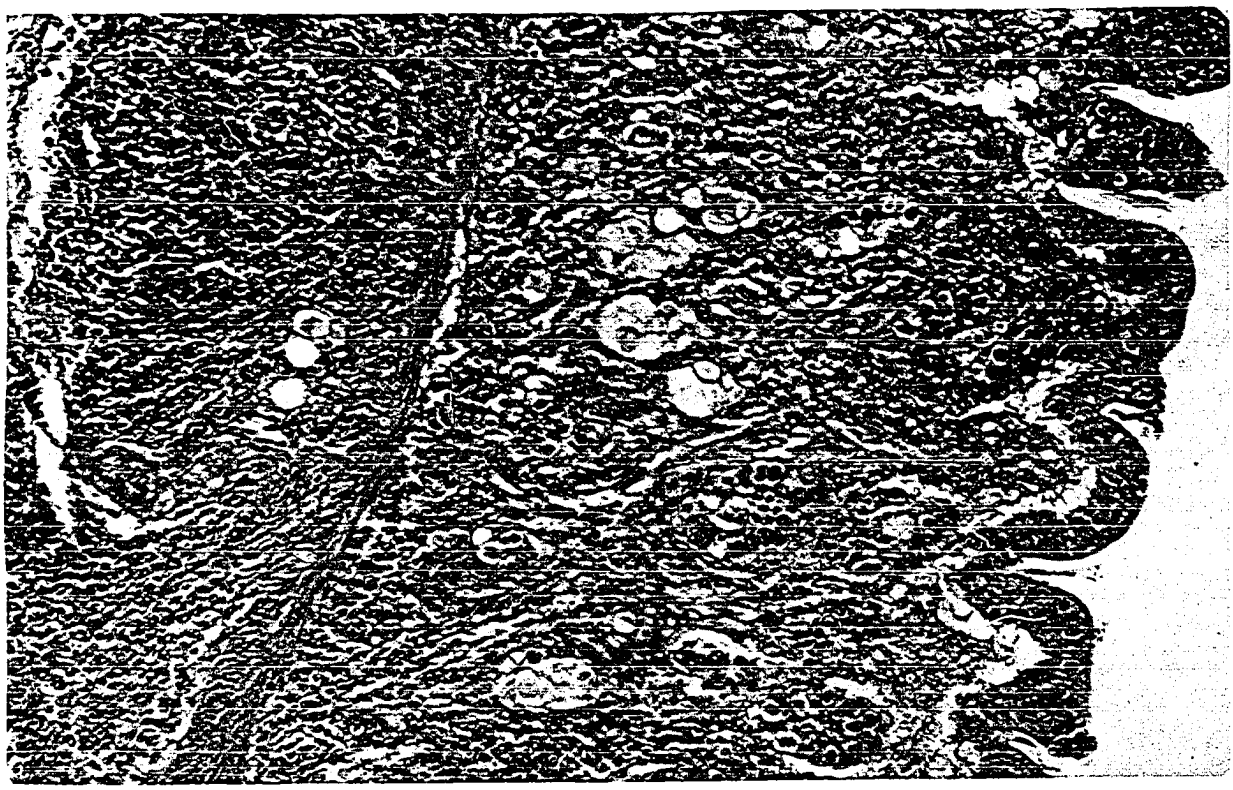


Figure 3. Photomicrograph. Treated infected bird number 580. This chicken was sacrificed on the eighth day following inoculation with E. tenella oocysts. Structurally the tissue appeared normal, except for the presence of a few parasitized epithelial cells, and some parasites in the tunica propria. The epithelial cells lining the lumen were intact. X100.

Figure 4. Photomicrograph. Non-treated infected bird number 509. The epithelial cells of the glands and those lining the lumen were apparently undergoing regeneration when the bird was sacrificed. These cells appeared as cuboidal or low columnar cells in the tissue sections. Parasitic forms and tissue debris could be seen in the lumen of the glands. X100.



