Miss. Helen E. Hem... Thesis 2333 - 1964

SUMMARY OF THESIS.

The pathogenic effects of the protozoan parasite Eimeria tenella were studied in the domestic fowl, Gallus domesticus. Observations were then made on the effect of X-irradiation on the pathogenicity and immunogenic potential of the sporulated oocysts of Eimeria tenella.

The findings established that the severity of an infection can be determined satisfactorily by consideration of the haemoglobin concentration, the clinical signs and the mortality during the acute stage of the disease, together with the results of the mean weight gains, the post mortem findings, and the total oocyst production of the chickens during the patent phase of the infection. The pathogenicity of the parasite cannot be assessed accurately from consideration of only one aspect of the disease.

The disease was reproduced with consistent pathogenicity by administration of a standard dose of sporulated oocysts.

A definite relationship was suggested between the age of the bird, the number of oocysts inoculated and the severity of the disease, although no significant difference in susceptibility to infection was shown which could be directly attributed to the age of the chicken.

Marked differences were recorded in the severity of the disease after administration of doses ranging from 1,000 to 500,000 oocysts per bird. The deleterious effects of relatively low levels of infection were reflected in the less satisfactory weight gains and the high total oocyst production during the patent phase of the disease. No significant variation in mortality or haemoglobin concentration was recorded between birds given doses ranging from 32,000 to 500,000 oocysts, the increasing severity of infection being indicated by the higher morbidity, the lower weight gains and the marked fall

in oocyst production. The reproductive potential of the parasite decreased significantly as the dose of oocysts was increased, suggesting a correlation with the severity of the caecal lesions at high levels of infection.

A significant relationship between the feeding regime before infection and the subsequent pathogenicity of the parasite was demonstrated, the most severe pathogenic effects occurring in birds given free access to food before infection.

A significant difference in susceptibility to infection was demonstrated between Broiler and Leghorn Type Hybrid chickens, the severity of the disease being greater in the Leghorn Hybrid chicks.

The pathogenic effects of a standard dose of oocysts was influenced by the diet of the experimental chickens. The influence of the ration also varied significantly in the Broiler and Leghorn Type Hybrid chickens.

Resistance to reinfection was conferred by a single dose of sporulated oocysts but this method of immunisation was contraindicated by the pathogenic effects of the parasite during vaccination.

Observations made on levels of X-irradiation, selected on an arbitrary basis, ranging from 5,000 Rontgens to 80,000 Rontgens demonstrated significant differences between the pathogenicity of normal and irradiated oocysts.

Exposure to 5,000 Rontgens reduced morbidity and haemorrhage during the acute phase of the disease, attenuation being marked after exposure to 7,500 Rontgens.

The findings demonstrated conclusively that sporulated oocysts must be exposed to a minimum dose of 10,000R to avoid deterimental effects during immunisation, the only evidence of infection being shown by the production of a relatively small number of oocysts. After exposure to 12,500R, 15,000R or 20,000R only very small numbers of oocysts were recorded, while no indication of infection was indicated after administration of oocysts exposed to levels of X-irradiation ranging from 25,000R to 80,000R.

Observations on susceptible chickens reared with vaccinated birds on deep litter suggested that the oocyst production associated with immunisation does not introduce a cycle of continuous reinfection leading to the build up of a heavy challenge infection under intensive management.

No significant variation was recorded in the oocyst production after vaccination between chickens given one or two doses of vaccine. Significant differences were demonstrated in the effect of certain levels of X-irradiation on the immunogenic potential of the oocysts.

The highest level of immunity was conferred by oocysts exposed to 10,000R. Good immunity was also developed after administration of oocysts exposed to 12,500R or 15,000R. A significant difference was demonstrated between the immunogenicity of oocysts exposed to 15,000R and 20,000R, resistance being significantly lower in birds vaccinated with oocysts exposed to 20,000R, 25,000R or 30,000R. A marked difference was also shown between the effect of 30,000R and 40,000R, immunity being negligible after exposure of oocysts to 40,000R, 50,000R or 60,000R. Chickens given oocysts exposed to 80,000R appeared fully susceptible on reinfection indicating that the oocysts were completely inactivated by exposure to 80,000R.

Single vaccination failed to confer satisfactory immunity against a high challenge infection. Resistance increased as the immunising dose of oocysts was raised by the results demonstrated conclusively that the highest degree of immunity was conferred by double vaccination.

Satisfactory immunity was demonstrated against reinfection in 11 week old broiler chickens.

The practical implications of the X-irradiated vaccine were indicated by the high degree of resistance to reinfection consistently reproduced in chicks without any pathogenic effect during immunisation, and by the development of immunity at an early age, suggesting the presence of adequate protection before infection would be established under field conditions.

A significant effect of vaccination was also indicated on the epidemiology of the disease by the low oocyst production of the immunised chickens after challenge compared with the high production of oocysts from the surviving susceptible birds.

Observations suggested that the decrease in the pathogenicity of the parasite after X-irradiation was not due to a simple reduction in the number of viable oocysts in the inoculum alone.

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OBSERVATIONS ON THE PATHOGENICITY AND IMMUNOGENIC POTENTIAL OF NORMAL AND X-IRRADIATED OOCYSTS OF *EIMERIA TENELLA* IN THE DOMESTIC FOWL (*GALLUS DOMESTICUS*).

by

HELEN E. HEIN, B.V.M.S., M.R.C.V.S.

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INDEX

GENERAL INTRODUCTION.

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"Coccidiosis", a disease due to infection with the protozoan parasite *Eimeria sp.*, can be responsible for considerable economic loss in the Poultry Industry.

Immunological studies have established that resistance is developed following infection with the parasite (Tyzzer, 1929; Pierce, Long and Horton-Smith, 1962). Immunity due to planned exposure of chicks to *Eimeria sp.* has been investigated under field conditions leading to the institution of a method of vaccination with normal oocysts in the U.S.A. (Edgar, 1958). Prevention of the disease by this method is subject to the following limitations:- (i) Satisfactory immunisation depends on natural reinfection during the patent phase of the initial infection which may not occur if conditions are unsuitable for sporulation of the oocysts. (ii) The unattenuated vaccine necessitates the simultaneous administration of a coccidiostat during vaccination to control the pathogenic effects of the parasite.

Earlier work had indicated that the pathogenicity of *Eimeria tenella* was reduced by X-irradiation (Albanese and Smetana, 1937) and that immunity was conferred against reinfection (Waxler, 1941), although marked evidence of infection occurred during vaccination. As recent work had demonstrated that certain helminth infections of domestic animals can be controlled successfully by the administration of an irradiated larval vaccine without evidence of any deleterious effects during immunisation (see Review by Urquhart et al., 1962), it was decided to initiate investigations on the effect of X-irradiation on *Eimeria tenella*. The primary objective of this work was to study the pathogenicity and the immunogenic potential of irradiated oocysts with the ultimate aim of determining the practicability of developing an irradiated vaccine against "Coccidiosis".

It was essential to ensure that the disease could be reproduced with a standard dose of normal oocysts before investigating the effect of X-irradiation on the parasite. Therefore the first series of experiments were designed (i) to study the pathogenic effects of normal oocysts of Eimeria tenella in the domestic fowl (Gallus domesticus) and determine criteria for the evaluation of the severity of the disease: (ii) to establish the significance of the age, breed, diet and feeding habits of the chickens on the pathogenicity of the parasite.

The second series of experiments was designed to study the effects of X-irradiation on Eimeria tenella. Preliminary observations were made to establish the levels of X-irradiation where changes in the pathogenicity of the parasite are first recognised and where the parasite is completely inactivated. Investigations were then carried out (a) to determine the Rontgen dose which reduced the pathogenic effect of the parasite to a minimum without inhibiting the development of immunity; (b) to determine the optimum number of irradiated oocysts for immunisation and the number of doses of vaccine required to confer resistance against a high challenge infection; (c) to establish that immunity could be developed in young chicks before the introduction of a heavy challenge infection under field conditions and show that resistance was adequate throughout the life of a broiler chicken.

SECTION I

DESCRIPTION OF THE DISEASE.

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The Organism Eimeria tenella

Eimeria tenella is a protozoan of the genus Eimeria of the subfamily *Eimeriinae*, a division of the family *Eimeriidae*. This family is a member of the suborder *Eimeridea*, a division of the order Coccidia which belongs to the *Class Sporozoa*.

Two genera, *Eimeria* and *Isospora* of *Eimeriidae* are of particular economic importance in both birds and mammals. Their life cycle is divided into two phases, one involving asexual multiplication (schizogony) and the second sexual multiplication (sporogony). The pathogenic effects of the parasite are usually related to the phase of schizogony. When the life cycle is direct the infective sporozoites are found in a sporocyst within the oocyst, providing additional protection against adverse environmental factors. Each genus of this family can be characterised by its oocyst; the number of contained sporocysts and their sporozoites varies with the species. *Eimeria* are recognised by four sporocysts, each containing two sporozoites seen in the oocyst. In most instances each species is host specific, although more than one species can occur in one host. In the chicken eight species are found; *Eimeria tenella* is one of the most pathogenic, being responsible for the disease "Caecal Coccidiosis".

The species of *Eimeria* are all obligatory intracellular parasites. They are found in the cells of the alimentary tract in the chicken. The entire life cycle of *E. tenella* is completed in the caecum of the fowl, with the exception of sporogony which takes place outside the host.

The Life Cycle.

The life cycle of E. tenella is composed of three phases; namely sporogony, which takes place after the oocyst has been passed in the faeces, and schizogony and gametogony which both occur in the caecum of the fowl (Becker 1934).

The life cycle is self limiting in the absence of reinfection (Tyzzer 1929). The prepatent period of seven days (Becker 1934) is unaffected by the size of the dose of oocysts given to a fully susceptible bird (Fish 1931). Recent work has shown that the first fertile oocyst can be passed out by a chick, given a slight infection, at between 5½ and 6 days (Edgar 1954). The longest recorded prepatent period of nine days occurred after infection with oocysts, stored for 687 or 781 days at 3°C (Dickenson 1946). The patent period is said to extend for between seven and 11 days after a slight infection and is not influenced by the size of the original infection (Fish 1931). However, in survivors of a severe infection, viable oocysts can be recovered from the caecal mucosa seven and a half months later (Herrick, Ott and Holmes 1936).

Sporulation of the oocysts of E. tenella has been recorded at a temperature ranging from 12°C to 33°C (McCullough 1952). It takes place in fortyeight hours at room temperature (Becker 1934). Under optimum conditions at 29°C in the laboratory, sporulation has been noted to take place after 15 to 18 hours of incubation, with maximum sporulation taking place between 24 to 27 hours. Therefore the complete life cycle can take place within seven days (Edgar 1954/1955).

The oocyst is colourless and has no apparent micropyle. There is no residual mass of cytoplasm in either the oocyst or in the sporocyst (Davies, Joyner and Kendall 1961).

The oocyst is a broad ovoid shape measuring 22.9u x 19.1 u. (14.2 u - 31.2 u x 9.5 u - 24.8 u) (Becker 1934, 1959). The wall of the oocyst is a double membrane composed of an inner thin endocyst, which is a continuous envelope enclosing the zygote, and the outer smooth surfaced ectocyst (Goodrich 1944; Davies, Joyner and Kendall, 1961). The unsporulated oocyst is practically filled with the zygote which has a limiting cytoplasmic membrane with a slightly irregular border (Edgar 1954). Within a few hours of being passed to the exterior in the faeces of the host the protoplasm of the zygote contracts away from the cyst wall, leaving a clear space. The oocyst can remain dormant for long periods in this state, although in a suitable environment sporulation occurs (Davies, Joyner and Kendall 1961).

The phase of asexual multiplication represents the division of the zygote to form four sporocysts, each containing two sporozoites. There is no visible distinction between the nuclear material and cytoplasm when the zygote divides to give four spherical refractile sporoblasts which elongate slightly and secrete individual refractile envelopes in the sporocysts. The protoplasm inside each sporocyst divides and elongates to form two sporozoites. Each sporocyst measures 11 u x 7 u and has a hyaline plug at one end. The sporozoites are banana-shaped and measure approximately 10 u x 2 u. The nucleus is located in the centre of the sporozoite. The sporozoites have granular cytoplasm; each contains a large eosinophilic refractile globule at one end and a smaller one at the other end (Becker 1934; Davies, Joyner and Kendall 1961).

After three hours incubation in the laboratory at 29°C the zygote has retracted from the wall of the oocyst and appears round with a clear band across the protoplasm. At six hours the zygote occupies half the area of the oocyst, the clear band being very distinct. The first signs of division are seen at nine hours when the zygote is either distinctly divided into four cells or has a four lobed appearance. At twelve hours 50% of the oocysts contain four distinct round or wedge-shaped cells; these appear like mature sporocysts at fifteen hours when the sporozoites begin to develop. At this time the oocysts are not infective. However at eighteen hours nearly all the oocysts contain sporocysts, many containing mature sporozoites (Edgar 1954).

Infection of the fowl follows ingestion of food or water which has been contaminated with faeces containing sporulated oocysts (Becker 1959). The excystation process in which the sporozoites are liberated from the oocyst has been observed to occur within five minutes of oral infection. The sporocysts pass through the oocyst wall before liberation of the sporozoites. This latter process has not been observed (Pratt 1937). Excystation is said to be facilitated by the body temperature and the digestive juices of the fowl (Becker 1959). It has been observed to occur in both the crop and the duodenum and the conclusion was drawn that enzymes from the lower part of the digestive tract were not essential for the process (Pratt 1937). However in later experiments infection was unsuccessful in birds which had had their pancreatic duct ligated, although inoculation with sporozoites led to infection. This led to the conclusion that pancreatic secretion is required for excystation (Levine 1942). Later studies confirmed these observations (Ikeda 1956).

Sporozoites have been observed in the surface epithelial cells of the caecum two to four hours after massive infections of five to twenty million oocysts in two to four week old chickens which had been fasted overnight before infection. It has been suggested that after penetrating the cells of the surface epithelium the sporozoites pass to the lamina propria where they are engulfed by macrophages and subsequently transported by these cells to the cells of the glands of Lieberkuhn (Challey and Burns 1959).

The sporozoite is usually located in the basal portion of the gland cell below the nucleus. It can be recognised in stained sections by its eosinophilic globule. The sporozoite which is developing into a trophozoite appears as an ovoid or rounded body; it then gives rise to the first generation schizont by repeated binary division (Becker 1934). The cytoplasm segments around each newly formed nucleus to produce first stage merozoites. The spherical merozoites elongate into fusiform organisms (Davies, Joyner and Kendall 1961). The developing first stage schizonts can be recognised in sections between twentyfour and fortyeight hours after infection. The mature schizont measures approximately 24 μ x 17 μ and contains about 900 sickle-shaped merozoites, each measuring 2 μ - 4 μ x 1 μ - 1.5 μ. As the schizont grows the parasitised cell increases in size and becomes squeezed out into the lumen of the gland (Becker 1934). This takes place between the 48th and 72nd hour following infections (Davies 1954). The first stage schizont can be differentiated from the second stage schizont both by the presence of the eosinophilic globule, which is not seen in the 2nd stage schizont and also by its location in the gland cell.

The first generation merozoites which are liberated from the schizont in the lumen of the gland penetrate epithelial cells in the fundus of the gland and begin to develop above the nucleus. The parasitised cell increases markedly in size and actively migrates into the subepithelial tissue where development of the second generation schizont proceeds until it contains 200 - 350 second generation merozoites (Becker 1934). The developing second generation schizonts can be demonstrated first in sections of the caecum seventytwo hours after infection (Davies, Joyner and Kendall 1961). A wide variation in size is seen among the 2nd stage schizonts ranging from

25/u - 34/u x 23/u - 40/u. The second generation merozoites are also larger than the first generation, being an average of 16/u x 2/u when mature.

In a severe infection the lamina propia between the glands is completely disorganised; the entire space between the glands may become packed with second stage schizonts developing in the migrating gland cells giving the appearance of a syncytium. As these schizonts approach maturity, usually about ninetysix hours after infection, the affected mucosa begins to slough, freeing the enclosed schizonts and their merozoites. This is accompanied by avariable degree of haemorrhage, which may be due to the degeneration of the blood vessels following interference with the circulation associated with pressure from the developing parasites.

The liberation of the second generation merozoites may be followed either by the formation of further schizonts containing 4 - 30 merozoites measuring 6.8/u x 1/u, or by the development of gametocytes. During gametogony the parasitised cells do not develop migratory tendencies (Tyzzer 1929).

The mature male microgametocyte measures approximately 12.4/u x 8.7/u and contains numerous microgametes which are formed by multiple division of the protoplasm. The microgametes are fusiform motile organisms which have two flagella attached at one end; they measure approximately 5/u long. The macrogamete is slightly smaller than the mature oocyst. Each one is formed from a single second generation merozoite. The macrogametocyte has granular cytoplasm, the nucleus being central in position. Large mucoprotein granules are present near the periphery of the macrogamete which disappear after fertilisation. It is assumed that they are incorporated in the wall of the oocyst. The first oocysts are present in the faeces on the seventh day after infection (Davies, Joyner and Kendall 1961). The prepatent period is said to be shorter when a very small infective dose is administered to chicks, fertile oocysts being recovered between 5¾ and 6 days after inoculation (Edgar 1954).

The total number of oocysts produced during the patent period is related to the size of the infecting dose (Fish 1931). Other significant factors include the inherent reproductive potential of the parasite to reproduce in the fully susceptible bird; this is high with E. tenella. This can be reduced in the case of a host which has been previously infected with the parasite, while strain differences in the host can influence susceptibility. Nutrition of the bird may also be significant. Experimental evidence shows that the number of oocysts produced per oocyst ingested decreases as the size of the inoculum increases, although up to a certain point the total output increases. It is suggested that this may be associated with a crowding effect.

The possible explanation has been given that it may be associated with the production of antibody initiated by the early stages in the life cycle which leads to an inhibition of the later stages, the level of antibody increasing with the size of the infective dose and exerting a more significant inhibitory effect on oocyst output (Brackett and Bliznick 1952).

The Pathogenic Effects of E. tenella.

The early phases in the life cycle of E. tenella exert no significant detrimental effects on the host. The pathogenic effects of the parasite are directly associated with the development of the second generation schizonts. These are relatively numerous even in light infections, since it is estimated that each first generation schizont liberates 900 merozoites. Therefore in a heavy infection very large numbers of cells are involved: this leads first to a marked disorganisation in the caecal glands as the cells migrate to the lamina propia; there the developing schizonts lead to widespread disruption of the tissue. These changes in the mucosa culminate in erosion of the tissues, together with haemorrhage, which can lead to death on the fifth and sixth days after infection. In some cases peritonitis is a feature and death may occur later in the course of the disease.

Experimental evidence suggests that a massive infection does not necessarily result in a high mortality, although it is associated with severe retardation of growth (Gardiner, 1955).

The exact pathogenesis of the haemorrhage has not been described. It has been suggested that the rapid development of the second generation schizonts leads to their encroachment upon blood vessels and interference with the circulation, so that eventually there is sufficient degeneration to cause haemorrhage from the vessels. Other factors implicated include the possibility that changes in the nutrition of the mucosa associated with the disorganisation of the circulation may be significant (Tyzzer 1929).

Clinical signs are not apparent until the third or fourth day after infection, when slight diarrhoea may be seen and perhaps a decreased food intake recorded. Marked symptoms occur towards the end of the fourth day or beginning of the fifth day when severe haemorrhage is present. In a heavy infection birds appear very depressed and may be found dead after having shown few premonitory signs. At this time copious blood is present in the faeces. If the bird does not die at this stage, marked weakness and thirst are evident and the comb and mucous membranes exhibit marked pallor. Birds which survive longer than seven days usually recover if the management is good. Survivors may show reduced weight gains, their magnitude depending on the degree of infection and the age of the bird; these are most significant in the older bird (Edgar 1955, 1958).

The typical lesions seen at post mortem examination of birds, which have died on the fifth or sixth day after infection, are severe haemorrhage in the caeca, which may be markedly distended with blood and erosion of the caecal mucosa. In a heavy infection the caecal wall may be very friable and may rupture on handling.

Changes associated with the haemorrhage include alterations in the haemoglobin level, haematocrit and erythrocyte count. These give a more accurate evaluation of the severity of the infection than consideration of mortality, the extent of lesions, or growth rate alone (Waxler 1941).

In less severe infections differences are more marked in haematocrit and haemoglobin levels than in the erythrocyte count. Changes in the haemoglobin level and haematocrit are first evident on the fifth day of infection, being at their lowest level on the sixth day. Their values are back to normal by the twelfth day (Joyner and Davies 1960).

In heavy infections there is a marked fall in the erythrocyte count on the fifth day; these become lower on the 6th day and reach a minimum on the 7th day, returning to normal limits fourteen days after infection (Herrick, Ott and Holmes 1936).

Comparisons of the effects of caecal coccidiosis with experimental haemorrhage on the erythrocyte count suggest that the decrease seen in the disease is due to loss of blood from the ruptured vessels (Natt and Herrick 1956).

During the haemorrhagic phase of the disease there is a decrease in the body weight, blood volume and corpuscular volume, but no change in the plasma volume (Natt and Herrick 1956).

It is suggested that compensation in the circulating blood volume during haemorrhage is in part associated with the withdrawal of fluid from the muscle plasma. There is an increase in the blood glucose level during the acute stage of the disease; it has been suggested that the source of this might be the muscles as there is a fall in muscle glycogen at this time (Pratt, 1940, 1941). There is also an increase of blood chloride on the sixth and seventh days, together with a very small decrease in the tissue chloride level (Waxler 1941).

Other changes recorded during infection include a reduced R.Q. of muscle (Daugherty and Herrick 1952) and early fatigue in the gastrocnemius muscle of infected chickens (Levine and Herrick 1954). Changes have also been observed in the oxygen consumption of the caecal mucosa. Measurements made before infection demonstrated a variation in the oxygen requirement of different regions of the caecum, the highest value being recorded in the central area of the mucosa where the most severe lesions were seen during the acute phase of the disease. Following infection the oxygen consumption decreased in both the central and distal regions of the mucosa, the values being more uniform throughout the caecum (Ripsom, Johnson and Herrick 1949).

The motility of the digestive tract is also impaired, leading to the retention or delayed passage of food in the crop on the 5th day of infection. In vivo observations show a decrease in the activity of the crop on the third day, with almost complete stasis on the 5th day. Crop activity does not return to normal for approximately fourteen days. The delay does not appear to be associated with the gizzard or small intestine (Schildt and Herrick 1955).

Epidemiology.

The disease can occur under any method of husbandry which permits the chickens access to faecal matter. It is most frequently seen amoung young birds kept under intensive management, although adults are susceptible if they have been reared under conditions which prevented infection with the parasite Under practical conditions infection can be maintained from year to year (Davies, Joyner and Kendall 1961).

Dissemination of the parasite is associated with the contamination of the food, water or litter with infected faeces. Therefore the introduction of the disease in some instances can be related to mechanical spread by the poultry attendant.

There is no record of infection in day old chicks hatched from eggs which had been contaminated with viable oocysts before incubation and incubated under normal conditions (Tyzzer 1932, Ellis 1938). However, after incubation for 21 days at a high relative humidity, contaminated egg shells fed to chicks led to infection, although the chicks hatched from the eggs remained free from infection (Herrick 1935).

Flies have been incriminated as mechanical vectors (Allen 1932) although other workers feel that adequate proof is absent, (Benger and Card 1935). However, it has been shown experimentally that oocysts can remain viable in the digestive tract of muscoid flies for up to 24 hours, and also in the insect faeces until they become dried up (Metelkin 1935).

The accepted method of infection is the ingestion of sporulated oocysts by a susceptible bird. Experimental transmission has been successful following cloacal injection of merozoites (Levine 1940).

Ingestion of small numbers of oocysts by susceptible birds does not lead to clinical disease, but it is a significant factor in the building up of infection in the rearing unit due to the high reproductive potential of E. tenella. The ground population of coccidia is also influenced by the number of birds present in the flock per unit areas and by factors associated with the degree of resistance in the birds. Small numbers of oocysts can be passed by partially resistant chickens, leading to outbreaks of disease among other susceptible birds should conditions favour optimum sporulation, before the birds have had sufficient time to develop resistance (Horton-Smith 1957).

The most important factors which affect the sporulation of oocysts in the field are temperature and humidity, others of less importance include lack of oxygen, the ammonia content of the litter and possible interference from bacterial or fungal activity. Rapid sporulation is favoured by a temperature of 29°C and a high relative humidity, while extremes of temperature or lack of moisture, leading to dessication, result in loss of viability (Horton-Smith 1957).

Experimental studies indicate that unsporulated oocysts are nonviable following exposure to either temperatures of 55°C or - 12°C to - 30°C for 48 hours and less than 96 hours respectively. Sporulated oocysts remain viable for fourteen days when subjected to the low temperatures but are killed on exposure to 40°C and 45°C in 96 and 5 hours respectively. Sporulation was observed between 12°C and 33°C. Unsporulated oocysts also appear slightly less resistant to unfavourable levels of humidity, although both sporulated and unsporulated oocysts are adversely affected when the relative humidity falls below 90% (McCullough 1962). Observations also suggest that the thick outer membrane of the oocyst is impervious to fluids, although it is easily damaged by dessication (Goodrich 1944).

Under range conditions in the field, low temperature is believed to be a limiting factor in the sporulation of oocysts (Horton-Smith 1957). Field observations made under the deep litter system of husbandry have demonstrated a direct relationship between the moisture content of the litter and the parasite content which indicated that moisture levels in the litter are probably a significant factor, limiting sporulation under this method of husbandry. These observations also suggested that the temperature of the litter in open pens may possibly favour sporulation of oocysts when adequate moisture is present (Davies and Joyner 1955). However, earlier investigations demonstrated temperatures in the litter which were suboptimal for sporulation (Horton-Smith and Long 1954). Field studies also suggest that the fermentation processes occurring in deep litter do not appear to be detrimental to oocysts, indicating the potential danger of a significant increase in the parasite population of litter when young chickens are reared consecutively on old litter (Kouts 1953).

The incidence of clinical disease may be influenced by the ration given to the chickens as there is a significant relationship between the diet of the birds and the subsequent pathogenic effects of the parasite after infection. Early experimental observations demonstrated a reduction in the mortality of chickens which were fed on a ration of buttermilk, grain and greens, compared with that in chickens reared on a standard dry mash diet after inoculation with oocysts of E. avium (Beach and C'Orl 1925). Subsequent work indicated that mortality also was reduced when the ration contained 40% dried skim milk or 20% lactose. It was suggested that the lower pH of the intestinal contents, associated with the inclusion of these products in the diet, might be responsible for an injurious effect on the sporozoites of the parasite (Beach and Davies 1926). These findings were in agreement with earlier observations which suggested that sour milk and buttermilk were of value in the control of coccidiosis (Fantham 1951). Later investigations also indicated a variation in the death rate of chickens which were fed on different rations after inoculation with oocysts of E. tenella. However, it was concluded that the inclusion of high levels of dried milk in the ration enhanced the pathogenic effect of the parasite (Becker and Waters 1938, 1939; Becker and Wilke 1938).

The least detrimental effects were recorded in the groups on a ration containing high levels of vitamins A and B and also a high protein level of 21% (Allen 1932). Subsequent studies failed to confirm a definite beneficial effect which could be conclusively attributed to high levels of either Vitamin A or protein in the diet, although the most satisfactory weight gains were recorded in the birds on the high protein rations in which the highest oocyst production was also seen. The exact level of Vitamin A was not specified, the sole source of the vitamin being derived from yellow corn meal which constituted 44% of the ration. The significance of the diet was emphasised when a marked difference was recorded between the clinical evidence of haemorrhage, the mortality and the oocyst production of chickens reared on three different rations. Later observations made on chicks placed on infected litter in a brooder house and reared on three different feeding regimes led to the conclusion that the disease was less severe in birds on a diet relatively high in carbohydrate and low in protein and fibre (Mann 1947).

Recent experimental work has demonstrated a clear relationship between the level of Vitamin A in the ration of chickens and the severity of caecal coccidiosis. Studies indicated that during the recovery period of the disease, feed consumption and growth rate were most satisfactory in birds receiving the highest level of Vitamin A in the ration after inoculation with oocysts of both E. tenella and E. acervulina. The death rate was also lower in birds receiving the lower levels of infection, although no difference was recorded when the inoculum was increased and administered to younger chickens. The results also demonstrated lower levels of Vitamin A in the liver of infected birds compared with non-infected control birds, while carotene appeared less efficient in promoting growth than vitamin A in both the control and the infected chickens, although the difference was most pronounced in the latter birds (Erasmus, Scott and Levine 1960). Subsequent observations made on birds kept intensively on deep litter under conditions which favoured the incidence of the disease demonstrated a significant decrease in mortality and higher weight gains in the experimental groups given supplementary Vitamin A in the ration or in the drinking water. The growth rate and food conversion were directly proportional to the level of the vitamin supplied to the chickens (Gerriets 1961).

The pathogenicity of E. tenella may also be enhanced when the diet is deficient in Vitamin K. Experimental findings indicated a significant difference in the mortality, clotting time, and prothrombin time after infection with the parasite between chickens on a basic diet low in Vitamin K, and similar chickens reared on a ration supplemented with either menadione sodium bisulfite of alfalfa leaf meal (Harms and Tugell 1956; Tugwell, Stephens and Harms 1957; Otto, Jorke, Frost and Perdue 1958).

The incidence of clinical disease may also be influenced by the feeding habits of the birds; those having access to food at all times appear less susceptible to low infections than birds which are fasted before infection (Edgar and Herrick 1944).

Another significant factor is the high virulence of E. tenella, the number of oocysts required to infect susceptible chickens and produce severe lesions being less than many other species. This factor, associated with the high reproductive potential, could explain the incidence of the disease among young birds, when the environment also provides suitable conditions of temperature and humidity.

Resistance.

Resistance is developed following infection with *E. tenella*. It is specific and does not confer immunity against any other species of coccidium (Tyzzer 1929).

Age is not considered to be a factor in the development of immunity (Mayhew 1934). Birds reared under conditions which prevent the ingestion of sporulated oocysts are susceptible to coccidiosis throughout their lives (Johnson 1927).

Experiments with birds aged from one to six weeks old indicated that chicks appear least susceptibe to infection at fourteen days old and most susceptible at four weeks (Gardiner 1955). Other experiments demonstrated no difference in susceptibility to infection between six month old chickens and younger birds (Horton-Smith 1947). These observations were confirmed by later work which concluded that susceptibility to coccidiosis increases up to four to six weeks of age, remaining high until eighteen months, the oldest age tested. Approximate five weeks was considered to be the age most susceptible to infection (Edgar 1958). However, it can be shown that chickens rigidly isolated from infection remain fully susceptible throughout their lives and that age per se has no influence on resistance (Davies, Joyner and Kendall 1961). Earlier work had led to the conclusion that the pathogenic effects were less marked in birds of twelve weeks old or older, but the number of birds in these groups was very small (Herrick, Ott and Holmes 1936).

Hereditary factors can be responsible for a variation in the susceptibility to coccidiosis. Careful selection of breeding stock has led to the isolation of strains of birds which are either highly susceptible or only slightly susceptible to infection (Mayhew 1934). Chicks bred from birds which are particularly resistant to *E. tenella* are far less susceptible to infection compared with chicks from unselected parents (Herrick 1934). It is suggested that multiple genetic factors are involved which do not exhibit dominance (Champion 1954). Similar results using another strain of bird and a different strain of E. tenella confirmed these findings (Rosenburg, Alicata and Palafox 1954).

When resistance to E. tenella was compared in chicks from immune and non-immune breeding stock it was suggested that parental immunity might be partially responsible for the lower susceptibility of chicks under two weeks of age (Edgar 1958). Observations on chicks from resistant and susceptible parent stock have not demonstrated the transfer of any maternal factor(s) responsible for resistance through the egg (Long and Rose 1962).

The degree of resistance which develops following infection depends on the method and magnitude of infection. A single infection with a small number of oocysts gives little protection to reinfection with large doses of oocysts (Tyzzer 1929, Horton-Smith 1947). At fourteen to twentyone days after a severe infection there is good resistance to challenge among survivors, but results show a decrease in protection at twentyeight days after infection (Horton-Smith, Beattie and Long 1961). Good resistance is developed after administration os several graded doses of oocysts (Farr 1943). Complete resistance has been obtained following three weekly graded infections in chickens, which showed no signs of diminished protection three months after immunisation (Pierce, Long and Horton-Smith 1962). Experiments in which pullets were immunised by four to six weeks of age demonstrated good protection to reinfection at six, twelve and eighteen months of age (Edgar 1958).

Immunity due to planned exposure of chicks to E. tenella has been investigated under field conditions. Three-day old chicks were given a small dose of oocysts in the drinking water and subsequently reinfected from seeded litter. After two or three subclinical infections which were controlled by continuous medication with coccidiostatic drugs in the food, a high degree of resistance was aquired to a challenge which gave a heavy mortality in non-infected control birds (Edgar 1958).

The administration of oocysts of E. tenella following exposure to various physical agents has been investigated with particular reference to possible changes in the pathogenicity of the parasite, and the development of resistance to challenge with unaltered oocysts. Good resistance associated with only slight haemorrhage during immunisation, followed the administration of three graded doses of oocysts exposed to a temperature of 48°C for fifteen minutes after sporulation (Jankiewicz and Schofield 1934). Less satisfactory results were given by a similar number of oocysts after exposure to -5°C for five days. Satisfactory resistance was not developed after the administration of oocysts subjected to 60°C or after exposure to either ultrasonic vibrations or gamma radiation (Urricchio 1953). Immunity is developed following the administration of oocysts which have been exposed to x-rays (Waxler 1941). These experiments are referred to in greater detail in Part Two.

The mechanism of acquired resistance to coccidiosis has not yet been explained. Experimental evidence suggests that humoral factors are important (Burns and Challey 1959; Horton-Smith, Beattie and Long 1961), but they have not been identified. Precipitins have been demonstrated in the serum of birds after infection. These appear identical with the precipitins produced by the injection of antigen made from schizonts of E. tenella. However, present evidence suggests that they are not directly associated with resistance (Pierce, Long and Horton-Smith 1962).

The effect of serum from resistant birds on the sporozoites and merozoites of *E. tenella* has been studied. The first successful results demonstrated the agglutination of merozoite suspensions with sera from chickens which had recovered from the disease (McDermott and Stauber 1954). Recent work has shown that second generation merozoites appear to become immobilised and lysed after incubation with serum from resistant birds. No oocyst production followed the introduction of these suspensions into susceptible chickens. Similar experiments using serum from normal birds demonstrated no change in the merozoites, which after administration to susceptible chickens led to the production of oocysts for a period of 24 yo 72 hours. No differences were seen in mortality or post mortem lesions between birds given sporozoites after incubation with serum from resistant or normal chickens before infection.

No evidence of passive immunisation has been produced by experiments in which serum from resistant birds was given to chickens before infection with *E. tenella* (Horton-Smith, Long, Pierce and Rose 1961; Tyzzer 1929).

Early experimental studies demonstrated that sporozoites failed to develop in the mucosa of resistant chickens. These observations were interpreted to indicate that resistance to reinfection was fundamentally associated with a local cellular response (Tyzzer 1932).

Differences have also been observed in the oxygen consumption of the caecal mucosa after infection with *E. tenella* together with a variation in the distribution of the parasite in the caecu on reinfection. These findings are believed to suggest that local physiological factors may be associated with the immune response (Ripon, Johnson and Herrick 1949).

Experimental observations indicate that the development of resistance is closely associated with the phase of schizogony in the life cycle of *E. tenella*.

Results from chemotherapy trials suggest that the very early stages in the life cycle are less significant in the production of resistance. This work clearly shows that complete suppression of schizogony results in a susceptible bird (Kendall and McCullough 1952). Recent work has shown that birds remain susceptible to reinfection after massive doses of second generation merozoites. This indicates that immunity does not appear to be influenced by gametogony (Horton-Smith, Long, Pierce and Rose 1963).

The development of *Eimeria tenella* in immune chickens has been studied. The results show that the life cycle appears to be interrupted before schizogony. Careful histological examination failed to demonstrate the presence of first generation schizonts or any later stage of the parasite in the caecum of completely resistant birds. Sporozoites were found in the cells of the glands of Lieberkuhn, twentyfour hours after infection, but there was no evidence of any subsequent nuclear division. Excystation of the sporozoites in the alimentary tract appeared to be normal (Horton-Smith, Long, Pierce and Rose 1963). Similar interference has been described in the life cycle of *Eimeria necatrix* in birds which were immune to this species (Tyzzer, Theiler and Jones 1932). The production of oocysts following merozoite passage has been investigated in resistant chickens. The oocyst production was far less from immune birds compared to that from susceptible birds given the same number of merozoites of *E. tenella*. Therefore it is suggested that stages in the life history of the parasite other than the early phases are affected by the immune response in the chicken (Horton-Smith, Long, Pierce and Rose 1963).

SECTION 2.

MATERIALS AND METHODS.

1. The Experimental Birds.

Broiler type hybrid day-old chicks were purchased from an accredited hatchery. Initially cockerels were selected from a single strain to minimise any variation in results due to genetic factors or difference related to sex, e.g., weight gains. Later both cockerel and pullet broiler chicks were used which were identical with the birds supplied to the broiler industry.

The chicks were placed in the isolation unit on arrival, and there they were reared in electrically heated brooders with wire floors. The chicks were selected at random, weighed and wing banded at the beginning of an experiment. The birds were kept in the isolation unit until the day before inoculation to ensure that they were fully susceptible to E. tenella. During the experimental period the chicks were transferred to either similar brooders or to metal cages with wire floors in the experimental unit situated in a separate building. The number of birds placed in each cage depended on the age of the chicks.

The birds were fed on proprietary chick or broiler crumbs without antibiotic or coccidiostatic supplements during the initial part of the research project. Later it was decided to "standardise" the ration to avoid any possible difference in the food associated with market variations. Therefore a chick mash was obtained which was prepared from a formula used by Joyner and Davies (1960).

2. Precautions to Prevent Extraneous Infection.

1. All accommodation and equipment were fumigated with ammonia before the beginning of each experiment.

2. Separate accommodation with different attendants was provided for birds before inoculation, giving complete isolation from the unit housing the birds under experiment.

3. Separate protective clothing was provided for each unit for all attendants and technicians visiting the birds.

4. The entrance to each unit was provided with a foot-bath containing an ammoniacal solution, which was changed at least once daily.

5. The chicks were all kept on wire floors to reduce access to faeces. The brooders and cages were scrubbed thoroughly each day during the patent period of infection to prevent any reinfection from the faeces.

6. Uninfected control birds were placed at random among the infected birds to detect any evidence of reinfection from the faeces. These birds were removed during the experiment to clean cages, where samples of faeces were taken for examination by salt flotation.

7. Faeces samples were examined each week from the controls and all groups in the isolation unit for the presence of oocysts.

3. PARASITOLOGY.

3. PARASITOLOGY.

(i) The Culture and Isolation of *Eimeria tenella* Oocysts.

The method used was based on that of Joyner and Davies (1960). Broiler hybrid chicks were kept in strict isolation until they were required for infection at fourteen days old. The chicks were reared in electrically heated, wire floored brooders. Samples of faeces were examined by salt flotation each week to ensure that the birds were not infected with coccidia before inoculation.

When the chicks were two weeks old they were transferred to similar brooders in the experimental unit; these had been previously fumigated with concentrated ammonia to exclude any extraneous infection with coccidia. Food was usually withheld for three hours before infection and returned one hour afterwards. The infecting dose was 1 ml. of water containing 2,000 sporulated oocysts; this was administered directly into the crop using an automatic syringe. During inoculation the stock of oocysts from which the syringe was filled was kept in suspension by decantation of the solution between two beakers.

The chicks were killed seven days later. The caeca were removed and weighted and cut up with scissors before being placed in the blender (Atomix..........M.S.E.) with 2% potassium dichromate solution. Each aliquot was left in the blender for not longer than thirty seconds. Care was taken to prevent overheating of the culture by the limited time of blending and by the use of adequate potassium dichromate solution. The final concentration of the suspension was one gramme of caecal material per 10 ml. of 2% potassium dichromate solution. After blending the culture was filtered through muslin into a beaker. Subsequently sodium bicarbonate was added to give a final concentration of 2%.

After overnight sedimentation the top third of the supernatant liquid was siphoned off and was discarded. The sediment and remaining liquid was then sieved through sieves (Endecot) of 100, 200 and 300 mesh per linear inch, using a specially constructed funnel which was fitted into a Buchner flask.

The filtrate was transferred to one litre cylinders and was left to sediment overnight. The top portion of 800 ml. was then siphoned off and was discarded. The remaining culture was centrifuged for five minutes at 2000g. per minute. The supernatant was then carefully discarded and the sediment was resuspended in 2% potassium dichromate.

A total oocyst count was made and the concentration was adjusted to not more than one million oocysts per ml. The culture was then transferred to shallow crystallising dishes and was placed in an incubator at 29°C to allow sporulation to take place.

A sporulation count was made each day and, when this was constant, the culture was stored in a conical flask or medical flat at 4°C. Sporulation normally required four to five days.

The age of a culture was calculated from the day on which the birds were killed.

(ii) Preparation of the Inoculum.

(a) The Total Oocyst Count.