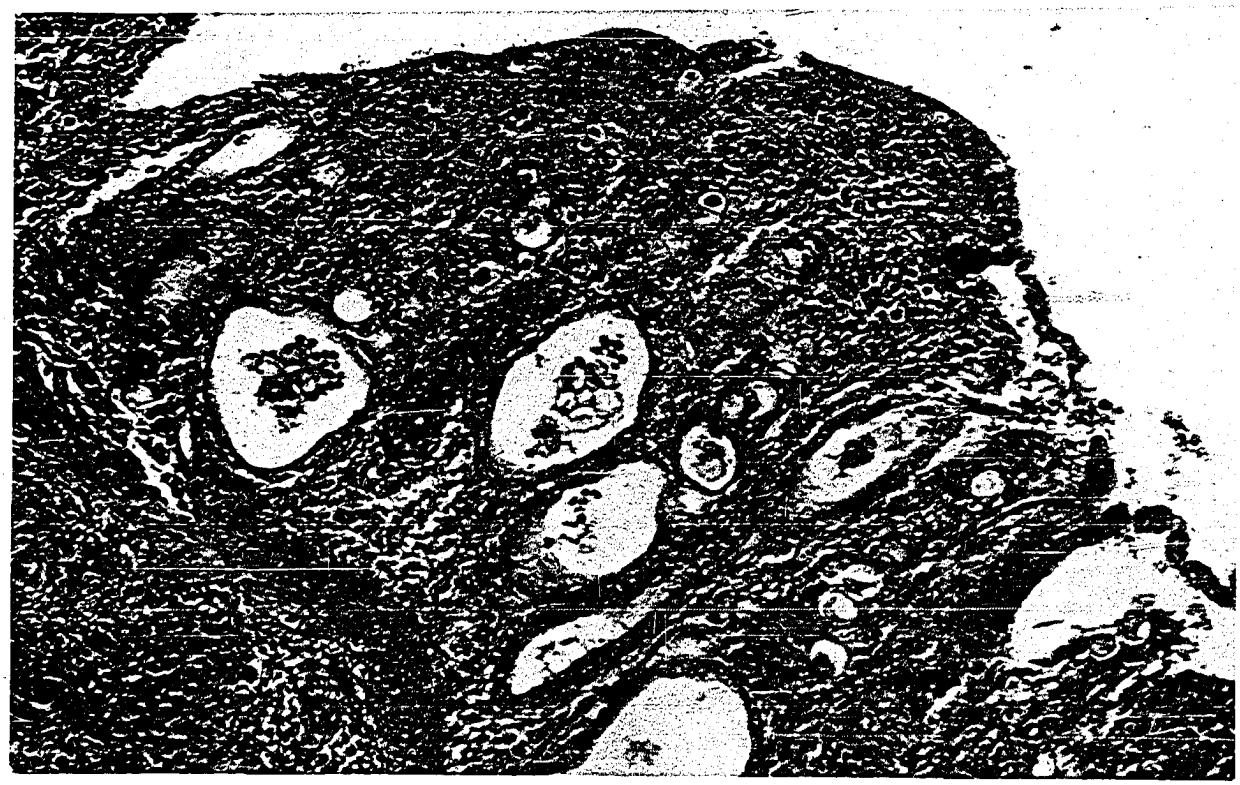
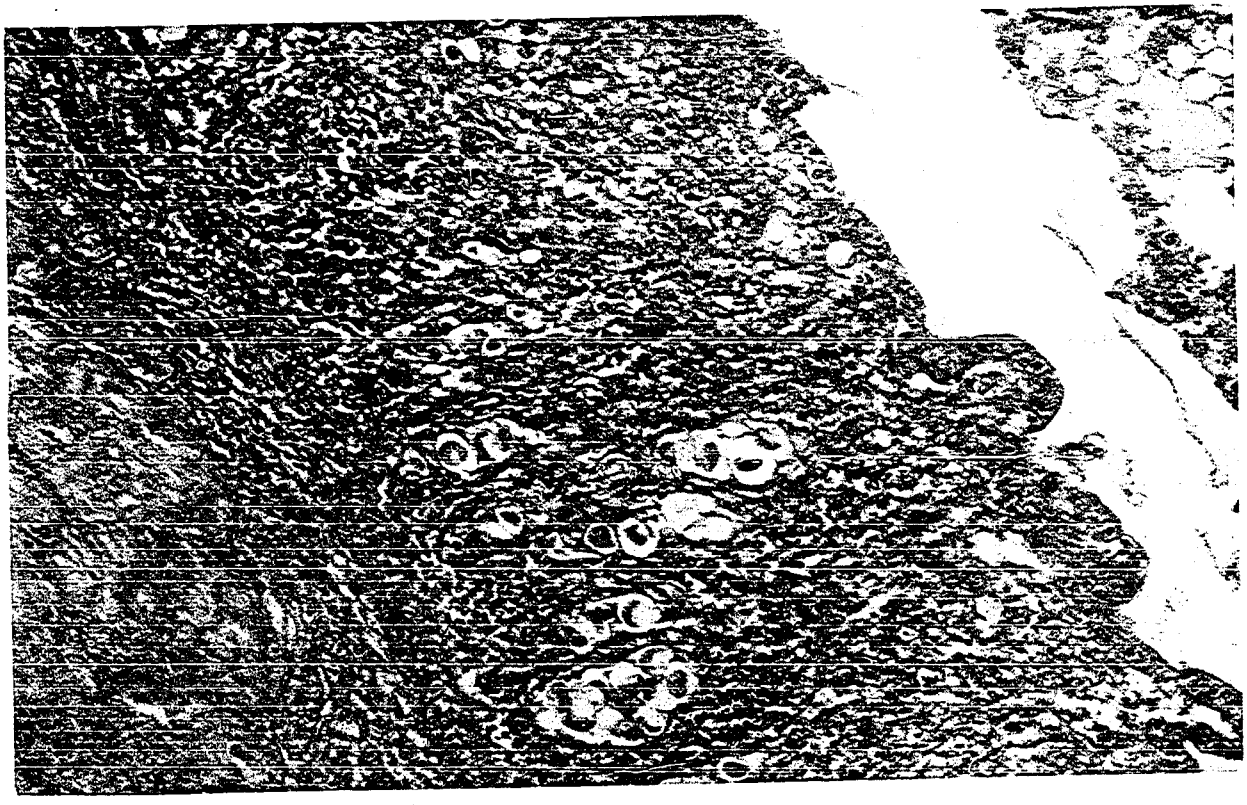