The oocysts were suspended in a 2% solution of potassium dichromate. The culture was mixed thoroughly by inverting the container several times in an attempt to attain a uniform suspension of oocysts. Samples were placed on a Neubauer haemocystometer (Hawksley, London), using a clean Pasteur pipette and the oocysts in each of the four corner squares were counted. The mean value of ten individual samples from each culture was used to estimate the total number of oocysts per ml. Precautions were taken to ensure that the oocysts were as evenly distributed as possible in the counting chamber by discarding any slide which contained an air bubble under the coverslip and by cleaning the slide carefully to facilitate the entry of the sample under the coverslip.

The accuracy of the final estimate of the number of oocysts per ml. in a culture is indicated by consideration of the results (Table 20) of a series of counts on a typical culture of oocysts.

Calculation of the 95% confidence interval for a series of ten total oocyst counts with the highest standard deviation shows that for a mean count of 191.5 and a standard deviation of ± 28.14 the interval is from 171 - 212, i.e. from 428,475 - 529,025 oocysts per ml. The estimated variation is in the order of 20%

Similarly, calculation of the 95% confidence interval for a series of ten total oocyst counts with the lowest standard devation shows that for a mean count of 186.9 and a standard deviation of ± 10.5, the interval is from 179 - 194, i.e. from 447,500 - 486,000 oocysts per ml. The estimated variation is in the order of 8%.

Table 2. 0.

The Results of a Series of Total Oocyst Counts made on Individual Samples taken from the same Stock Suspension of Oocysts to Show the Possible Range of Variation in the Estimated Dose of Oocysts for Administration to the Experimental Chickens.

	S.1	S.2.	S.3	S.4	S.5	S.6
	182	239	209	190	177	172
	176	172	229	190	239	201
	232	191	173	180	172	177
	215	191	182	191	183	182
	171	156	157	209	185	183
	150	172	190	232	209	182
	183	209	183	173	173	197
	239	156	182	197	157	209
	182	173	182	172	185	173
	185	176	182	182	156	157
mean	191.5	183.5	187	191.6	183.6	183.3
s *	±28.1	±25.4	±10.4	±18.0	±24.8	±15.4
$s_{\bar{x}}$ **	8.9	8.0	3.3	5.7	7.8	4.9
@95%***	191±28	184±18	187±8	192±13	184±18	183±11

* the standard deviation.

** the standard error.

*** the 95% confidence interval calculated with "t" = 2.26 for 9 d.f.

(b) The Sporulation Count.

The sporulation count was made on an aliquot of culture following concentration of the oocysts by salt flotation. A total of 200 oocysts were counted under oil immersion and the % of sporulated oocysts was calculated.

(iii) Administration of the Inoculum.

Dilutions were made to give the appropriate number of oocysts per ml. for each experimental group, by the addition of distilled water to an aliquot of the culture which was suspended in 2% potassium dichromate solution.

The infecting dose was given in 1 or 2 ml. of water, depending on the age of the experimental chickens. It was administered directly into the crop, using an automatic dosing syringe. During inoculation the stock of oocysts was kept in suspension by decanting the solution between two beakers.

(iv) The Estimation of the Average Total Daily Oocyst Output / Bird.

Preparation of Samples.

The complete daily output of faeces was collected on greaseproof paper placed under the wire floor of the brooder or cage, on the droppings tray. This sample represented the pooled output of one experimental group, which was normally composed of ten birds.

Individual group samples were put into a liquidiser (Kenwood) in appropriate portions and was made up to 1500 ml. with tap water. After it was mixed thoroughly the contents were emptied into a larger container, and the complete sample was mixed again.

Eight samples of 1 ml. were removed from the total sample and placed in clean dry universal bottles, using a pipette designed for this purpose, with a wide bore which facilitated accurate delivery into the bottles.

This procedure was repeated for each of the experimental groups. The equipment was carefully washed between each new sample. The samples were always prepared in the same rotation beginning with those which had the lowest number of oocysts.

The Oocyst Count.

The number of oocysts in the sample was estimated by means of salt flotation using a McMaster slide. Five 1 ml. samples were counted for each group. Each sample was diluted with a measured volume of saturated sodium chloride solution. The bottle was carefully inverted several times, avoiding the production of large numbers of bubbles. One chamber of the McMaster slide was immediately filled, using a clean Pasteur pipette for each group. It was then examined under a low power objective and the number of oocysts counted after allowing a few minutes for the oocysts to rise in the slide. The average count was then calculated from the mean value of five counts.

(v) The X-Irradiation of E. tenella Oocysts.

The cultures of E. tenella used for X-irradiation were prepared in the usual manner and suspended in 2% potassium dichromate solution. The total oocyst counts ranged from 500,000 to 1,800,000 per ml. and sporulation count of 75% to 95%, the value being constant for each session of X-irradiation.

A standard 15 ml. aliquot of the oocyst suspension was transferred to a Petri dish, with a diameter of 4.5 cm., giving a constant depth of 1 cm. for X-irradiation. This dish was placed on a phantom, resting on hard wood blocks, under the window of the X-ray machine. The space surrounding the perimeter of the dish was carefully packed with bolus, and aluminium/starch preparation, to reduce X-irradiation scatter.

The distance between the dish and the X-ray machine was adjusted to a point where the dose rate to the base of the dish was 591 R/min.

The Siemens X-ray machine used in these experiments was operated at 140 kV and 20 mA with external filtration of 0.1 mm. Cu and 1 mm. Al. Calibration of the X-ray machine was carried out using a Baldwin-Farmer Sub-standard dose meter. The doses recorded in the experiments are in each case those delivered to the bottom of the oocyst suspension under the conditions described.

When each aliquot of the oocyst suspension had received the appropriate Röntgen dose it was carefully transferred to a clean bottle, together with the additional 2% dichromate used to rinse out the dish, the quantity varying from 5 ml. to 15 ml., depending on the original concentration of the oocysts in the culture. The irradiated oocysts were stored at 4°C for a period not exceeding 48 hours before dilution with water and subsequent administration to the chickens.

4. HAEMATOLOGY.

(i) The Estimation of Haemoglobin in Chicken Blood.

The haemoglobin concentration in the blood was determined using a modification of the Oxyhaemoglobin Method for human blood (Dacie, 1958).

In this method the haemoglobin in the sample of blood is converted to oxyhaemoglobin on dilution with a weak solution of ammonium hydroxide, by exposure to atmospheric oxygen (Hainline, 1958). The colour intensity of the oxyhaemoglobin in the solution is measured in a photometer. The principle of this method is founded on Beer's Law which states the the transmittance of a solution containing light absorbing material depends on the nature of the substance, the amount of light absorbing material in the light path and the wavelength of the light. This law is valid for a solution in which there is a linear relationship between optical density and concentration of the solute at a given wavelength (Hawk, Oser and Summerson, 1961).

The photometric values of the samples are converted to grams of haemoglobin per 100 ml. of blood from a calibration curve. A standard solution of cyanmet-haemoglobin prepared from human blood containing 10 grams of haemoglobin per 100 ml. ± 0.1 gram, is used to prepare the calibration curve (Davis and Keeler).

The advantage of the oxyhaemoglobin method is that it is accurate, simple and quick. The oxyhaemoglobin formed with the ammonium hydroxide is stable for up to twentyfour hours at room temperature and the optical density of the solution is of the same order as that of other haemoglobin derivatives. The reliability of the method is not affected by moderate amounts of bilirubinaemia (Dacie 1958). This procedure has the advantage over the cyanmethaemoglobin method in that no poisonous reagents are required and, secondly, that the ammonia solution is more stable in the laboratory than cyanide solution which also requires three reagents to be measured, compared with one simple volumetric dilution for the ammonium hydroxide.

Limitations of the method occur when there is excessive lipaemia or severe leukaemia, and in the presence of methaemoglobin, carboxyhaemoglobin or sulphaemoglobin (Dacie, 1958; Hainline, 1958).

In the oxyhaemoglobin method described for human blood, 0.02 ml. of blood is washed into 4 ml. of 0.04% (v/v) ammonia, contained in a tube provided with a tightly fitting stopper. After mixing by inversion of the tube several times, the sample is read in a colorimeter with a green filter (540 m/u) (Dacie, 1958).

The method used for the estimation of the haemoglobin concentration of chicken blood was as follows:-

The blood sample was taken from the bird by venesection of a small venule which lies parallel with the third metacarpal. The blood was collected directly into a heparinised haemoglobin pipette from the wing. Digital pressure was applied to the brachial vein before venesection. 0.02 ml. of blood was delivered into a colorimeter tube containing 1.8 ml. of 0.04% ammonium hydroxide and 0.2 ml. of heparin (1,000 units per ml.). Then the blood and ammonium hydroxide were mixed immediately by inversion of the tube several times, and placed in a rack. Subsequently a colorimeter (EEL - Evans Electroselenium Ltd.) with a green filter (540 mμ) was used to make the readings of samples. The photometric values were converted into grammes of haemoglobin per 100 ml. of blood from a calibration curve; the latter was prepared for oxyhaemoglobin from a standard solution of cyanmethaemoglobin supplied by Davis and Keeler, Ltd.

The standard which was used was prepared from human blood, therefore the values obtained for avian blood in these experiments may not be considered as absolute values of haemoglobin in chicken blood. In order to determine that samples containing avian blood obey Beer's Law, serial dilutions of chicken blood were prepared in serum and measured in the colorimeter. The results (Table 2.1) indicated close agreement between the calculated value and the photometric measurement of the haemoglobin concentration of each sample, confirming that Beer's law was valid for the estimation of haemoglobin in avian blood.

<u>Table 2. 1.</u>

The Calculated and Recorded Values of the Haematocrit and the Haemoglobin Concentration in Chicken Blood after Dilution with Serum.

Sample	Blood ml.	Serum ml.	Dilution %	Haematocrit		EEL	*Haemoglobin	
				A	B		A	B
1	1	0	100	28.5	-	31	9.3	-
2	1	0.25	80	22.8	22.8	25	7.5	7.44
3	1	0.5	60	19	17.1	21	6.3	5.58
4	1	1.0	50	14.5	14.25	16.5	4.9	4.65
5	1	2.0	33	9.5	9.5	11	3.25	3.07

* Haemoglobin estimated as grammes per cent.

(ii) The Accuracy of the Method Used in Haemoglobin Estimation in Chicken Blood.

There were three possible sources of error:-

(1) The colorimeter itself, involving the light bulb, the photoelectric cell and the colorimeter tubes.

(2) The preparation of the samples and the reading of results.

(3) The collection of the sample from the bird.

Experiments were designed to determine the variation which can occur in the estimated haemoglobin concentration due to these factors.

(1) Errors Related to the Colorimeter.

The photoelectric cell and the light bulb were carefully checked for any variation during the initial adjustment of the colorimeter before use. The intensity of the light was first made constant by adjusting the galvanometer to 100% transmission with a tube of equal thickness as that containing the unknown sample, but filled with a blank solution of ammonium hydroxide and heparin, placed in the aperture. The "blank" sample was then replaced by a similar tube containing a standard solution of eosin which should give a constant deflection. Subsequently between each sample the light transmission was checked with the "blank" tube.

The colorimeter tubes were matched with a standard solution of eosin. Any tube which gave a reading with a difference greater than one division on the colorimeter scale compared to that given by the standard tube was discarded.

The same green filter was used for all colorimeter readings.

The above precautions ensure that no significant variation in the haemoglobin levels could be attributed to factors directly associated with the colorimeter.

(2) Errors Related to the Preparation and Reading of Samples.

Errors could be associated with the following factors.

(a) The Accuracy of Filling the Syringe.

An automatic pipetting syringe adjusted to deliver a pre-determined volume of liquid was used to measure the ammonium hydroxide and heparin and transfer it to a colorimeter tube.

Ten clean, dry conical flasks were weighed empty and again after delivering 1.8 ml. of water from the syringe into each one. A further 0.2ml. of water was then added to each flask and it was re-weighed.

The weight of the 2 ml. sample of water was calculated. The results (Table 2. 2) show that the mean value of 2.0 ml. of water is 2.0646 grammes and the standard deviation is ± 0.00245, therefore the coefficient of variation is 0.1187%.

Table 2.2.

The Weight of the Flask and of the Samples of Water Delivered by the Automatic Pipetting Syringe.

No.	Wt. of Flask*	Wt. of Flask +1.8ml.Water	Wt. of Flask + 2ml. Water	Wt. of 2 ml. Water
*1.	32.2502	34.0583	34.3187	2.0685
2.	36.3315	38.1327	38.3941	2.0686
3.	29.1348	30.9404	31.2004	2.0656
4.	33.2700	35.0748	35.3346	2.0646
5.	30.6700	32.4748	32.7358	2.0658
6.	29.3315	31.1389	31.3984	2.0669
7.	33.5157	33.3264	35.5851	2.0616
8.	29.4208	31.2238	31.4848	2.0610
9.	30.7665	32.5711	32.8289	2.0624
10.	34.0797	35.8860	36.1452	2.0655

* Weight in grammes.

(b) The Accuracy of Delivering 0.02 ml. of Blood into a Colorimeter Tube from a Haemoglobin Pipette.

A single sample of chicken's blood was collected for the experiment. Any variation, therefore, in the colorimeter readings was related to the difference in the amount of blood delivered from the haemoglobin pipette. The colorimeter tubes were prepared and the readings made by the same technician. The same haemoglobin pipette was used by each technician.

The technicians, who were normally involved with the work, each added 0.02ml. of blood from the haemoglobin pipette into ten colorimeter tubes.

The results (Table 2. 3) indicated that there was no significant difference in the accuracy of delivering 0.02 ml. of blood from a haemoglobin pipette into a colorimeter tube between the technicians who were normally engaged in this work.

Comparison of the estimated values of the haemoglobin concentration in Series 2 and Series 3 showed that on technician was not more erratic than the other. The ratio of the variance of these results, i.e. $\frac{(0.237)^2}{(0.205)}$, is only 1.336; even if it was 3.18 (looking under 9 and 9 degrees of freedom), this would only be significant 2x5 = 10% level of significance, i.e. even then it would not be significantly different at the usually accepted level (i.e. 5%).

Table 2. 3.

The Variation in the Estimated Haemoglobin Concentration of a Single Sample of Chicken Blood Which is Associated with the Accuracy of Delivering 0.02 ml. of Blood into a Colorimeter Tube from a Haemoglobin Pipette.

Tube.	Series 1*	Series 2	Series 3
1.	6.7	6.1	6.9
2.	6.1	6.6	6.9
3.	6.1	6.3	6.7
4.	6.1	6.1	6.3
5.	6.3	6.1	6.1
6.	6.6	6.4	6.4
7.	6.4	6.7	6.6
8.	6.4	6.3	6.6
9.	6.4	6.4	6.3
10.	6.6	6.3	6.3
Mean	6.37	6.33	6.46
	±0.221	±0.205	±0.237

* Haemoglobin expressed as grammes per 100 ml. blood.

(c) The Accuracy of Reading the Colorimeter Scale.

This experiment was designed to study any possible error in the colorimeter reading attributed to the time of reading the result. Two samples, designated (a) and (b), were collected from different birds and placed in colorimeter tubes prepared in the usual manner. Each sample was read every ten minutes for one hour.

Examination of the colorimeter readings indicated that there was no significant difference between the readings of the colorimeter for the samples over the period of one hour (Reference Table 2. 4).

Table 2. 4.

The Colorimeter Readings Recorded at Ten Minute Intervals for One Hour of Blood Samples (A) and (B).

Time	0 min.	10 min.	20 min.	30 min.	40 min.	50 min	60 min.
(A)	22	22	22	23	23	23	23
(B)	27	27	27	27	27	27	27

Samples of blood were taken from fifty different birds, and were prepared in the usual manner, before colorimeter readings were made by the same person. The Samples were then placed in the refrigerator and left overnight. Next morning they were all read in the colorimeter.

The results (Table 2. 5) demonstrated no significant difference between the mean colorimeter reading 25.47 and a standard deviation of ± 2.299 of the samples following collection and the mean colorimeter reading 25.16 and a standard deviation of ± 2.26 of the samples after storage at $4\,^{o}C$ for 24 hours. However, when the difference between each pair of readings was evaluated, it was found to be significant ($p = 0.001$) although this valuation was of no importance in terms of haemoglobin concentration.

Table 2. 5.

The Colorimeter Readings of Fifty Blood Samples (A) Following Collection, (B) Twentyfour Hours After Storage in the Refrigerator at 4°C.

Sample	(A)	(B)	Sample	(A)	(B)
1.	30	29.5	26.	25	25
2.	27	27	27.	23	22.5
3.	26	26	28.	25	24.5
4.	27	27	29.	24.5	24.5
5.	26	26	30.	26	26
6.	26	25.5	31.	25.5	25
7.	26	26	32.	25	24.5
8.	27	26.5	33.	27	26.5
9.	28	27	34.	28	27
10.	25.5	25.5	35.	28.5	28.5
11.	27	26.5	36.	29	28.5
12.	25.5	24	37.	28	29
13.	27.5	27	38.	25	25
14.	27	27	39.	25	25
15.	28	27	40.	24.5	24.5
16.	28	28	41.	24	24
17.	26	25	42.	25	25
18.	22.5	22.5	43.	21	21
19.	22.5	22	44.	24	23
20.	22	21	45.	23	23
21.	27	26.5	46.	20	20
22.	27	26	47.	20	20
23.	24	24	48.	21	21
24.	25	24.5	49.	26	26
25.	26	26	50.	27	26
			Mean	25.47 ± 2.299	25.16 ± 2.26

(3) Errors in the Collection of the Sample of Blood from the Bird.

The experiment was designed to estimate the limits of variation in the haemoglobin concentration of the blood in chickens associated with the collection of the sample.

Four blood samples were taken from each of 20 birds. Each sample was taken separately, two being taken from each wing by venesection of a small venule which lies parallel with the third metacarpal bone. The samples were collected at the one time. All samples were prepared and read by one person.

Subsequently four blood samples were taken from each of twenty birds in a replicate group of four different days. These samples were collected and examined in a similar manner and the results were compared with those of the samples collected at one time.

The results (Table 2. 6) showed that there was a difference in the estimated value of the haemoglobin concentration in repeated samples collected either at one time or on four different days from the same bird.

The mean variance of the haemoglobin concentration was calculated from the results (Table 2. 6 and Table 2. 7) with 60 degrees of freedom. This was 0.306 and 0.442 respectively. The mean concentration of the haemoglobin was 7.86 and 9.85 grammes per 100 ml. of blood and the standard deviation was ±0.557 and ±0.665 respectively so that the coefficient of variation is in the order of 7% in each case.

The results showed no significant variation between samples taken from either wing.

It was possible that part of the variation between samples taken from the same bird was related to changes in the blood flow when the sample was collected. This has been demonstrated in the dog where it is associated with the application of pressure when the sample was taken. Pressure was also applied to the brachial vein at the proximal end of the wing before venesection in the chicken.

Table 2. 6.

The Estimated Haemoglobin Concentration in Four Blood Samples Taken From One Bird at One Time.

Bird	Sample 1*	Sample 2	Sample 3	Sample 4
1	9.6**	8.65	8.3	8.65
2	8.3	8.3	8.3	8.65
3	9.0	8.2	8.65	8.2
4	8.8	8.65	9.0	9.3
5	8.3	7.2	7.2	7.05
6	8.65	8.65	7.7	8.0
7	8.0	7.4	8.0	7.85
8	8.3	8.2	8.0	8.65
9	9.0	9.1	9.3	9.0
10	8.0	8.0	7.85	7.7
11	8.0	6.7	7.7	7.4
12	6.4	6.4	6.6	6.6
13	8.3	8.65	8.65	8.3
14	7.7	6.7	8.0	7.85
15	7.7	6.7	8.0	7.85
16	7.4	7.2	6.7	6.9
17	6.7	6.7	7.4	6.7
18	7.4	7.05	7.05	7.4
19	8.3	8.65	7.5	7.5
20	6.7	6.4	7.05	6.7

* Sample 1 and 2 taken from the left wing and sample 3 and 4 taken from the right wing.

** Haemoglobin expressed as grammes per 100 ml. of blood.

Table 2. 7.

The Estimated Haemoglobin Concentration in Four Blood Samples Taken from Each Experimental Chicken on Four Different Days.

Bird	Sample 1	Sample 2	Sample 3	Sample 4
1	9.9*	9.9	10.95	9.6
2	10.75	10.2	10.65	10.8
3	9.75	10.65	10.65	10.8
4	9.75	10.95	10.8	10.95
5	9.45	10.05	9.45	10.05
6	11.4	10.95	9.9	11.28
7	8.85	9.3	9.3	9.15
8	9.6	10.65	10.35	10.35
9	8.85	9.75	9.75	10.05
10	10.35	10.05	9.6	11.28
11	10.05	9.9	9.75	9.75
12	10.35	10.05	10.5	10.8
13	9.75	9.75	9.6	9.6
14	8.55	9.45	9.15	9.3
15	10.95	11.55	11.28	11.1
16	10.05	11.55	9.3	10.03
17	9.75	10.05	10.2	10.35
18	10.95	10.2	11.4	11.55
19	10.35	10.65	10.65	10.65
20	9.9	10.35	9.9	10.65

* Haemoglobin expressed as grammes per 100 ml. of blood.

SECTION 3.

EXPERIMENTAL WORK - PART I.

"OBSERVATIONS ON THE PATHOGENIC AND IMMUNOGENIC EFFECTS OF NORMAL OOCYSTS OF EIMERIA TENELLA ON THE DOMESTIC FOWL."

INTRODUCTION.

The first series of experiments was designed to study (a) the pathogenic effects of Eimeria tenella in the fowl, and (b) the resistance to reinfection. Before investigating the effect of X-rays on the parasite, it was essential to ensure that the disease could be reproduced with consistent pathogenicity with a standard dose of oocysts.

Initially estimations were made of the changes in the haemoglobin concentration of the blood during the course of the disease. These were related to the clinical signs, post mortem lesions and mortality. Subsequently the total oocyst output and the weight gains were also recorded. These results were used to determine criteria for the evaluation of the severity of the disease.

The susceptibility to infection was studied in birds of different ages to demonstrate any significant difference in the pathogenic effect of Eimeria tenella which could be related to the age of the bird. It was also desirable to find the appropriate age group for the particular aspect of disease under consideration.

Observations were necessary to establish the minimum number of oocysts which could lead to severe clinical disease in completely susceptible birds. This information was required to avoid over challenge of the birds during the preliminary investigations on the production of immunity by the administration of either normal or irradiated oocysts.

The pathogenicity of different numbers of oocysts was studied, together with the degree of resistance to reinfection conferred on the survivors.

The assessment of immunity to Eimeria tenella, based on the evidence of disease produced by a heavy challenge dose of oocysts, is complicated by the fact that lesions from the initial infection may interfere with the entry of sporozoites into the caeca on reinfection. Therefore the minimum interval of time between the inoculation and the challenge infection was determined after the duration of the lesions in the caeca had been established, by serial post mortem examination of chicks surviving from a severe infection.

The relationship between the severity of the disease and the interval of starvation before inoculation was studied following the administration of both high and low levels of infection.

Experiments were also included to investigate any possible variation in the pathogenic effects of a standard dose of oocysts of *Eimeria tenella*, which could be associated with either the breed of the experimental chicken selected for infection, or due to the ration on which the experimental birds were fed during the experiment.

EXPERIMENT ONE.

THE PATHOGENIC EFFECT OF A STANDARD DOSE OF SPORULATED OOCYSTS OF EIMERIA TENELLA IN CHICKENS AGED 3 to 6 WEEKS OLD.

EXPERIMENTAL AIMS.

The experiment was planned to study the pathogenic effects of E. tenella in chickens kept under laboratory conditions. It was necessary to establish criteria to measure the pathogenicity of the parasite; and also show that consistent results could be reproduced in birds of the same age with a standard dose of sporulated oocysts.

Therefore observations were made during the course of the disease with particular reference to:-

(a) Changes in the haemoglobin concentration of the blood.

(b) Mortality.

(c) Any variation of susceptibility to infection associated with the age of the bird.

MATERIALS AND METHODS.

Experimental Birds.

Broiler type hybrid cockerel chicks were used in this experiment. They were reared in complete isolation and were transferred to the experimental unit on day 0, where they were kept in metal cages with wire floors. A proprietary commercial chick food, (Baby Chick Crumbs, British Oil and Cake Mills, Ltd.) was available ad lib. to the birds.

Parasitology.

The culture of E. tenella used for the infection of groups 1, 2 and 3 was five days old on day 0, with a total oocyst count of 60,750 per ml. and sporulation count of 75%. The same culture was used to inoculate the duplicate group 3/2 and group 4 fourteen days later, when the total oocyst count was 89,610 per ml. and sporulation count of 87%.

Administration of Inoculum.

The infecting dose contained approximately 128,000 sporulated oocysts and was suspended in 2.1 ml. of 2% potassium dichromate; this was administered directly into the crop using an automatic dosing syringe. During inoculation the stock of oocysts from which the syringe was filled was kept in suspension by decanting the solution between two beakers.

The birds were not starved before inoculation, with the exception of group E.3/2 and group E.4, when food was withdrawn two hours before infection. Groups E.1, E.2 and E.3 were dosed in the evening, while group E.3/2 and group E.4 were infected in the morning.

Haematology.

Haemoglobin estimations were made on all the experimental birds on day 0 + 1, 3, 4, 5, 6, 7, 12, 13 and 14.

Experimental Design.

Fifteen birds were selected at random from each age group and divided into an experimental group of ten birds, with a corresponding non-infected control group of five birds which remained in isolation during the experiment.

On day 0 the birds in Group E.1 were three weeks old, group E.2 were four weeks old, group E.3 were five weeks old and group E.4 were six weeks old. The group E.3/2 was a replicate of group E.3, which was inoculated sixteen days later to observe if similar results could be reproduced with a standard dose of oocysts before investigating other factors related to the disease.

Following the administration of 128,000 sporulated oocysts to all the experimental chickens, haemoglobin estimations were made to evaluate the changes in haemoglobin concentration associated with the haemorrhage during infections.

Clinical signs and mortality were also recorded. Post mortem examination was carried out on all birds which died during the course of the disease on day 0 + 6; survivors were killed for examination on day 0+14.

RESULTS.

(1) Haematology.

The results (Table 3.1.) of the haemoglobin estimations in the control birds did not demonstrate any significant variation, either in the haemoglobin concentration of these birds during the course of the experiment, or between the different age groups of chickens three to six weeks old.

On day 1, 3 and 4 the results (Table 3.1.) did not indicate any difference between the experimental groups and their corresponding control groups with the exception of E.4.

The first significant decrease in haemoglobin concentration was seen on day 5 in E.3, E.3/2 and E.4, their lowest values being recorded on day 6 when there was an average fall of approximately 4 grammes of haemoglobin per 100 ml. of blood. Similar changes took place in groups E.1 and E.2 on day 6 and 7.

The haemoglobin concentration began to increase on day 8 in groups E.3/2 and E.4, the only birds sampled at that time (Table 3.2.). The earliest recorded fall in the haemoglobin level of an individual bird was seen on day 4 (Appendix 1 Table A1.1).

The results (Table 3.2.) of the replicate groups E.3 and E.3/2 were very similar, with the exception of those on day 5 when the decrease was more marked in Group E.3/2.

Table 3. 1.

The Mean Haemoglobin Concentration in the Blood of Control and the Experimental Chickens after Inoculation with 128,000 Sporulated Oocysts of *Eimeria tenella*.

Group	Day After Inoculation.							
	(1)	(3)	(4)	(5)	(6)	(7)	(12)	(14)
C.1	8.7* ±0.9**	8.7 ±0.9	8.0 ±0.7	8.6 ±1.1	7.9 ±0.8	8.6 ±0.6	8.4 ±1.3	9.0 ±1.1
E.1.	8.7 ±1.2 p=.25***	9.8 ±1.1 p=.25	8.0 ±2.2 p=.25	7.2 ±2.9 p=.25	5.4 ±2.2 p=.01	4.6 ±1.3 p=.001	7.9 ±1.3 p=.25	7.3 ±1.1 p=.02
C.2	9.2 ±0.5	9.2 ±0.7	8.4 ±0.7	8.9 ±1.2	8.4 ±0.7	8.7 ±0.8	9.1 ±1.0	9.0 ±0.4
E.2	8.5 ±0.9 p=.25	9.3 ±0.7 p=.25	9.7 ±1.0 p=.25	6.2 ±1.4 p=.25	5.6 ±2.4 p=.01	4.7 ±1.5 p=.001	7.6 ±0.6 p=.02	7.9 ±0.4 p=.02
C.3	8.6 ±0.6	8.7 ±0.2	8.3 ±0.5	8.1 ±0.2	8.7 ±0.5	8.9 ±0.6	8.9 ±0.8	9.2 ±0.7
E.3	8.1 ±1.1 p=.25	8.4 ±0.8 p=.25	8.4 ±0.5 p=.25	6.6 ±1.4 p=.05	4.0 ±1.2 p=.001	4.3 ±0.9 p=.001	6.8 ±1.5 p=.02	7.5 ±1.1 p=.05
C.3/2	9.0 ±1.1	8.5 ±0.5	9.1 ±0.9	9.6 ±0.6	8.0 ±0.8	9.0 ±0.7	8.9 ±0.7	8.2 ±1.1
E.3/2	8.6 ±0.8 p=.25	8.2 ±0.2 p=.25	8.2 ±0.9 p=.25	5.3 ±2.1 p=.001	4.1 ±1.0 p=.001	4.6 ±0.8 p=.001	6.8 ±1.3 p=.05	7.3 ±0.6 p=.25
C.4	9.0 ±0.4	8.6 ±0.2	9.0 ±0.4	9.2 ±0.3	8.7 ±0.3	8.9 ±0.9	8.7 ±0.7	8.4 ±0.5
E.4	8.0 ±1.0 p=.1	7.9 ±0.4 p=.05	8.1 ±0.6 p=.05	5.5 ±2.1 p=.001	4.1 ±1.2 p=.001	4.4 ±1.1 p=.001	7.7 ±0.4 p=.05	7.9 ±1.1 p=.25

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

** The standard deviation.

*** The probability calculated by the "t-test".

Table 5.2

The Individual Results of Haemoglobin Concentration in Replicate Groups of Five Week Old Chickens, (E.3 and E.3/2), After Inoculation With 128,000 Sporulated Oocysts of Eimeria tenella.

Group E.3. — Day After Inoculation.

Bird No.	(1)	(2)	(4)	(5)	(6)	(7)	(8)	(14)
1.	8.3*	8.3	8.66	6.5	3.1	4.15	-	6.65
2.	9.75	10.3	8.55	7.4	3.4	died	-	
3.	6.8	8.3	8.3	5.5	4.45	3.85	-	6.2
4.	7.4	7.7	7.7	5.3	died			
5.	7.4	8.0	8.85	5.8	4.9	5.6	-	8.85
6.	7.7	8.65	9.6	7.4	died			
7.	7.7	8.55	8.7	6.2	3.25	4.0	-	7.1
8.	9.15	8.3	8.1	6.2	4.45	4.45	-	8.15
9.	7.1	7.25	7.4	6.5	3.85	2.8	-	6.05
10.	9.75	8.65	8.85	8.65	4.65	5.2	-	8.65
Mean	8.11 ±1.00	8.44 ±0.82	8.4 ±0.53	6.55 ±1.44	4.03 ±1.22	4.29 ±0.92	-	7.51 ±1.13

Group E.3/2

Bird No.	(1)	(2)	(4)	(5)	(6)	(7)	(8)	(14)
1.	7.7	8.0	8.6	5.85	3.75	4.45	7.0	7.6
2.	9.15	8.0	8.4	3.6	died			
3.	9.65	7.4	8.9	3.6	5.3	5.55	7.3	7.1
4.	8.1	8.4	6.4	6.55	died			
5.	8.7	9.8	7.25	3.36	4.6	died		
6.	9.75	8.9	9.5	9.4	5.6	4.45	6.1	7.5
7.	9.0	7.8	8.5	6.15	2.8	3.5	4.2	6.35
8.	7.5	8.5	7.8	3.05	died			
9.	9.0	7.7	8.9	4.7	3.05	5.4	7.0	8.1
10.	8.6	7.25	7.5	7.4	4.3	4.15	6.4	7.1
Mean	8.64 ±0179	8.18 ±0.24	8.17 ±0.92	5.27 ±2.06	4.11 ±0.95	4.59 ±0.77	6.33 ±1.13	7.28 ±0.58

* Haemoglobin expressed as grammes per 100 ml. of blood.

Table 3. 3.

The Pathogenic Effects of a Standard Dose of 128,000 Sporulated Oocysts of Eimeria tenella in Chickens Aged three to six weeks Old.

Group	Age of Chick on Day 0	Haemoglobin* Concentration		Haemoglobin Decrease	Mortality	Lesion Score ***
		Day 1	Day 6	Day 1-6		Day 14
E.1.	3 weeks	8.7	4.6**	4.1	1	++
E.2	4 weeks	8.5	4.7**	3.7	3	++
E.3	5 weeks	8.1	4.0	4.1	3	++++
E.3/2	5 weeks	8.6	4.1	4.5	4	++++
E.4	6 weeks	8.0	4.1	3.9	1	++

* The group mean value of haemoglobin concentration expressed in grammes per 100 ml. of blood.

** Haemoglobin value on day 7, when lowest result recorded

*** Lesion Score

\+ Very slight lesions, lumen of caecum patent in all birds.

++ Slight fibrosis of caecal wall, with lumen of caecum patent in the majority of birds.

+++ Moderately severe lesions in the caeca, including the absence of normal contents in the caecal lumen, in the majority of the chickens.

++++ Very severe lesions in the caecum, with many birds showing complete occlusion of the caeca.

+++++ Severe lesions in the caecum which were also associated with severe peritonitis in some birds. Complete occlusion of the lumen of the caecum in many chickens.

(2) Clinical Findings.

The first clinical signs were seen on day 4, when the birds in the infected groups appeared slightly dejected and stood with drooping wings and ruffled plumage; their corresponding control birds appeared normal.

On day 5 and 6 morbidity was marked, the birds had pale combs, copious blood was present on the trays of the cages and depression was severe in all the surviving experimental birds. During this period their water intake was increased while food consumption fell.

The survivors appeared brighter on the seventh day and thereafter improved daily, although there was a marked difference in weight between the experimental and control groups on handling the birds. No evidence of haemorrhage was seen after the seventh day.

(3) Mortality.

Deaths occurred on days 5 and 6. It was greatest in group E.3/2 (40%), and E.2 and E.3 (30%); it was least in E.1 and E.4 (10%), (Table 3.3).

(4) Pathology.

Birds which died had typical lesions of acute caecal coccidiosis at post mortem examination.

The caeca were enlarged, being from two to four times larger than the caeca of non-infected control birds killed on the same day.

Signs of haemorrhage were apparent before incision of the caeca; they were dark red, due to the presence of blood in the lumen. Evidence of haemorrhage was also indicated by the marked palor of the viscera and by staining of the feathers around the vent.

The caeca were very friable, great care being required to prevent rupture during their removal from the abdominal cavity.

Numerous white circular lesions, less than half a millimeter in diameter, were present in the caecal wall under the serosa, associated with groups of second stage schizonts. Small pin point haemorrhages were often visible in the centre of these lesions. Widespread areas of haemorrhage were evident on the caecal mucosa, related to severe erosion of the epithelium and the submucosa.

No variation was seen in the severity of the lesions between the caeca in the same or different birds, although examination suggested that the changes were most marked in the middle third of each caecum.

Examination immediately after death demonstrated a wide variation in the physical state of the caecal contents. This ranged from the presence of uncoagulated blood, which escaped from the caecum on incision of the wall, to solid material composed of cellular debris and altered blood, which formed a cast of the caecal lumen. In the latter instance the dark red material was occasionally adherent to the wall and was difficult to remove with forceps.

Post mortem examination of the surviving birds on day 14 demonstrated severe lesions in groups E.3 and E.3/2.

The caecal walls showed varying degrees of thickening associated with fibrosis. In the caecum of non-infected control birds the mucosa was arranged in longitudinal and horizontal folds, which were completely absent in the caeca of the severely affected birds. The caecal lumen contained cellular debris and extravasated blood in nine of the thirteen birds examined in these groups. This material varied in colour from white to dark brown, depending on the stage of the breakdown of the haemoglobin pigment present in the debris. The caeca of six birds were distended to twice the size of those in the corresponding control pens by solid masses of this material which completely occluded the lumen. In other birds small aggregates of this debris was found in the caeca on incision. In some instances, where distension was severe, the caecal wall was very thin, appearing fibrosed on section.

Lesions were less severe in the survivors of groups E.1, E.2 and E.4. The walls of the caeca were less thickened in the majority of birds and fewer caeca were distended by necrotic debris.

Only two birds in E.1 and one in E.4, out of nine in each group, exhibited total occlusion of the caeca with casts of cellular debris and extravasated blood.

A lesion score was allotted to each group (Table 3. 3.).

DISCUSSION.

The results (Table 3. 1.) showed no evidence of a decrease in the level of haemoglobin in the blood of the control birds during the experiment. This indicated that the daily collection of blood samples was not associated with detrimental effects which could be responsible for a significant fall in the concentration of the haemoglobin in the control or experimental birds. The difference between group E.4 and the corresponding control (Table 3. 1.) could be explained by the variation which occurs in the value of the haemoglobin concentration in normal chickens. The individual results were within the accepted normal limits.

The changes in the concentration of haemoglobin which took place during the course of the infection clearly demonstrated the value of this parameter as one criterion for the evaluation of the pathogenicity of E. tenella. This is in agreement with Waxler (1941). The similarity between the results of groups E.3 and E.3/2 (Table 3. 2.) both emphasised the validity of this criterion and also indicated that consistent results could be reproduced with a standard dose of 128,000 oocysts. The earlier decrease in the haemoglobin concentration, recorded on day 5 in group E.3/2 and E.4 was probably due to the difference in the time of inoculation.

The decrease in the haemoglobin (Table 3. 3.) of approximately 4g. per 100 ml. of blood was similar to that recorded by Waxler (1941) in five week old birds inoculated with 200,000 sporulated oocysts of Eimeria Tenella.

Natt and Herrick (1955) recorded differences in the haematocrit and the total erythrocyte count of five week old chickens after infection with 50,000 sporulated oocysts of E. tenella. The time of the changes in the level of haemoglobin in this experiment was similar.

The clinical symptoms appeared with the onset of haemorrhage; the consumption of water increased and the weight of the experimental birds was less than those in the corresponding control pens. These facts were probably due to the changes related to the restoration of the blood volume.

There seemed to be no correlation between mortality and clinical signs; birds died suddenly while others, which appeared equally depressed, survived. The results of the haemoglobin estimation in individual birds (Appendix 1, Tables A1. 1-4) did not show any direct relationship between mortality and the initial haemoglobin level or the subsequent decrease after infection. There was no difference in mortality between birds which had haemoglobin levels at either limit of the range of values recorded before the effects of haemorrhage became apparent. Similarly, the actual decrease in haemoglobin did not indicate any definite difference between survivors and the birds which died, as some of the lowest values were recorded among survivors; some of the blood samples were taken immediately before death.

The difference in mortality (Table 3. 3.) between groups E.1 and E.4 on the one hand, and E.2, E.3 and E.3/2 on the other, could possibly have implied that birds of four and five weeks old were more susceptible to the pathogenic effects of E. tenella. This would have been in agreement with the conclusions of Gardiner (1955) and Edgar (1958). Those workers based their opinions on the observation of mortality, post mortem lesions and differences in weight gains. However in this experiment, although the lesion score bore some relationship to the mortality, the results (Table 3. 3.) of haemoglobin estimations failed to confirm a definite difference between the groups.

Therefore it was concluded that the results of this experiment provided insufficient evidence to state that there was any significant variation in the susceptibility between the groups to infection with a standard dose of 128,000 sporulated oocysts of <u>E. tenella</u>, which could be attributed to the difference in the age of the birds.

The severe lesions found in some of the surviving birds clearly confirmed the observations of Kalatsky and Hughes (1949) who indicated that an incorrect assessment of immunity to <u>E. tenella</u> could be made on evidence, based on the results of a high challenge dose of oocysts, in cases where persistent lesions from the initial infection prevented entry into the caecum of the sporozoites from the challenge infection. Thus post mortem examination showed that, after inoculation with 128,000 sporulated oocysts of <u>E. tenella</u>, reinfection with the same species to evaluate resistance was contraindicated for at least a fortnight after the initial infection.

EXPERIMENT TWO.

THE PATHOGENIC EFFECT OF DIFFERENT NUMBERS OF SPORULATED OOCYSTS OF EIMERIA TENELLA IN CHICKENS AGED 6 WEEKS OLD.

EXPERIMENTAL AIMS.

The experiment was designed to study the pathogenic effects of E. tenella in six week old chickens after inoculation with different numbers of sporulated oocysts. The range of doses included low and high levels of infection for comparison. This information was necessary to illustrate the relationship between the number of oocysts administered and the severity of the disease. Observations were made on the changes in concentration of haemoglobin, on mortality and on growth rate. Serial post mortem examinations were carried out on birds given low doses of oocysts during the acute phase of the disease and, in addition, on birds from the experimental groups. These results were also important for the determination of criteria to evaluate the pathogenic effects of E. tenella.

Observations were necessary to establish the minimum number of oocysts which would lead to severe clinical disease in completely susceptible birds. This information was required to avoid over challenge of birds during preliminary investigations on the production of immunity to E. tenella. Birds of six weeks old were chosen for the experiment so that the results would be directly applicable for reinfection of birds of this age.