Figure 5. Photomicrograph. Treated infected bird number 519. This bird was also sacrificed on the eighth day. The epithelial lining of the lumen was intact, but the individual cells were less clearly defined than normally. A few parasitic forms were present in the thickened tunica propria. X100.

Figure 6. Photomicrograph. Non-treated infected bird number 507. This bird was sacrificed on the eighth day after inoculation. Microscopically, normal tissue or normal histological relationship of tissue structures were difficult to determine in the tissue sections. Apparently no regeneration of tissue had occured in the cecum of this bird. X100.



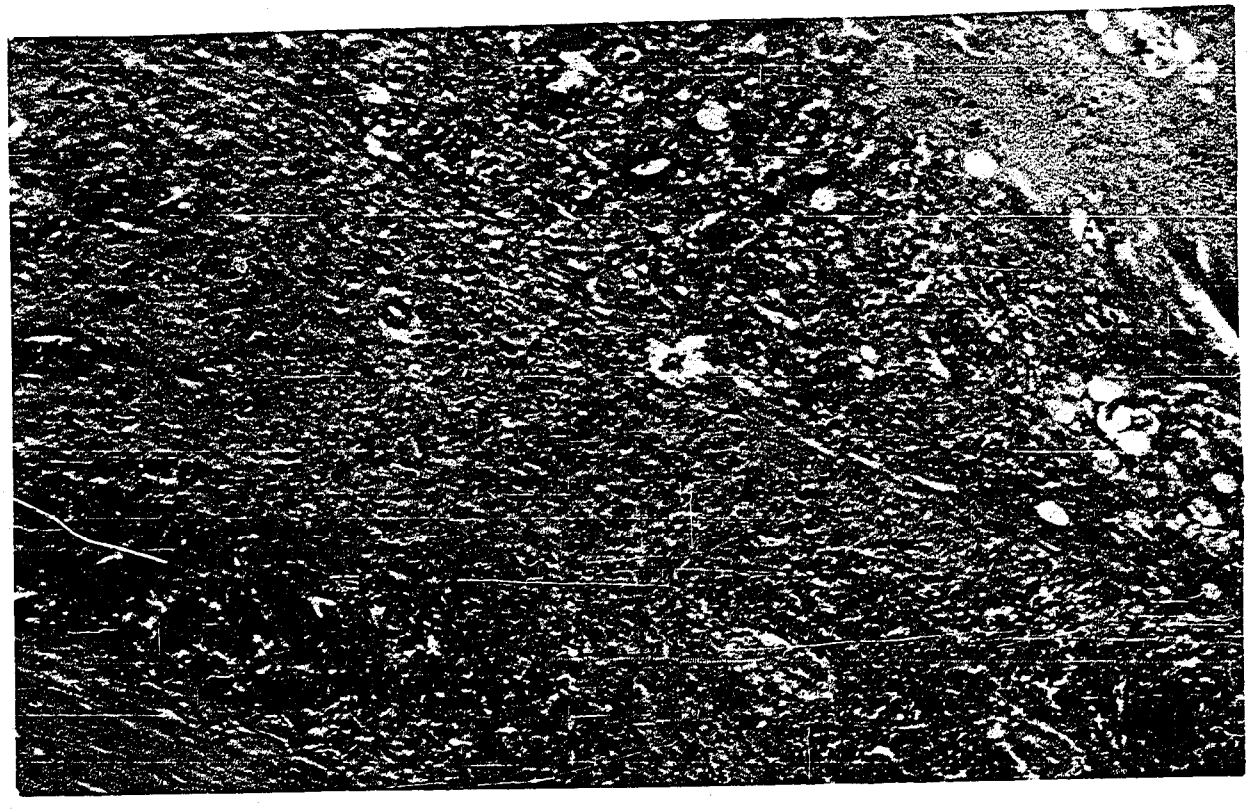
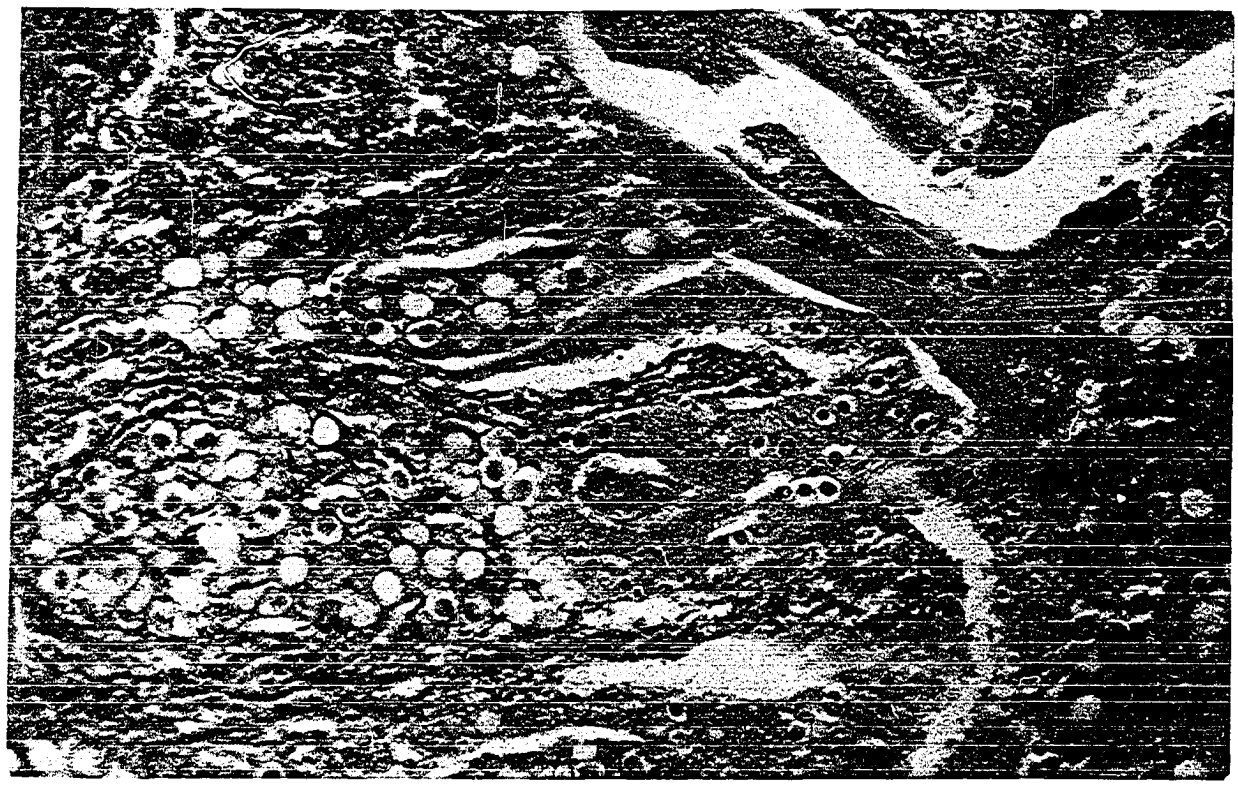


Figure 7. Photomicrograph. Treated infected bird number 621. This bird was sacrificed on the eighth day. The sections showed that hemorrhage had occurred in the cecum. The blood had apparently clotted and retraction of the clot underway when the bird was sacrificed. The epithelial cells appeared to have lost their immediate contact with the underlying tissue of the tunica propria. Numerous parasitic bodies, apparently oocysts, were scattered throughout the tunica propria. X100.