MATERIALS AND METHODS.

Experimental Birds.

Broiler type hybrid cockerel chickens were used in this experiment. They were reared in complete isolation and were transferred to the experimental unit on day 0, where they were kept in metal cages with wire floors. A proprietary commercial chick food (Baby Chick Crumbs, British Oil and Cake Mills, Ltd.) was available ad lib. to the birds.

Parasitology.

The culture of E. tenella used for the infection of all the experimental birds was 41 days old on day 0, with a total oocyst count of 128,250 per ml. and sporulation count of 80%. Serial dilutions were made to give the appropriate number of oocysts per ml. for each experimental group, by the addition of distilled water to an aliquot of the culture which was suspended in 2% potassium dichromate solution.

Administration of Inoculum.

The infecting dose was given in 2 ml. of water for groups E.1 - E.4 and in 2.5 ml. for group E.5. It was administered directly into the crop using an automatic dosing syringe. During inoculation the stock of oocysts was kept in suspension by decanting the solution between two beakers.

The birds were all infected in the morning. Food was withdrawn for three hours before inoculation.

Experimental Design.

Ten birds were selected at random for each experimental group and transferred to the experimental unit. Ten similar birds were left in the isolation unit to act as non-infected controls. The birds were 46 days old on Day 0.

The experimental groups designated E.1 - E.5 received a total of 16,000; 32,000; 64,000; 128,000; and 256,000 sporulated oocysts per bird respectively on day 0.

Also infected were birds intended for post mortem examination on days 4, 5, 6, 7 and 8. The groups were designated A, B, C and D, and received a total of 4,000; 8,000; 16,000; and 32,000 sporulated oocysts per bird respectively.

Haemoglobin estimations were made on all experimental and control birds on day 0 - 1 and 0 + 4, 5, 6, 7, 8, 11 and 14.

Clinical signs and mortality were also recorded. Post mortem examination was carried out on day 6 on all birds which died on day 5; survivors were killed for examination on day 14.

The birds were weighed two days before day 0, 0 - 2, and again on day 12.

RESULTS.

(1) Haematology.

The results (Table 3. 4) of the haemoglobin estimations in the control birds did not demonstrate any significant variation in the haemoglobin concentration of these birds during the course of the experiment.

On day 0 - 1 and on day 4, the results did not indicate any difference between the control and the experimental groups. On day 0 - 1 each experimental group was compared with the control group; the results of the "t-test" gave "p" equal to 0.25 in each case.

The first significant decrease in the haemoglobin concentration was recorded in all experimental groups on day 5. In groups E.1, E.2 and E.3, the haemoglobin concentration remained unaltered on day 6 and 7, the results being similar to those recorded for each group respectively on day 5. In group E.4., the level of haemoglobin continued to decrease on day 6 and 7, reaching its lowest value on day 7. In group E.5, the decrease on day 5 was approximately 1 g. of haemoglobin less than the other groups at that time. The lowest values in E.5 were seen on day 6 and 7.

The average decrease in the haemoglobin concentration was 3 g. in groups E.1 and E.2; and nearly 4 g. in groups E.3, E.4 and E.5, per 100 ml. of blood respectively (Table 3. 6).

The haemoglobin level began to increase in all groups on day 8; and was approaching normal values on day 14 (Table 3. 4).

Table 3. 4.

The Mean Haemoglobin Concentration in the Blood of Control and Experimental Chickens after inoculation with Different Numbers of Sporulated Oocysts of *Eimeria tenella*.

Group	Day After Inoculation.							
	(1)	(4)	(5)	(6)	(7)	(8)	(11)	(14)
Control	7.5* ±1.5**	7.2 ±0.7	6.9 ±1.4	7.6 ±0.5	7.1 ±1.2	7.3 ±0.8	7.8 ±0.7	7.6 ±0.6
E. 1.	7.5 ±1.2	7.2 ±0.9	4.5 ±0.6	4.9 ±0.7	4.3 ±0.8	4.8 ±0.8	6.0 ±0.8	7.0 ±0.7
E. 2.	7.6 ±0.9	7.7 ±1.3	4.8 ±1.4	4.9 ±0.6	4.6 ±1.4	5.2 ±1.1	5.9 ±0.8	6.7 ±0.5
E. 3.	7.9 ±0.9	7.3 ±1.2	3.7 ±0.5	4.2 ±0.8	4.2 ±0.4	4.8 ±0.8	5.8 ± 0.5	6.9 ±1.0
E.4.	7.4 ±0.5	7.2 ±0.6	4.4 ±0.8	4.2 ±0.9	3.6 ±0.7	4.5 ±0.9	5.6 ±1.1	6.8 ±0.7
E. 5.	7.9 ±0.8	7.2 ±0.4	5.5 ±0.5	4.0 ±1.0	3.8 ±1.0	4.6 ±1.3	5.2 ±1.1	6.3 ±0.6

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

** The standard deviation.

(2) Clinical Findings.

The first indication of haemorrhage was present on day 4, when a little blood was seen on the trays of the experimental groups E.2 to E.5. No blood was found in Group E.1 or in the Pens A and B. At this time there were no other signs of disease; all the experimental birds appeared bright and showed no evidence of thirst or anorexia.

On the morning of day 5, clinical signs were most pronounced in E.3 and E.4; haemorrhage was very severe, the birds had pale combs (Fig. 1) and were very depressed. Morbidity and haemorrhage appeared slightly less in groups E.2 and E.5. Only a little blood was present on the trays of group E.1, but this increased during the day and there was little difference from E.2 by the evening. However, morbidity was less in E.1.

On day 6 the survivors of E.1 showed no signs of morbidity in contrast to the chickens of E.2, E.3 and E.4, where approximately fifty per cent of the birds in each group had pale combs and appeared depressed. Morbidity was greatest in E.5, all the birds were equally dejected and were lighter in weight compared to the other experimental groups, this difference still being evident on day 8 when the birds were bled for the estimation of haemoglobin.

On day 7, morbidity was less in E.3 and E.4 and completely absent in E.2. Thereafter the birds in these groups improved daily. Recovery was slower in group E.5., where the chickens still appeared very depressed on day 8.

A little blood was seen on the trays from all the experimental groups on days 6 and 7. None was present on day 8.

The birds of groups A and B showed slight evidence of haemorrhage on days 5, 6 and 7; no other clinical signs were seen in these birds. The groups C and D were similar to E.1 and E.2 respectively.

FIGURE 1

THE CLINICAL SYMPTOMS IN ACUTE CAECAL COCCIDIOSIS.

Note the dejected appearance and the marked pallor of the comb in the affected bird (left), in contrast to the alert appearance and red comb in the normal bird (right), on Day 5.

(3) Mortality.

Mortality occurred only on day 5. It was least in E.1 and E.6 (10%). and E.4 (20%). It was greatest in E.2 (30%) and E.3 and D (40%).(Table 3. 6.).

Table 3. 5.

The Mean Weight in Grammes of the Experimental and Non-infected Control Chickens on Day 0 - 2 and Day 0 - 12 to Illustrate the Decrease in Growth Rate Associated with Different Levels of Infection with *E. tenella*

Group	Day 0-2	Day 0-12	Weight Gain	Weight Gain As % Control
Control	865	1458	593	100
E. 1.	865	1043	178	33
E. 2.	898	1129	231	36
E. 3.	912	968	56	8
E. 4.	906	983	77	11
E. 5.	900	974	74	11

(4) Weight Gain.

The results (Table 3. 5.) showed no significant variation in weight between the control and the experimental groups on day 0-2.

There was a marked difference between the control and the experimental birds when they were reweighed on day 0-12. There was also a wide variation between the groups E.1 and E.2 on the one hand, which gained approximately 200g. per bird, and E.3, E.4 and E.5. on the other, which gained an average of less than 70g. per bird. The control group gained approximately 600g. per bird, clearly indicating a severe retardation of growth in all the experimental birds, which was least in E.1 and E.2 and greatest in E.3, E.4 and E.5.

Table 3. 6.

The Pathogenic Effects of Different Numbers of Sporulated Oocysts of *Eimeria tenella* in Chickens aged Six Weeks Old on Day 0.

Group	Dose Oocysts	Haemoglobin* Decrease Day 1-7	P**	Mortality	Weight Gain	Lesion Score Day 14
Control	nil	nil	0.25	nil	593***	
E. 1.	16,000	3.2	0.001	1	178	♦♦♦♦****
E. 2.	32,000	3.0	0.001	3	231	♦♦♦
E. 3.	64,000	3.7	0.001	4	56	♦♦♦♦♦
E. 4.	128,000	3.8	0.001	2	77	♦♦♦♦
E. 5.	256,000	4.1	0.001	1	74	♦♦♦♦♦

* Haemoglobin decrease expressed as grammes per 100 ml. blood.

** t-test carried out on mean value of haemoglobin concentration of each group on the results of day 0-1 and day 0-6.

*** Weight gain expressed in grammes. Result equal to Day 12 - Day 0 - 2.

**** Lesion Score.

♦♦♦ Moderately severe lesions in caeca, marked fibrosis caecal wall, necrotic debris in lumen of some birds.

♦♦♦♦ Very severe lesions in caeca, marked fibrosis caecal wall and occlusion of lumen with necrotic debris in many birds.

♦♦♦♦♦ Very severe caecal lesions with peritonitis in some birds.

(5) Pathology.

(i) Post mortem findings on Day 4, 5, 6, 7 and 8 of Groups A, B, C and D.

Two birds from each group were killed for examination during the acute phase of the disease. Group A received 4,000: B, 8,000: C, 16,000: and D, 32,000 sporulated oocysts of E. tenella on day 0. Group C and D corresponded to E.1 and E.2 respectively.

On day 4 (Figs. 2 & 3) slight thickening was present in small areas of the caecal wall in A, B and C. There was no evidence of haemorrhage in A or B. The contents in the caeca of these birds appeared quite normal. A very little blood was evident in the contents of the caeca in group C, indicating slight haemorrhage. In contrast to A, B and C, marked changes were found in Group D. The caecal walls were thickened and small haemorrhages were present on the mucosa. The contents of the caeca were pink in colour due to the presence of blood.

On day 5 (Figs.4 & 5) the caecal walls were thickened in group A and B. Blood was present in the lumen, the haemorrhage being slightly more pronounced in group B. The lesions were far more severe in group C, with widespread erosion of the mucosa and severe haemorrhage, which was responsible for distension of the caeca with extravasated blood and cellular debris. In group D, lesions were very severe with even greater haemorrhage and erosion of the mucosa than in C. These birds had died shortly before examination.

On day 6 (Figs.6 & 7) one bird in group A exhibited rather marked lesions with obvious thickening of the caecal wall, together with erosion of the epithelium and numerous haemorrhages on the mucosa. Extravasated and cellular debris were found in one caecum only, the other containing a little blood-stained mucus. The second bird in group A resembled those of group B. The caecal walls were thickened but erosion of the epithelium appeared less severe and small haemorrhages were present on the mucosa which was thrown into longitudinal ridges presenting a corrugated appearance. The caeca contained only blood-stained mucus in these birds. Lesions were more marked in group C than in A or B. The caecal walls were thickened and the caecal lumen contained blood in one bird which caused marked distension of the caeca; in the second bird the lumen was empty. Severe lesions were present in Group D, resembling those found on day 5. The mucosa was very thickened and thrown into longitudinal folds.

On day 7 the caeca appeared contracted in group A. The caecal walls were pink and white in colour under the serosa, which gave a marble-like effect. The walls were severely thickened with the mucosa thrown into longitudinal ridges presenting a corrugated appearance. Small haemorrhages were widespread on the mucosa. The lumen of the caeca were empty. In group B the caecal wall was also thickened but it was not thrown into longitudinal folds. The caeca of one bird in this group were distended by necrotic debris which was black and viscous and resembling tar in appearance in one caecum, while in the other it was formed into a solid cast which completely filled the lumen. The caeca were empty in the second bird. The caecal walls were also thickened in group C and varying amounts of necrotic material was present in the lumen of the caeca. In group D the caeca appeared contracted. The caecal walls were less thickened than in the other groups. Erosion of the epithelium was very severe with marked haemorrhage present on the mucosa. The lumen of the caeca were filled with necrotic debris which was adherent to the mucosa in one caecum. In this bird peritonitis was present, being associated with a necrotic lesion in the wall of the caecum.

On day 8 similar lesions were found in groups A and B, with the exception of the thickening seen in the walls of the caeca, which was more pronounced in Group A. Small haemorrhages were evident on the mucosa and necrotic debris was present in the lumen of the caeca, varying in amount from small aggregates of material to casts of debris completely filling the lumen. The caeca of the birds in group C were distended by large masses of necrotic debris. The caecal walls were very thin, with numerous small pin point haemorrhages visible on the mucosa. In group D, erosion of the mucosa was widespread and very severe, with no evidence of haemorrhage present on the caecal wall which was very thin and white in colour, indicating fibrosis. The caeca of one bird were filled with typical necrotic material; in the second bird, the contents of the caeca were black and very viscous, resembling tar in consistency.

(ii) Post mortem Examination of the Birds which Died on Day 5.

Examination of these birds revealed typical lesions of acute caecal coccidiosis.

There was no variation in the severity of the lesions in the birds from the different groups.

(iii) Post mortem Examination of the Surviving Birds from the Experimental Groups E.1, E.2, E.3, E.4, and E.5, on Day 14.

Changes were present in the caeca of the majority of the birds. In group E.1 and E.2 (Figs.8 & 9) the caeca were contracted and appeared smaller than those of the non-infected control birds. In E.1 the caecal walls were thickened with evidence of severe fibrosis, the mucosa was thrown into ridges presenting a corrugated appearance. In E.2 the thickening of the caecal wall was less severe. The majority of the caeca were patent; only three birds from the sixteen survivors of E.1 and E.2 had caeca which were completely occluded by necrotic debris. Severe lesions were present in one bird of each group; that from E.1 showed evidence of peritonitis associated with perforation of the distal end of the caecum.

The most severe lesions were present in group E.3 (Fig.10) and E.5. The walls of the caeca were severely thickened with evidence of fibrosis. The majority of the survivors had the lumen of the caeca occluded with large masses of necrotic material which was associated with considerable distension of the caeca. Severe peritonitis with widespread adhesions between the caeca and the intestines was present in five of the birds from E.3 and E.5 (Fig.11).

In group E.4 the caecal walls also showed evidence of marked fibrosis (Fig.10). Howevere, there were no signs of peritonitis. The majority of the caeca contained necrotic debris in the lumen which caused complete occlusion of the caeca in three birds. The lesions appeared slightly more pronounced in the survivors of this group in comparison with the survivors of the same age in the previous experiment which had also received a similar number of sporulated oocysts of E. tenella.

A lesion score was allotted to each group (Table 3. 6).

FIGURE 2 - FIGURE 11.

THE PATHOGENICITY OF DIFFERENT NUMBERS OF NORMAL OOCYSTS OF EIMERIA TENELLA IN SIX WEEK OLD CHICKENS - CHANGES IN THE CAECA OF SIX WEEK OLD CHICKENS KILLED FOR EXAMINATION ON DAYS 4, 5, 6 AND 14.

FIGURE 2.

POST MORTEM FINDINGS ON DAY 0+4

(A) Note that caecal contents appear normal in birds given 4,000, 8,000 or 16,000 oocysts (L-R), in contrast to the slight evidence of haemorrhage, indicated by discolouration of the caecal contents, in birds given 32,000 oocysts.

FIGURE 5.

POST-MORTEM FINDINGS ON DAY 0+4.

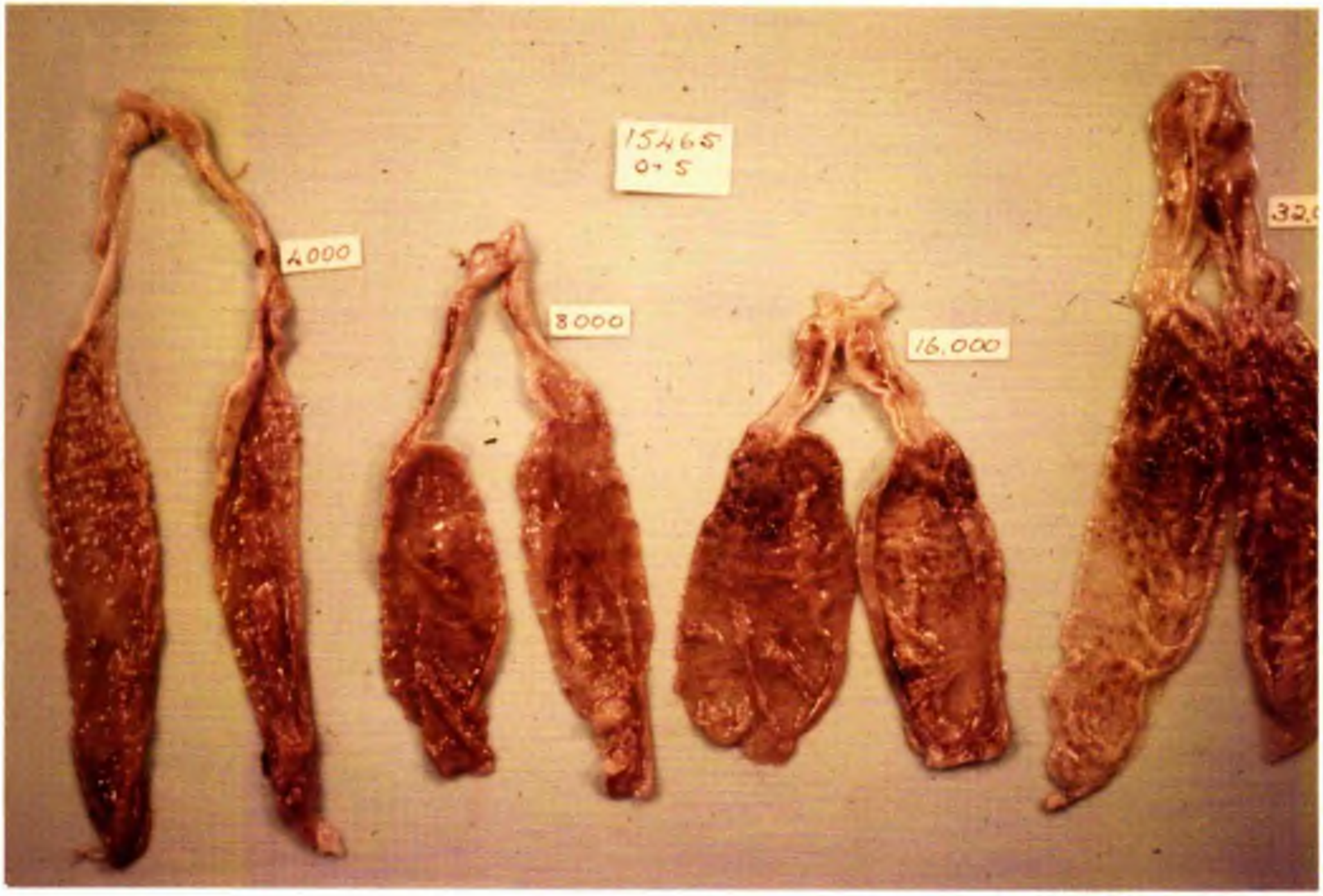
(B) The caecal mucosa appears thickened and oedematous in each bird, the lesions increasing in severity as the size of infective dose is increased from 4,000 - 32,000 normal oocysts (L-R). Note the presence of small haemorrhages on mucosa of birds given 32,000 oocysts.

FIGURE 4.

POST MORTEM FINDINGS ON DAY 0•5

(A) Note marked evidence of haemorrhage in birds receiving only 4,000 or 8,000 normal oocysts per bird. Haemorrhage appears significantly greater in birds given 16,000 or 32,000 oocysts per bird, the most pronounced changes being recorded in the bird (extreme right) given the latter dose.

FIGURE 5



POST MORTEM FINDINGS ON DAY 0+6

(B) Note pronounced lesions in the caecal wall, illustrated by the erosion of the mucosa and the small haemorrhages which are significantly greater in birds given 16,000 or 32,000 oocysts per bird.

FIGURE 6.

POST-MORTEM FINDINGS ON DAY 0+6.

(A) Note that evidence of haemorrhage appears slightly less compared with that on Day 5. Lumen contains extravasated blood and tissue debris. Lesions are most marked in birds given 16,000 or 32,000 oocysts, although the caecal wall is markedly thickened in birds receiving lower doses of 4,000 or 8,000 oocysts.

FIGURE 7

POST MORTEM FINDINGS ON DAY 0+6

(B) Note that the caecal mucosa appears markedly thickened and oedematous in birds given only 4,000 or 8,000 per bird. Small haemorrhages can also be detected in caecal wall. Lesions most pronounced in survivors given 32,000 oocysts (extreme right).

FIGURE 8.

POST MORTEM FINDINGS ON DAY 14.

(A) Note the presence of necrotic debris in the caecal lumen, indicating that reinfection with a challenge dose of oocysts is contra-indicated at this time.

FIGURE 9.

POST MORTEM FINDINGS ON DAY 14.

(B) Note marked evidence of fibrosis of caecal wall in birds after administration of 16,000 (left) or 32,000 (right) normal oocysts.

FIGURE 10.

CAECAL LESIONS IN SURVIVORS FROM A HIGH DOSE OF

64,000 OR 128,000 NORMAL OOCYSTS ON DAY 14.

Note complete occlusion of caeca by massive plugs of tissue debris and also the marked fibrosis of the caecal wall.

FIGURE 11.

TYPICAL CAECAL LESIONS ON DAY 14 IN BIRD SURVIVING

AFTER INFECTION WITH 256,000 NORMAL OOCYSTS.

Note marked evidence of peritonitis and the presence of large masses of necrotic debris in the caeca.

DISCUSSION.

The results (Table 3. 4.) showed no significant variation in the concentration of haemoglobin in the blood of the control and the experimental groups on day 0-1 and day 4. The first decrease in the level of the haemoglobin occurred on day 5 in the experimental birds. In groups E.1, E.2 and E.3 the concentration of haemoglobin remained unaltered on day 5, 6 and 7; in E.4 and E.5 the value was lower on day 6 and 7 than on day 5. The results indicated that the maximum decrease in the concentration of haemoglobin could be measured from the difference in the values of an estimation made between day 0-1 and day 4, and one made on either day 6 or 7, after inoculation with doses of sporulated oocysts of E. tenella ranging from 16,000 to 256,000 oocysts per bird in fully susceptible six week old chickens.

The changes in the haemoglobin level of the birds in group E.4. were similar to those of an identical group, E.4 in experiment 1. This observation confirmed that consistent changes in the haemoglobin level could be reproduced with a standard dose of 128,000 sporulated oocysts in fully susceptible six week old chickens.

The results (Table 3. 6.) giving the decrease in the concentration of haemoglobin which occurred in the experimental groups on day 7 suggested a possible variation between E.1 and E.2 on the one hand, and E.3, E.4. and E.5 on the other, but the difference was not statistically significant.

However, the observations on the growth rate (Table 3.5) clearly demonstrated a marked difference between these experimental groups.

The significance of the pathogenic effect due to E. tenella on the gain in weight of the experimental birds was emphasised by a comparison of the gain in weight expressed as a percentage of the gain recorded for the control birds during the same period. E.1 and E.2 each attained 33% and E.3, E.4 and E.5 only 10% of the mean weight gain achieved by the control pen. These results clearly demonstrated their value as a criterion for the evaluation of the pathogenic effects of E. tenella in six week old chickens.

Mortality did not increase progressively in each group of experimental birds (Table 3. 6.). There appeared to be little correlation between mortality and the size of the infective dose; neither was any direct relationship evident between mortality and the changes recorded in the concentration of haemoglobin and in the growth rate. These facts suggested that mortality alone appeared of doubtful value as a useful criterion for the evaluation of the pathogenicity of different doses of oocysts of E. tenella in six week old chickens.

Waletzky and Hughes (1949), studied the effect of different numbers of oocysts in chickens aged three to seven weeks old; they observed that there was little difference in the severity of the disease, assessed on mortality, produced by the administration of either 100,000 or 500,000 sporulated oocysts.

These results were in agreement with the work of Gardiner (1955) who found that mortality in six week old birds was greater after infection with 50,000 oocysts than that following ingestion of 100,000 or 200,000 oocysts. He also studied growth rate, recording the least effect after 50,000 oocysts per bird; the effect of 100,000 approached that of 200,000 oocysts, which were responsible for the greatest retardation of growth. The observations on growth rate were not strictly comparable, as the weight gains were measured over a shorter period of time than in the present experiment; day 8 and day 12 respectively. The difference might account for the variation between the results based on weight gains following infection. On day 8 the weight of the birds may still be influenced by anorexia and changes in the total body fluid associated with haemorrhage; which can be responsible for considerable variation in body weight, depending on the severity of the disease. On day 12 these changes are unlikely to remain in evidence; therefore at this time weight gains may show less variation between experimental groups where marked differences were due to these factors.

Clinical observations and post mortem findings demonstrated a marked difference between the pathogenicity of 8,000 and 16,000 sporulated oocysts of E. tenella in six week old chickens. Clinical signs were negligible following infection with 4,000 and 8,000 oocysts; in these birds evidence of haemorrhage was far less than that seen in birds receiving 16,000 oocysts. Although morbidity was slow in each of these groups, serial post mortem examination revealed marked lesions in the caeca of the birds given only small numbers of oocysts. These findings illustrated the value of post mortem examination as one criteria for the evaluation of the pathogenicity of E. tenella, when morbidity was not a marked feature in the course of the disease.

Detrimental effects appeared slightly less severe after a dose of 16,000 oocysts, compared with one of 32,000 oocysts in six week old birds. The changes in the level of haemoglobin and the growth rate (Table 3.6.) were similar in these birds, but morbidity was less in the former group. Pronounced lesions were present on day 4 in the caeca of birds receiving 32,000 oocysts, unlike those given the lower dose in which changes were not marked until day 5. Subsequently on day 6, 7 and 8, lesions seemed slightly greater at the higher level of infection; on day 14 thickening of the caecal wall was more pronounced in the survivors of E.1, other lesions being similar in the majority of these birds. Mortality was 10% and 30% respectively (Table 3.6.) but the significance of this difference was doubtful.

There was a very significant difference between the pathogenicity of 32,000 and 64,000 oocysts per bird; this was shown most clearly by observations on the growth rate (Table 5.5). The results indicated conclusively that the pathogenic effects of 64,000 oocysts were far greater than 32,000 oocysts. This was supported by clinical and post mortem findings.

The results (Table 5.6) did not demonstrate any significant difference in the pathogenicity of 64,000, 128,000 or 256,000 oocysts per bird. Clinical symptoms were severe in each group, but morbidity persisted longer in the birds which received 256,000 oocysts compared to those given the lower doses of oocysts.

EXPERIMENT THREE.

THE PATHOGENIC AND IMMUNOGENIC EFFECTS OF DIFFERENT NUMBERS OF SPORULATED OOCYSTS OF E. TENELLA IN CHICKENS AGED FOUR WEEKS OLD.

EXPERIMENTAL AIMS.

The experiment was designed to study the effects of different numbers of sporulated oocysts of *E. tenella* in four week old chickens; with particular reference to (a) the pathogenicity of the different levels of infection, and (b) the degree of resistance to reinfection conferred on survivors.

The range of doses for the first inoculum included low and high levels of infection. Three weeks later a standard challenge dose of sporulated oocysts was administered to the experimental birds. The pathogenicity of the challenge infection was determined by inoculation of fully susceptible chickens, which had been kept in strict isolation until the day of infection. Observations were made on the changes in the concentration of haemoglobin, on mortality, on clinical signs and on growth rate after each inoculation. Serial post mortem examinations were made after the first infection to compare the lesions in replicate groups of chickens, selected to represent low, moderate and very high levels of infection. Chickens were examined also after challenge from the groups given low and moderate levels of infection.

The total oocyst production of each group was recorded during the patent phase of the disease as this factor would be significant in the epidemiology of the disease, under intensive methods of husbandry in the field.

These results were required to study the relationship between the pathological and immunological effects of E. tenella. The results were also necessary (i) to illustrate the relationship between the level of infection and severity of the disease and also the number of oocysts ingested and the total oocyst output per bird: and (ii) to indicate appropriate criteria for the evaluation of the pathogenic effects of E. tenella in four week old birds given different levels of infection

This information was also essential for the determination of a standard dose of oocysts for administration to chickens in the preliminary investigations on the effect of X-irradiation on the pathological and immunological effects of E. tenella.

MATERIALS AND METHODS.

Experimental Birds.

Broiler type hybrid cockerel chickens were used in this experiment. They were reared in complete isolation, being transferred to the experimental unit on the day before infection; where they were kept in metal cages with wire floors.

A proprietary commercial chick food (Baby Chick Crumbs, British Oil and Cake Mills, Ltd.) was available ad lib. to the birds.

Parasitology.

The culture of E. tenella was used for the infection of the experimental birds on day 0 and was 27 days old, with a total oocyst count of 519,000 per ml. and a sporulation count of 86%. The same culture was used twentyone days later for the challenge infection, when the total oocyst count was 162,000 per ml. and the sporulation count 75%. This culture was also used to inoculate the replicate groups on day 12 when the total oocyst count was 150,000 per ml. and the sporulation count 80%. Serial dilutions were made to give the appropriate number of oocysts per ml. for each experimental group, by the addition of distilled water to an aliquot of the culture which was suspended in 2% potassium dichromate solution.

Administration of Inoculum.

The infecting dose was given in 2 ml. of water to each experimental bird. It was administered directly into the crop using an automatic dosing syringe. During the inoculation the stock of oocysts from which the syringe was filled was kept in suspension by decanting the solution between two beakers.

The birds of experimental groups E.1 to E.10 and Ch. were inoculated in the morning. Food was withdrawn seventeen and four hours before the initial and challenge infections respectively. The replicate groups E.12, E.13 and E.14 were dosed in the afternoon, food being withheld seven hours before inoculation.

Experimental Design.

Ten birds were selected at random from each experimental group and transferred to the experimental unit the day before inoculation, with the exception of the non-infected control group which were left in the isolation unit. The birds were 27 days old on day 0. Birds of the same age selected at random from a later consignment of chicks for the replicate groups E.6/2, E.7/2, E.8/2, and appropriate non-infected control group C.2.

The experimental groups designated E.1 to E.10 received a total of 1,000; 2,000; 4,000; 8,000; 16,000; 32,000; 64,000; 128,000; 256,000; and 500,000 sporulated oocysts per bird respectively on day 0. The surviving birds from these groups were inoculated on day 21 with a standard challenge dose of 64,000 sporulated oocysts. The pathogenicity of the challenge infection was determined by inoculation of ten fully susceptible chickens, designated group Ch., which had been retained in isolation until day 20.

The experimental groups E.6/2, E.7/2 and E.8/2 were replicate groups corresponding to E.6, E.7 and E.8. These were infected twelve days later to observe whether the same results, with particular reference to mortality and changes in the concentration of haemoglobin could be reproduced following the administration of 32,000; 64,000 and 128,000 sporulated oocysts per bird respectively.

Serial post mortem examinations were made on replicate groups of chickens corresponding to E.3, E.7 and E.10, given 4,000, 64,000 and 800,000 sporulated oocysts on day 0. These groups were selected to represent low, moderate and very high levels of infection. Two birds were killed from each group at 24, 48, 60, 72, 84, 96, 108, 120, 132 and 144 hours after inoculation. Chickens were also examined from the replicate groups given 4,000 and 64,000 oocysts on day 0 after reinfection with the standard challenge dose of 64,000 oocysts on day 21. Two birds were killed from each group at times similar to those after the initial infection.

Haemoglobin estimations were made on all experimental and control chickens after each infection. The initial series of estimations were made on day 0-1 and 0 + 1, 4, 5, 6, 7, 8 and 14. The second series of estimations associated with the challenge infection on day 21 were carried out on day C-2 and C + 4, 5, 6, 7 8 and 12.

Haemoglobin estimations were also made on the replicate groups E.6/2, E.7/2, E.8/2 and C.2 on day 0-2 and after infection on day 4, 5, 6, 7, 8, 14 and 21.

The birds were weighed two days before day 0 and again on day 11; day 19, i.e. C-2; and finally on day 32, i.e. C + 11

The total oocyst production of each group was recorded each day during the patent period of the disease, commencing on day 7 and finishing on day 21 when the birds were reinfected.

Post mortem examination was carried out on the birds which died on day 5 and 6; survivors were killed for examination on day 32. Birds from the replicate groups which survived were killed for examination on day 21.

Clinical signs and mortality were also recorded.

RESULTS.

(1) Haematology.

The results (Tables 3. 7 and 3.8) of the haemoglobin estimations in the control birds did now show any significant variation in the haemoglobin concentration of these birds during the course of the experiment.

The haemoglobin concentration in the blood of the experimental groups was within accepted normal limits on day -1, + 1, 4; and day -2 and + 4 at the time of the initial a nd challenge infections respectively (Tables 3.7, 3.8 and 3.10).

The first significant change in the level of haemoglobin was seen on day 5 in the experimental groups E.3 - E.10; the lowest values were recorded on day 6. There was a fall of 1 g. per 100 ml. in the level of haemoglobin of E.1 and E.2. on day 6 and 7.

The results (Table 3. 15) indicated a decrease in the haemoglobin concentration of 2 g. in E.3 and E.4. There was a difference between these groups and E.5, where the decrease was 3 g. of haemoglobin. The changes were more marked in E.7, E.8 and E.9, ranging from 3.8 g. to 4.5 g. of haemoglobin. The greatest decrease occurred in E.6. and E.10., where it was 4.9 and 5.5 g. of haemoglobin per 100 ml. of blood respectively.

Similar changes were recorded in the replicate groups (Table 3. 8. and 9) with the exception of E.6 where the fall in the level of haemoglobin was less marked in the replicate group, being 4.9 and 4.0 g. respectively.

The haemoglobin level began to increase in all groups on day 8 and was approaching normal values on day 14.

After reinfection on day 21, the first change in the concentration of haemoglobin was seen on day 5 in the fully susceptible birds of group Ch. (Tables 3. 10. and 15). The level decreased further on day 6 and 7, when the lowest values were recorded; representing a fall of 3.4 g. of haemoglobin per 100 ml. of blood.

The haemoglobin concentration of the experimental groups E.1 and E.2 was unaltered on day 5; a decrease of approximately 2 g. of haemoglobin in each group was recorded on day 6 and 7. A decrease of 1 g. of haemoglobin was seen in E.3 on day 7. The concentration of haemoglobin increased in these groups on day 8, returning to normal values by day 12, with the exception of group Ch., where the level of haemoglobin was slightly lower than the other experimental groups.

No significant decrease was seen after reinfection in groups E.4 to E.10. A small change was indicated in E.7 on day 6 and 7, but evidence of haemorrhage was not confirmed by clinical findings.

<u>(2) Mortality.</u>

After the initial infection on day 0, deaths occurred on day 5 in E.5 and E.6 (10%); E.7 (70%); E.8 and E.9 (40%); in E.10 (50%) deaths occurred on day 5 and 6. (Table 3. 15). No birds died in groups E1 - E.4. There was a marked difference in the mortality of E.6 and E.7 and the corresponding replicate groups of 10 and 70%; and 70 and 20% respectively (Table 3.9). The deaths were approximately similar in E.8 and E.8/2 (40 and 50%).

Table 5. 7.

The Mean Haemoglobin Concentration in the Blood of the Experimental Chickens after infection with Different Numbers of Oocysts of E. *tenella*.

Group	Day After Inoculation.							
	(-1)	(+1)	(4)	(5)	(6)	(7)	(8)	(14)
C.1	7.6 ±0.7	9.2 ±1.0	9.3 ±0.5	9.0 ±0.6	8.9 ±0.6	9.1 ±0.9	9.4 ±1.1	8.8 ±0.6
E.1.	8.7 ±0.4 p=.25	8.1 ±0.8 p=.02	8.0 ±0.6	8.0 ±1.2 p=.25	7.5 ±1.2 p=.25	7.6 ±0.8 p=.25	7.5 ±0.5	8.7 ±0.4
E.2.	8.9 ±0.7 p=.01	8.6 ±0.2 p=.1	8.9 ±0.7	8.2 ±1.2 p=.25	7.6 ±1.2 p=.02	7.5 ±1.2 p=.02	8.7 ±1.2	8.8 ±0.8
E.3.	8.7 ±0.4	8.6 ±0.8 p=.25	8.7 ±0.6	7.6 ±1.5 p=.1	6.6 ±1.7 p=.01	7.0 ±1.1 p=.01	8.0 ±1.1	9.0 ±0.5
E.4	8.3 ±0.4	8.2 ±0.6 p=.05	8.2 ±0.3	7.1 ±1.5 p=.05	5.9 ±1.8 p=.001	6.7 ±1.2 p=.01	6.8 ±1.1	8.6 ±0.5
E.5	9.0 ±0.6	8.9 ±0.4 p=.25	8.5 ±0.5	6.9 ±2.2 p=.01	5.7 ±1.8 p=.001	6.0 ±0.8 p=.01	7.3 ±0.8	8.6 ±1.1
E.6	8.7 ±0.8	8.3 ±0.7 p=.05	8.9 ±0.5	6.2 ±1.0 p=.001	3.7 ±1.0 p=.001	4.5 ±1.0 p=.001	6.6 ±1.4	9.1 ±0.5
E.7.	7.6 ±0.7 p=.25	7.9 ±0.6 p=.01	8.2 ±0.6	4.6 ±1.6 p=.001	4.2 ±0.7 p=.001	4.5 ±0.6 p=.001	6.6 ±0.9	7.9 ±0.8
E.8.	8.1 ±0.9 p=.25	8.4 ±0.6 p=.1	8.3 ±0.9	4.1 ±1.6 p=.001	3.8 ±1.0 p=.001	4.4 ±0.4 p=.001	6.8 ±1.1	8.0 ±0.7
E.9	8.6 ±0.7 p=.05	8.8 ±0.2 p=.25	8.3 ±0.3	4.4 ±2.2 p=.001	4.2 ±1.7 p=.001	4.7 ±1.2 p=.001	6.8 ±1.5	8.3 ±0.6
E.10.	8.6 ±0.7 p=.05	9.2 ±0.6 p=.25	8.7 ±0.9	5.8 ±2.0 p=.001	3.1 ±1.0 p=.001	3.4 ±1.1 p=.001	4.3 ±0.7	8.0 ±1.1

Table 3. 8.

The Mean Haemoglobin Concentration in the Blood of the Experimental Chickens E.6, E.7, E.8 and the Replicate Groups E.6/2, E.7/2 and E.8/2, after Infection with 32,000; 64,000 and 128,000 Sporulated Oocysts per Chicken Respectively, of *Eimeria tenella*

Group	Day After Inoculation (1)	(4)	(5)	(6)	(7)	(8)	(14)	(20)
C.1.	7.0* ±0.7**	9.5 ±0.5	9.0 ±0.6	8.9 ±0.6	9.1 ±0.9	9.4 ±1.1	8.8 ±0.6	8.2 ±0.8
C.2.	8.6 ±0.5	8.6 ±1.0	7.9 ±0.5	8.4 ±1.0	8.2 ±0.5	8.5 ±0.6	8.6 ± 0.7	7.9 ±0.5
E.6.	8.7 ±0.8	8.9 ±0.5	5.2 ±1.0 p=.001	3.7 ±1.0 p=.001	4.5 ±1.0 p=.001***	6.6 ±1.4	9.1 ±0.5	10.1 ±0.3
E.6/2	8.7 ±0.5	8.9 ±0.6	5.6 ±2.0 p=.001	5.0 ±1.1 p=.001	4.6 ±1.0 p=.001	5.9 ±1.3	8.1 ±0.9	8.0 ±0.7
E.7	7.6 ±0.7	8.2 ±0.6	4.6 ±1.6 p=.001	4.2 ±0.7 p=.001	4.5 ±0.6 p=.001	5.6 ±0.9	7.9 ±0.8	8.5 ±0.9
E.7/2	9.2 ±1.4	8.1 ±0.9	5.2 ±1.6 p=.001	4.1 ±0.7 p=.001	4.5 ±1.2 p=.001	5.3 ±1.0	5.9 ±0.7	7.7 ±1.0
E.8	8.1 ±0.9	8.3 ±0.9	4.1 ±1.6 p=.001	3.8 ±1.0 p=.001	4.4 ±0.4 p=.001	5.8 ±1.1	8.0 ±0.7	9.8 ±1.1
E.8/2	8.9 ±0.5	8.1 ±0.7	5.8 ±1.6 p=.001	4.3 ±1.2 p=.001	4.4 ±0.9 p=.001	4.7 ±1.2	7.6 ±0.4	8.6 ±0.5

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

** The standard deviation.

*** The probability calculated by the "t-test".

Note the haemoglobin estimation of the replicate groups was made initially two days before day 0 and finally on day 21.

Table 3. 9.

The Mortality and the Decrease in the Concentration of the Haemoglobin of the Experimental Groups E.6, E.7, E.8 and the Replicate Groups E.6/2, E.7/2 and E.8/2, after Inoculation with 32,000, 64,000 and 128,000 Sporulated Oocysts of *Eimeria tenella* respectively on Day 0.