Figure 8. Photomicrograph. Non-treated infected bird number 677. This bird was sacrificed on the eighth day. The tissue sections showed that rather severe tissue destruction had occurred, apparently as a result of the coccidial infection. The portion seen in the photomicrograph showed a large area in which the tunica propria had been sloughed. The debris and blood present in the lumen were not evident in this particular portion of the section. X100.



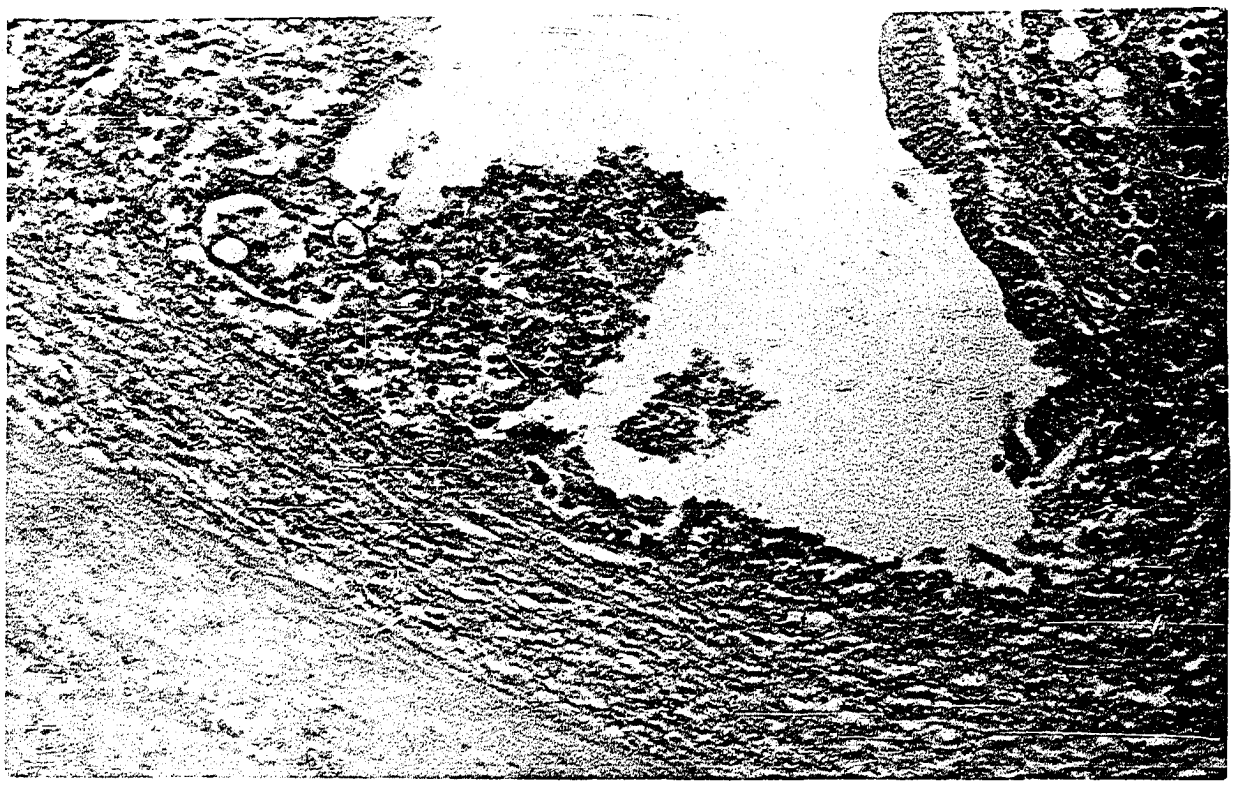


Figure 9. Photomicrograph. Treated infected bird number 545. This bird died on the seventh day following inoculation. The photomicrograph was made of the tunica propria to show that the integrity of the tissue had been maintained. X450.

Figure 10. Photomicrograph. Infected non-treated bird. This bird also died on the seventh day. The photomicograph was made of the tunica propria of the cecum. The integrity of the tissue has been disrupted and the individual cells are less distinct than in above photomicrograph. X450.