Group	Dose of Oocysts*	Haemoglobin** Decrease	Mortality
Control 1.	nil	nil	nil
Control 2.	nil	nil	nil
E.6.	32,000	4.9	1
E.6/2	32,000	4.0	7
E.7	64,000	3.8	7
E.7/2	64,000	4.4	2
E.8	128,000	4.3	4
E.8/2	128,000	4.2	3

* The same culture of *Eimeria tenella* was used for the infection of all the experimental chickens. The replicate groups were inoculated 12 days after E.6, E.7 and E.8.

** The decrease in the concentration of the haemoglobin expressed as grammes per 100 ml. of blood. The decrease represents the difference between the mean value of day -1 and day 4, on one hand, and the lowest value recorded on day 6 or day 7.

Table 8. 10.

The Mean Haemoglobin Concentration in the Blood of the Experimental Chickens after Reinfection with a Standard Number of Oocysts of *Eimeria tenella*.

Group	Day After Inoculation.						
	(-2)	(4)	(5)	(6)	(7)	(8)	(12)
C.1.	8.2 ±0.8	8.5 ±0.6	8.8 ±0.6	8.1 ±0.6	8.2 ±2.1	8.0 ±1.1	9.4 ±0.6
Ch.	9.1 ±1.0 p=.1	8.4 ±0.8 p=.25	7.1 ±1.6 p=.01	5.5 ±1.9 p=.001	5.0 ±1.4 p=.001	6.0 ±1.6	7.8 ±0.6
E.1.	8.6 ±0.5 p=.25	8.6 ±0.4 p=.25	8.6 ±0.3 p=.25	7.4 ±1.0 p=.05	6.9 ±1.4 p=.001	7.8 ±0.8	8.7 ±0.2
E.2.	8.3 ±0.1 p=.1	8.8 ±0.4 p=.1	8.1 ±0.3 p=.25	6.6 ±2.2 p=.01	7.0 ±1.5 p=.05	7.4 ±0.5	9.3 ±0.7
E.3.	8.8 ±0.5 p=.25	8.7 ±0.5 p=.25	9.3 ±0.5 p=.1	8.2 ±0.2 p=.1	7.5 ±0.6 p=.25	8.7 ±0.5	8.9 ±1.1
E.4.	8.4 ±0.7 p=.25	8.5 ±0.4 p=.25	8.7 ±0.6 p=.25	8.2 ±0.9 p=.25	7.9 ±0.8 p=.25	8.3 ±0.7	9.2 ±0.8
E.5.	8.2 ±0.8 p=.25	7.9 ±1.0 p=.25	8.7 ±1.2 p=.25	8.3 ±1.0 p=.25	8.7 ±0.5 p=.25	8.5 ±1.3	9.5 ±0.8
E.6	10.8 ±0.8 p=.001	8.8 ±0.6 p=.01	9.5 ±1.0 p=.25	8.2 ±1.9 p=.01	8.1 ±1.3 p=.01	8.6 ±1.2	8.8 ±0.4
E.7.	8.5 ±0.9 p=.25	8.0 ±0.4 p=.25	7.9 ±1.1 p=.25	6.9 ±0.8 p=.25	7.1 ±0.1 p=.25	8.0 ±0.7	8.7 ±0.7
E.8.	9.8 ±1.1 p=.01	8.9 ±0.7 p=.25	8.8 ±0.6 p=.25	8.3 ±0.9 p=.05	8.4 ±0.9 p=.05	8.7 ±0.8	9.1 ±0.3
E.9.	9.9 ±1.3 p=.01	8.7 ±0.4 p=.1	9.7 ±1.4 p=.25	8.3 ±0.7 p=.05	8.5 ±0.8 p=.05	8.7 ±0.3	9.6 ±0.7
E.10.	9.4 ±0.5 p=.1	8.8 ±0.4 p=.25	9.8 ±0.8 p=.25	8.1 ±0.7 p=.05	8.4 ±0.8 p=.25	9.3 ±0.6	9.6 ±0.5

Following reinfection on day 21, one death (10%) occurred on day 26, i.e., C + 5, in the fully susceptible chickens of group Ch. No birds died in the experimental groups E.1 to E.10.

(3) Clinical Findings.

The first clinical symptoms were seen on day 4 after the initial infection, when severe haemorrhage was evident in the groups E.8, E.9 and E.10. A little blood was also found on the trays of groups E.6 and E.7.

On day 5, no evidence of morbidity was apparent in the birds of E.1 - E.4, although a little blood was present on the trays from these pens, being most pronounced in E.4. The first indication of morbidity was seen in E.5 and E.6, where some of the birds were very depressed; haemorrhage was marked in these groups. Morbidity was very high in E.7 - E.10, being most severe in E.10; haemorrhage also appeared greater in these groups, being most pronounced in E.9 and E.10. At this time the survivors from E.10 appeared far lighter in weight compared with the other experimental birds.

On day 6 haemorrhage was still severe in E.9 and E.10, being less marked on day 7. A little blood was also present on the trays of groups E.4 - E.8 on day 6; none was seen in groups E.1 - E.3. No evidence of haemorrhage was found in any of the experimental groups after day 7; morbidity also became less obvious, the birds improving daily, although recovery was protracted in group E.10.

The clinical picture in the replicate groups was similar to that of the corresponding groups E.6, E.7 and E.8.

Following the challenge infection on day 21, typical signs of acute caecal coccidiosis were seen in the fully susceptible birds of group Ch. The first evidence of haemorrhage was present on the 4th day after inoculation; on day 5 haemorrhage was severe, and depression was very marked in contrast to chickens of E.1 - E.10, who showed no sign of morbidity. However, haemorrhage was quite marked in the experimental groups E.1 and E.2; a little blood was also seen in groups E.3, E.4 and E.5. On day 6, haemorrhage was quite severe in groups Ch., E.1 and E.2; traces of blood were also present on the trays of E.3, E.4 and E.5. In the evening a very little blood was also seen in groups E.6, E.7 and E.9. On day 7 only, slight evidence of haemorrhage persisted in the groups Ch., E.1 and E.2. No indication of haemorrhage was seen after challenge in either group E.8 or E.10.

(4) Weight Gains.

The results (Table 3. 11) showed no significant variation in weight between the non-infected control group and the experimental groups E.1 - E.10, or group Ch. (Table 3. 12) before the initial or challenge infections respectively.

On day 11, following the initial inoculation, the highest weight gain attained by any of the infected groups, in E.2, was 70 g. less than the mean weight gain of approximately 400 g. recorded in the control group (Table 3. 11). The mean weight gains of E.1 to E.7 measured between 250 to 340 g., representing 60 - 80% of the gain in the control group. There was a marked variation between these groups and E.8 and E.9, where severe retardation of growth occurred; the gain in these groups was approximately 160 - 180 g., representing only 40% of that in the controls. The greatest retardation was shown in E.10, where the birds only gained 50 g., equal to 10% of the gain in the non-infected chickens.

On day 19, two days before reinfection, the growth rate between the non-infected control group and that of E.1 to E.7 still indicated less satisfactory weight gains in the infected birds although the variation was less marked; the gain of these chickens represents approximately 82% of the total weight gain of 70. g. recorded in the non-infected control group. The difference between E.1 - E.7, on one hand and E.8 and E.9 on the other, was also less; the weight gain of the latter groups represent approximately 70% of the control group, i.e., 10% less than E.1 - E.7. However, severe retardation of growth was still evident in E.10, where the weight gain of 270 g. represented only 60% of that in the control birds. On the eleventh day after reinfection, i.e., day 32 (Table 3.12), the results indicated a marked retardation in the growth rate of the fully susceptible challenge group which attained only 60% of the weight gain of 360 g. recorded in the non-infected control chickens. The gain in weight of E.1 - E.6 ranged between 70 and 95% of that attained by the control group. The gain in E.7 was exactly twice that in the controls, while that in E.8 - E.10 was approximately similar to the control chickens.

Table 3. 11.

The Mean Weight Gains of the Experimental Chickens on Day 11 and Day 19 to Illustrate the Effect of Infection with Different Numbers of Sporulated Oocysts of Eimeria Tenella.

Group	Day 0-2	Gain On Day 11	Weight Gain As % Control	Gain On** Day 19	Weight Gain As % Control.
Control	400*	413	100	701	100
E.1.	373	278	67	599	85
E.2.	383	338	82	559	80
E.3.	375	315	76	624	89
E.4.	378	327	79	596	85
E.5.	381	290	70	573	82
E.6.	378	253	62	559	80
E.7.	381	276	67	541	77
E.8.	383	186	45	510	73
E.9.	362	162	39	472	67
E.10.	362	52	13	270	39

* Weight in grammes.

** The value on day 19 represents the total weight gain; i.e., day 19 - day 0-2.

Table 3. 12.

The Mean Weight Gains of the Experimental Chickens on Day 32 to Illustrate the Effect of a Standard Challenge Infection of 64,000 Sporulated Oocysts of *Eimeria tenella* on day 21.

Group	Day 19	Gain On Day 32	Weight Gain As % Control	Total Gain	Weight Gain As % Control
Control	1101*	358	100	1060	100
Ch.	1060	224	62	954	87
E.1.	972	317	88	916	86
E.2.	942	366	99	915	86
E.3.	999	256	71	880	82
E.4.	973	309	86	904	84
E.5.	964	346	96	919	86
E.6.	957	300	84	859	80
E.7.	922	728	202	1269	119
E.8.	873	428	119	918	90
E.9.	854	375	104	847	79
E.10.	652	364	101	654	69

* Weight in grammes.

The results (Table 3.12) showed that the total weight gain of the infected birds on day 32, with the exception of E.7, was still less than that of the control group. The mean gain in weight of the experimental groups E.1 - E.9 and Ch. was approximately 86% of that recorded in the non-infected chickens. The retardation of the growth rate was still severe in E.10, where the final weight gain represented only 60% of that in the control group.

(5) Oocyst Production.

No oocysts were found in the samples of faeces which were collected from each group on the morning of day 6.

The results (Table 3.13) showed that the maximum oocyst output occurred in all the groups during the first three days of the patent period. The oocyst output remained quite high until day 14, when there was a marked decrease in the daily counts from all groups with the exception of E.7 where the number fell on day 19. Very small numbers of oocysts were present in the samples from the majority of the groups until day 21 when the last sample was examined before reinfection.

The daily oocyst production fluctuated widely; it appeared to have no relationship with either the number of oocysts inoculated or with any specific day in the patent period after infection.

The total average oocyst production was relatively similar in groups E.1 - E.4 and E.8 - E.10; it ranged between 85 - 100 million oocysts per bird. There was a significant variation between these groups and E.5 - E.7, where the output ranged from 160 - 180 million oocysts per bird.

The results (Table 3.14) indicated a marked difference between each group when the number of oocysts produced per oocyst inoculated was calculated. The number decreased significantly as the infective dose increased.

Table 3.13.

The Average Total Daily Oocyst Production of the Experimental Chickens, expressed in Millions of Oocysts per Bird, after Inoculation on Day 0 with Different Numbers of Sporulated Oocysts of *Eimeria tenella*

Day	E.1.	E.2.	E.3.	E.4.	E.5.	E.6.	E.7.	E.8.	E.9.	E.10.
7	57.5	24.9	59.3	41.0	60.9	33.8	95.4	54.0	32.9	38.9
8	7.9	48.3	11.7	11.5	19.7	20.6	14.2	7.4	22.4	28.4
9	21.2	8.0	6.3	4.4	10.5	16.1	38.3	5.9	15.0	10.7
10	8.4	8.9	7.5	6.3	8.8	2.8	6.9	2.8	3.6	6.2
11	9.6	7.1	8.9	13.1	13.1	14.0	1.1	4.0	1.8	1.3
12	4.7	3.5	3.8	3.4	21.6	9.9	1.6	4.6	.2	.4
13	3.1	.4	1.5	3.8	13.6	4.3	3.9	5.1	.4	.1
14	2.2	.4	2.3	.7	.5	.8	.7	3.2	.7	.1
15	.6	*	.6	.3	.7	1.3	8.4	1.5	.2	.1
16	.4	*	.4	.1	.1	1.6	7.3	.9	.5	.1
17	.1	*	1.2	*	.1	.7	5.8	2.2	*	*
18	*	*	.3	.8	.1	.6	1.0	.9	.1	*
19	.1	*	.1	.1	.1	.2	.1	.3	-	*
20	.1	*	*	.6	.2	.2	.2	*	*	.1
21	.1	*	.2	.1	*	.2	.1	*	.1	.1
Total** per Bird	98	102	84	86	149	182	180	91	78	86

* Indicates that oocyst production was less than 50,000 per bird on that day.

** The total production of oocysts per bird calculated to nearest million oocysts.

Table 3. 14.

The Oocyst Production of the Experimental Chickens after Inoculation on Day 0 with different Numbers of Sporulated Oocysts of *Eimeria tenella*.

Group	Dose of Oocysts On Day 0	No. Oocysts Produced/ Oocyst Inoculated	Total Oocyst Production/Bird
Control	nil	nil	nil
E.1.	1,000	96,000	96*
E.2.	2,000	51,000	102
E.3.	4,000	21,000	84
E.4.	8,000	10,750	86
E.5.	16,000	9,375	149
E.6.	32,000	5,000	162
E.7.	64,000	2,500	160
E.8.	128,000	710	91
E.9.	256,000	306	78
E.10.	500,000	170	85

* The average total oocyst production per bird on day 7 to day 21 inclusive, calculated to the nearest million oocysts.

Table 3. 15.

The Pathogenic Effects of Different Numbers of Sporulated Oocysts of *Eimeria tenella* in Chickens aged 4 Weeks Old on Day 0.

Group	Dose Oocysts	Haemoglobin* Decrease	Mortality	Weight Gain Day 11	Weight Gain Day 19	Total Oocyst Production Per Chicken
Control	nil	nil	nil	415	701	nil
E.1.	1,000	0.8**	nil	278***	590	96****
E.2.	2,000	1.0	nil	338	559	102
E.3.	4,000	2.0	nil	316	624	84
E.4.	8,000	2.3	nil	327	596	86
E.5.	16,000	3.2	1	290	573	149
E.6.	32,000	4.9	1	253	559	162
E.7.	64,000	5.8	7	276	541	160
E.8.	128,000	4.5	4	186	510	91
E.9.	256,000	4.4	4	162	472	78
E.10.	500,000	5.5	5	52	270	85

* Haemoglobin expressed as grammes per 100 ml. blood; decrease Day 1-6.

** The probability calculated on the "t-test" of E.3. was 0.01, and of E.4 - E.10 was 0.001 on the mean haemoglobin value on day 1 and 6.

*** Weight gain expressed in grammes. Values = Day 11 - Day 0-2 and Day 19 - Day 0-2.

**** The average total oocyst production per bird on day 7 to day 21 inclusive, calculated to nearest million oocysts.

Table 3. 16.

The Pathogenic Effects of a Standard Dose of 64,000 Sporulated Oocysts of *Eimeria tenella* in Chickens aged 4 weeks old on Day 0, following reinfection on Day 21.

Group	Dose of Oocysts on Day 0.	Haemoglobin* Decrease	Mortality	Weight Gain** Day 32 - 21
Control	nil	nil	nil	369
Ch.	nil	3.4	1	224
E.1.	1,000	1.7	nil	317
E.2.	2,000	2.0	nil	366
E.3.	4,000	1.2	nil	256
E.4.	8,000	0.6	nil	309
E.5.	16,000	nil	nil	346
E.6.	32,000	nil	nil	300
E.7.	64,000	nil	nil	728
E.8.	128,000	nil	nil	428
E.9.	256,000	nil	nil	375
E.10.	500,000	nil	nil	364

* The decrease in the concentration of the haemoglobin expressed as grammes per 100 ml. of blood. The decrease represents the difference between the mean value on day -2 and 4, and that of the lowest value recorded on day 6 or 7.

** Weight gain expressed in grammes.

Table 3. 17.

The Pathogenic Effects of Eimeria tenella in Chickens Following Inoculation with Different Numbers of Sporulated oocysts on Day 0; and subsequently after Reinfection on Day 21 with a Standard Dose of 64,000 Oocysts per Bird.

Group	Dose of Oocysts Day 0	Haemoglobin* Decrease		Mortality		Weight** Gain		Total Weight Gain	Oocyst*** Output
	-	(1)	(2)	(1)	(2)	(1)	(2)		-
Control	-	-	-	-	-	100	100	100	-
Ch.	-	-	3.4	-	1	-	62	87	-
E.1.	1,000	0.8	1.7	-	-	67	88	86	96
E.2.	2,000	1.0	2.0	-	-	82	99	88	102
E.3.	4,000	2.0	1.2	-	-	76	71	82	84
E.4.	8,000	2.3	0.6	-	-	79	86	84	80
E.5.	16,000	3.2	-	1	-	70	96	86	149
E.6.	32,000	4.9	-	1	-	62	84	80	102
E.7.	64,000	3.8	-	7	-	67	202	119	160
E.8.	128,000	4.5	-	4	-	45	119	90	91
E.9.	256,000	4.4	-	4	-	39	104	79	78
E.10.	500,000	5.5	-	5	-	13	101	59	85

* Haemoglobin expressed as grammes per 100 ml. of blood. (1) and (2) represent initial and challenge infections respectively.

** The gain in weight expressed as percentage of the gain of the non-infected control group, which was attained in the 11 day period following each inoculation.

*** The average total oocyst production per bird on day 7 to day 21 inclusive, calculated to the nearest million oocysts.

(6) Pathology.

(1) The Post mortem Findings Following the Initial and Challenge Infections in the Replicate Groups of E.3, E.7 and E.10.

Two birds were killed from each group after the initial infection; chickens were not examined from group E.10 following reinfection on day 21. Examinations were made at 24, 48, 60, 72, 84, 96, 108, 120, 132 and 144 hours after each inoculation in the appropriate groups, which were selected to represent low (4,000), moderate (64,000) and high (500,000) levels of infection.

Group E.3. - 4,000 Sporulated Oocysts/Bird.

Post-mortem examinations made at 24, 48, 60, 72 and 84 hours after infection on day 0 revealed no evidence of macroscopic lesions in the caeca. The first indication of infection was seen at 96 hours when there was a slight suspicion of one or two very small haemorrhages present on the mucosa. At 108 hours the caecal walls were slightly thickened and small haemorrhages were present on the mucosa. There was no significant difference between the lesions in birds killed at 120 or 132 hours. The caecal walls were thickened and small haemorrhages were seen on the mucosa, together with slight erosion of the epithelium. The caecal contents appeared normal in one bird, in another slight haemorrhage was evident, while in the remaining two, marked haemorrhage was present and the caeca contained a small amount of clotted blood. On the sixth day, 144 hours, only slight lesions were found in the caeca. The mucosa was a little thickened and the caecal contents appeared blood-stained.

No significant lesions were seen in birds examined at 24, 48, 60 or 72 hours after reinfection on day 21 with 64,000 sporulated oocysts. A small mass of necrotic debris was present at the distal end of one caecum, where it was walled off from the lumen of the caecum by fibrous tissue. This appeared to represent a residual lesion from the initial infection on day 0. At 96 hours, slight thickening was present in small areas of the mucosa; this was more pronounced at 108 hours when it was also evident in the longitudinal folds of the mucosa. Quite severe lesions were present on day 5. The caecal walls were thickened, numerous small haemorrhages were evident on the mucosa, together with slight erosion of the epithelium. Blood was present in the lument, although the haemorrhage was less severe than that in the fully susceptiblechickens of group Ch. There was no significant difference in the lesions at 120 and 132 hours, with the exception of the material present in the lumen of the caeca, which in the later examination contained only traces of blood and appeared dehydrated. On day 6, 144 hours, the caecal walls were markedly thickened and the mucosa was thrown into longitudinal ridges, presenting a corrugated appearance. One or two small pin-point haemorrhages were present on the mucosa. The caecal contents of one bird appeared slightly pink, suggesting slight haemorrhage.

<u>Group E.7.</u> - 64,000 Sporulated Oocysts/Bird.

No macroscopic lesions were found in the caeca of birds which were examined at 24, 48, 60 and 72 hours after the initial inoculation on day 0.

The first indication of infection was seen on the third day when the caecal wall appeared slightly thickened in the birds examined at 84 hours. Lesions were more marked at 96 hours when small round thickened areas which were raised above the level of the epithelium were present in the caecal wall; small haemorrhages were evident on the mucosa and traces of blood were seen in the caecal contents which appeared relatively normal. Severe changes were found in the birds examined at 108 hours. The mucosa was very thickened and raised into longitudinal ridges presenting a corrugated appearance; numerous pin-point haemorrhages were present on the mucosa, together with evidence of slight erosion of the epithelium. A small amount of blood was seen in the lumens of the caeca. On the fifth day, the lesions were very severe in the birds examined at 120 hours. The caecal walls were markedly thickened; there was widespread erosion of the mucosa and very severe haemorrhage, which was associated with tremendous distension of the caeca in one bird where the lumen was filled with clotted blood. In the second bird, the caecal contents appeared dehydrated and were adherent to the mucosa. Examination at 132 hours suggested complete erosion of the mucosa from the caecal wall. The lumen contained cellular debris and clotted blood which was easily removed with forceps. Birds examined at 144 hours on the sixth day presented less marked changes; the caecal wall was slightly thickened, haemorrhages were not evident on the wall, although the lumen contained blood and necrotic debris. The caeca were not enlarged.

Birds examined after the challenge infection of 64,000 oocysts on day 21 showed no evidence of reinfection, with reference to any indication of haemorrhage or changes in the caecal wall which could be attributed to a recent infection. However, lesions were present in some birds which appeared to be associated with the initial inoculation on day 0. Many of the caeca appeared contracted and showed evidence of fibrosis. All the caeca were fully patent and none contained necrotic debris in the lumen.

Group B.10. - 500,000 Sporulated Oocysts.

No lesions were found in the birds examined 24, 48, 60 and 72 hours after inoculation on day 0. The caecal wall appeared slightly thickened in the chickens examined at 84 hours. There was also evidence of small haemorrhages present on the mucosa. Very marked changes were found at 96 hours. There was widespread erosion of the mucosa and numerous haemorrhages were present on the walls of the caeca. The caeca of one bird contained unclotted blood; cellular debris and extravasated blood were present in the caeca of the second bird and were adherent to the mucosa. The birds examined at 108 hours on the fourth day had very contracted caeca. Numerous haemorrhages were present on the mucosa associated with widespread erosion of the epithelium. The caeca contained dark red necrotic debris which was closely adherent to the caecal wall. Very severe changes were seen at 120 and 132 hours on the fifth day. The caecal walls were either slightly thickened or very thin and friable when associated with tremendous haemorrhage which led to marked distension of the caeca. Erosion of the mucosa was very severe, extending to the muscle layer in one bird. The lumens of all the caeca contained large amounts of blood. On the sixth day the caeca were very small, showing evidence of severe erosion of the mucosa which appeared to have been completely lost. The caecal contents were tar-like and viscous in consistency, being washed from the wall quite easily.

(ii) Post mortem Examination of the Birds which died on Day 5 and 6.

Examination of these birds revealed typical lesions of acute caecal coccidiosis. There was no variation in the severity of the lesions in the birds from the different groups.

(iii) Post mortem Examination of the Birds which died after the Challenge Infection

Examination of the bird which died in group Ch. on the fifth day after infection revealed typical lesions of acute caecal coccidiosis.

(iv) Post mortem Examination of the Surviving Birds from the Replicate Groups on Day 21.

Slight lesions were found in the caeca of the birds of R.6/2. The caecal walls were thickened, indicating slight fibrosis. The lumen of one caecum contained a little necrotic debris at the distal end. All the caeca were patent.

Severe lesions were present in the majority of the birds from E.7/2. Marked fibrosis was evident in the walls of the caeca in four birds, one bird also showing evidence of peritonitis. Four birds of the seven examined had necrotic debris present in one caecum, the other being patent in each case. Two birds had no necrotic debris in their caeca; the caeca of the remaining chickens were completely occluded by necrotic material.

Very marked changes were also found in the caeca of the chickens from E.8/2. The walls were severely thickened in the majority of birds; the lumen contained large cores of necrotic debris which completely occluded the caeca in three of the seven birds examined. Peritonitis was also evident in these three chickens. The caeca of the other four birds were patent.

(v) Post mortem Examination of the Surviving Birds from Groups E.1-E.10 and Ch. on Day 33.

In group Ch., the caecal walls appeared slightly thickened and,in four of the nine birds examined, necrotic debris was found in one caecum of each bird, the other caecum being patent.

No lesions were evident in E.1 or E.4. The caeca also appeared normal in the majority of the chickens from E.2, E.3, E.5, E.6 and E.7. One or two birds from each group showed evidence of slight thickening in the caecal wall. A little necrotic debris was found in the lumen of the caeca in one bird from each of the groups E.2, E.3, E.6 and E.7.

No lesions were evident in five of the six survivors from E.8. Severe lesions were found in one bird which appeared similar to those seen in colibacillosis.

The caeca from the chickens of groups E.9 and E.10 appeared contracted and showed evidence of thickening in the caecal walls, indicating fibrosis, in three of the eleven birds examined from these groups.

The caecal lumen was patent in all the survivors examined from E.1 to E.10 with the exception of the one bird described above in E.8.

DISCUSSION.

The results (Table 3. 7) showed no significant variation in the concentration of haemoglobin in the blood of the control and the infected groups on day 0-1, day 1 and day 4. The first decrease in the level of haemoglobin occurred on day 5, the lowest values being recorded on day 6 in all the experimental groups except group E.6/2, where the value was slightly lower on day 7 (Table 3. 8). This difference between the value recorded on day 6 and day 7 was not significant. The replicate groups were infected later in the day than groups E.1 - E.10 which could possibly explain the difference. These results indicated that the maximum decrease in the concentration of haemoglobin could be measured from the difference in the values of the estimation made between day 0-1 and day 4, and one made on day 6, after inoculation with doses of sporulated oocysts of E. tenella, ranging from 1,000 to 500,000 per bird in fully susceptible four week old chickens. Similarly, the results (Table 3. 10) indicated that the maximum decrease in the concentration of haemoglobin could be measured from the difference in the values of an estimation made between day 0-2 and day 4 and one made on day 6 or day 7, after a standard challenge infection of 64,000 sporulated oocysts of the same species, given three weeks after an initial infection, ranging from 1,000 to 500,000 oocysts in four week old birds.

The results (Table 3. 15), giving the decrease in the level of haemoglobin in the birds after the initial infection, showed that a small decrease of 1 g. occurred in birds given 1,000 or 2,000 oocysts, but the change was not statistically significant. However, the detrimental effects of a low infection were demonstrated by the observations made on the growth rate (Tables 3. 11

and 3. 12) which showed less satisfactory weight gains in those groups. These were still evident when the birds were slaughtered at the end of the experiment.

There was a significant fall in the haemoglobin level of 2 g. in birds given 4,000 or 8,000 oocysts, indicating a marked difference between the pathogenicity of these levels of infection on the one hand, and that of 16,000 oocysts on the other, where the decrease was 3 g. of haemoglobin. The difference between 8,000 and 16,000 oocysts was also reflected by the clinical findings and by mortality. The importance of low levels of infection was also demonstrated by the oocyst production of the groups which clearly emphasised the significance of this factor in the epidemiology of the disease. The total output per bird was relatively similar after infections of 1,000 to 8,000 oocysts, ranging from 84 to 100 million oocysts per bird. There was a significant variation between the output of these groups and that which received 16,000 oocysts, where the output was 150 million per bird.

The results showing the maximum decrease in the level of haemoglobin (Table 3. 16) after inoculation on day 0, suggests that 16,000 oocysts were less pathogenic than a dose of 32,000 oocysts in four week old birds. The decrease was 3 and 4.0 - 4.9 g. respectively. This was not confirmed conclusively by either observations on the growth rate, mortality or oocyst production.

The results of the haemoglobin estimation did not demonstrate any significant difference in the pathogenicity of doses ranging from 32,000 to 256,000 oocysts in four week old birds. However, the consistent results obtained in the replicate groups (Table 3. 9) again demonstrated the value of this criterion in the evaluation of the pathogenicity of E. tenella over this range of infection.

These results also indicated the doubtful value of mortality alone as a significant parameter for the evaluation of differences in the pathogenic effect of various levels of infection with E. tenella, ranging from 16,000 to 500,000 oocysts per bird in fully susceptible four week old chickens. The lower haemoglobin level recorded in E.6 (Table 3. 8) on day 6, compared with that of group E.6/2, may have been related to the difference in mortality. The inference is that the birds in E.6/2 which had low haemoglobin levels died, while those in E.6 survived, the difference in the mortality rate being associated with biological variation.

The results (Tables 3. 9 and 15) did not demonstrate a significant difference in the pathogenic effect between 32,000 and 64,000 oocysts in four week old birds. This was confirmed by the results of the replicate groups which emphasised the pathogenicity of the lower level of infection. The difference in mortality between E.6 and E.7 was clearly shown to be of no significance.

There was a definite difference between the pathogenic effect of 64,000 and 128,000 oocysts in four week old chickens, demonstrated by the variation in the growth rate, clinical findings and the total oocyst production of each group (Tables 3. 11 and 15). There did not appear to be any difference in the pathogenic effect of infection with either 128,000 or 256,000 oocysts per bird in four week old chickens. However, observations on the growth rate (Tables 3. 11, 12 and 15) and changes in the concentration of haemoglobin (Tables 3. 7 and 16) indicated a marked difference in the pathogenicity of these doses and one of 500,000 oocysts per bird. The lowest mean value of haemoglobin concentration and the most severe retardation of growth were recorded in birds given 500,000 oocysts. The highest morbidity was also seen in this group. There was no significant difference in mortality between birds given doses of oocysts ranging from 32,000 to 500,000 per bird.

Gardiner (1955) studied the pathogenic effects of 50,000, 100,000 and 200,000 oocysts of E. tenella in four week old chickens. He concluded that there was no significant difference in mortality between the three levels of infection. However, he observed a definite variation in the growth rate between 50,000 oocysts on the one hand and 100,000 and 200,000 oocysts on the other, the most severe retardation occurring in the latter groups. These results were similar to those recorded in groups E.7, E.8 and E.9 in which birds received 64,000, 128,000 and 256,000 oocysts of E. tenella respectively.

The results (Table 3. 14) giving the oocyst production of the groups after the initial infection, showed that there was little variation between the total output of the birds receiving low levels of infection one the one hand, E. 1 - E.4, and those given very high levels of infection on the other, E.8 - E.10. It would appear reasonable to suggest that the lower oocyst production of E. 8 - E.10, compared with that of groups E.5 - E.7, may be directly associated with the severity of the lesions in the caeca. It is possible that the tremendous disorganisation of the mucosa and the severe haemorrhage associated with schizogony could interfere with oocyst production in two ways, (i) due to a severe decrease in the number of epithelial cells after widespread erosion of the mucosa, and (ii) a loss of second generation merozoites when haemorrhage is severe. Another significant factor could be the "crowding effect" described by Brackett and Bliznick (1952).

The fall in the reproductive potential of E. tenella as the infective dose increased was shown clearly by the comparison of the infective dose and the total oocyst production per bird (Table 3. 14), giving the number of oocysts produced per oocyst ingested. This was in agreement with the observations of Brackett and Bliznick (1952).

The retardation of growth associated with doses of oocysts ranging from 16,000 to 256,000 oocysts per bird was less severe in four week old birds than in birds aged six weeks old (Tables 3. 5 and 11). These findings were in agreement with the results of Edgar (1955) who found that retardation of growth increased in severity with the age of the chicken between two - six weeks of age, while birds infected at six, eight and ten weeks of age suffered similar effects when the depression of growth was measured from the weight gains attained at twelve weeks of age.

The first lesions were found on day 3 in group E.10 at serial post mortem examination following the initial inoculation. Changes were not seen in E.3 and E.7 until 24 and 12 hours later respectively. On the morning of day 4, very severe changes were present in E.10 only, although on day 5 and 6 there was little difference between this group and E.7. The lesions present in the caeca of group E.3 were not marked until day 5 and 6, indicating that post mortem examination should be made at this time to demonstrate the significance of lesions associated with low levels of infection. The severity of the changes found in group E.3 emphasised the pathogenicity of small numbers of oocysts of E. tenella in four week old chickens which had also been indicated by the retardation of growth and the high oocyst production recorded in groups E.1 - E.4.

Following reinfection on day 21 with a standard challenge dose of 64,000 oocysts per bird, quite marked lesions were found in group E.3 in contrast to E.7, where no lesions were seen which could be attributed to the challenge inoculum. This observation indicated that birds are not fully resistant to a high challenge dose of oocysts after a low level of infection. However, the lesions appeared to be of far greater severity in the fully susceptible birds from group Ch. which were also examined on day 5 after the second inoculation. Birds were not kept for examination after challenge on day 21 from E.10, due to the high pathogenicity of the initial inoculum of 500,000 oocysts.

The time for reinfection was selected after preliminary investigations had been made to establish the duration of lesions in chickens of the same age, after infection with doses ranging from 1,000 to 256,000 oocysts per bird. The results indicated that reinfection on day 21 would not be complicated by the presence of necrotic debris in the lumen of the caeca, which would interfere with the entry of the sporozoites from the challenge infection. These findings were confirmed in the present experiment by the observations made at serial post mortem examination after challenge on the birds of group E.7, when it was shown that the caeca were patent, none containing necrotic debris in the lumen. However, severe lesions were found in the caeca of the survivors of the replicate groups E.7/2 and E.8/2 which were killed on the 21st day after inoculation. Careful examination of the lesions in these birds indicated that the caeca were not patent in one bird of E.7/2 and in three birds of E.8/2, out of a total of seven survivors from each group. Therefore it was possible that similar residual lesions could have been present in some of the birds of E.8 - E.10 when they were reinfected on day 21. However, post mortem examination of these birds on day 33 showed no evidence of changes associated with persistent lesions which could be responsible for occlusion of the caecal lumen. This evidence suggested that reinfection was probably satisfactory in these groups on day 21.

The selection of the challenge dose of 64,000 sporulated oocysts per bird was determined by the results of Experiment 2 which indicated that this level of infection was associated with severe pathogenic effects in 45 day old chickens. These included a marked fall of 3.7 g. in the level of the haemoglobin, high morbidity, 40% mortality and a very severe retardation in growth (Table 3.6).

Typical signs of acute caecal coccidiosis were seen in the fully susceptible chickens of group Ch., which confirmed the pathogenicity of the challenge inoculum administered on day 21. Severe haemorrhage was present on day 5 and 6 after infection when there was a fall in the level of haemoglobin of 3.4 g. (Table 3. 10), which was similar to that recorded in Group 3 given the same dose of oocysts in the previous experiment (Table 3.6). The growth rate of these birds was also affected (Table 3. 12), their weight gain being only 62% of that attained by the non-infected control group over the same period of time. This was less severe than that recorded in E. 3 of Experiment 2 (Table 3. 5.). Morbidity was high in these birds on day 5 and 6 after inoculation, although mortality (10%) was low. The difference in weight gain recorded in group Ch. and that in group E.3 of Experiment 2 might be associated with two factors, (i) a difference in the pathogenicity of the culture used for the inoculum and (ii) the different number of birds in each cage after the acute phase of the disease, due to mortality. The age of the culture at the time of the challenge infection was 48 days, while that in Experiment 2 was 41 days old, which does not appear to be a significant variation. The pathogenicity of the culture used for reinfection on day 21 was demonstrated on administration to the replicate groups on day 12, when severe mortality occurred in E.7/2 (Table 3. 9.).

After challenge on day 21, no evidence of morbidity was seen in any of the chickens in groups E.1 - E.10. in marked contrast to group Ch. Slight haemorrhage occurred in all these groups with the exception of E.8 and E.10. A small decrease in the level of haemoglobin was recorded in E.1 - E.4 and E.7 on day 6 and 7; 24 hours after the first decrease in haemoglobin was seen in the fully susceptible birds of group Ch., which occurred on day 5. The change in the concentration of haemoglobin was significant in E.1 - E.3 and group Ch. The difference recorded in E.7 was not confirmed by the presence of blood in the cage on clinical observation (Tables 5. 10 and 16).

There was a slight variation in the growth rate of some groups between day 19 and day 32 (Table 5. 12). This could be associated with two factors, (i) a difference in the susceptibility to reinfection related to the immunological effect of the inoculum on day 0 and (ii) the variation in the number of birds in each cage due to losses in certain groups during the acute phase of the disease. The results (Table 5. 17) did not show any definite correlation between the magnitude of the infective dose and the variation in the growth rate. However, there did appear to be a relationship between the number of birds in each group and their weight gain, very marked in E.7, where the weight gain was approximately double that attained by the non-infected control group. This observation showed that a variation in the number of birds in a cage at this age could have a significant effect on the growth rate. There was no indication that this factor influenced the growth rate in the period between the initial and challenge infections (Table 5. 11) when the birds were smaller.

The results (Table 5.17), giving the total weight gain attained in the period day 0-2 - day 32, indicated clearly that the retardation of growth which occurred after the initial infection was still evident on day 32. The weight gains of the chickens in E.1 - E.10, with the exception of E.7, was from 10% to 40% less than those in the non-infected control group.

The observations made after the administration of the challenge infection demonstrated a significant degree of resistance to reinfection in all the chickens of groups E.1 - E.10. The results (Table 5. 16) indicated that the birds which received an initial inoculum of 1,000 - 4,000 oocysts per bird on day 0 were slightly susceptible to reinfection. This was reflected in the changes in the level of haemoglobin on day 6 and 7 after challenge and was confirmed in E.3 by the lesions found at serial post mortem examination. The chickens which received doses of oocysts ranging from 8,000 - 500,000 oocysts per bird on day 0 showed no significant evidence of any detrimental effects after challenge although the slight haemorrhage seen between day 5 and 7 in some of the groups indicated that these birds were not completely resistant to reinfection.

The results (Table 3. 17) suggest that the most successful immunising dose of oocysts from the series investigated was 8,000 oocysts per bird. This level of infection did not cause mortality or severe morbidity after inoculation and was not associated with deterimental effects following challenge on day 21. However, the high oocyst production and the retardation of growth, which were recorded in this group after inoculation, demonstrated conclusively the disadvantages associated with low levels of infection with particular reference to epidemiology and the down-grading of birds; which could be significant aspects of the disease in the field under intensive methods of husbandry.

Horton Smith (1947) studied the immunological effect of different numbers of oocysts of E. tenella in young chickens with particular reference to the degree of resistance demonstrated against a large challenge dose of oocysts administered 21 days after the initial infection. The levels of infection examined were 100, 200, 30,000, 60,000 and 120,000 sporulated oocysts per bird; the magnitude of the challenge inoculum was not stated. The results, which were on mortality, showed that very slight protection was given by the very low doses of oocysts, while better protection was demonstrated in the groups receiving the higher levels of infection, although some mortality was recorded in these groups after challenge. These results suggested a marked difference in the pathogenicity of 60,000 and 120,000 oocysts per bird, shown by mortality, which was 25% and 50% after the initial infection respectively. No variation was apparent in the pathogenic effect of 30,000 and 60,000 oocysts per bird, mortality being 25% in each group. However, a significant difference was recorded in the mortality after challenge in these groups, when approximately 30% of the survivors died in the group given 30,000 oocysts, compared with only 10% in the birds receiving either 60,000 or 120,000 oocysts on day 1. Mortality in the groups given 100 or 200 oocysts per bird was approximately 50%, compared with 75% in the fully susceptible control chickens which were infected at the same time to confirm the pathogenicity of the challenge inoculum.

The results in the present experiment are in agreement with the observations made on the variation in the pathogenicity over the range of infection of 30,000, 60,000 and 120,000 oocysts per bird. This was similar to the conclusion that no significant variation could be shown between 32,000 and 64,000 oocysts per bird, although there was a marked increase in the pathogenic effect of 128,000 oocysts. The fact that no difference was demonstrated between these groups on reinfection may be explained by a variation in the magnitude of the challenge infections. The results may also not be strictly comparable as the age of the chickens probably differed in each experiment

EXPERIMENT FOUR.

THE PATHOGENIC EFFECTS OF 4,000 AND 32,000 SPORULATED OOCYSTS OF EIMERIA TENELLA IN 14 DAY OLD CHICKENS WHEN FOOD IS WITHDRAWN 0, 3 and 12 HOURS BEFORE ADMINISTRATION OF THE INFECTIVE DOSE.

EXPERIMENTAL AIMS.

The experiment was designed to study the effect on 14 day old chickens of different pre-infection feeding regimes on the pathogenicity of *Eimeria tenella*. It was necessary to establish the significance of this factor in relation both to the severity of the disease and the epidemiology of the parasite. The pathogenic effects of low and high levels of infection were compared in three groups of chickens given ad lib. and restricted feeding before inoculation. Observations were made after both short and long periods of starvation, food being withdrawn 0, 6 and 12 hours before infection.

The severity of the disease in each group was determined from observations made on the changes in the concentration of haemoglobin, on mortality, on clinical signs, on growth rate and on oocyst production during the patent phase of the infection. Preliminary investigations suggested that slight differences in the pathogenicity of *E. tenella* were not indicated clearly by a marked variation in the severity of the lesions in the caeca, therefore birds were not killed for post mortem examination during the experiment.

MATERIALS AND METHODS.

Experimental Birds.

Broiler type hybrid "as hatched" cockerel and pullet chickens were used in the experiment. They were reared in complete isolation and were transferred to the experimental unit on the day before infection, where they were kept in metal cages with wire floors. A chick mash, prepared from a formula used by Joyner and Davis (1960), was available ad lib. to the birds.

Parasitology.

The culture of E. tenella used for the infection of the experimental birds on day 0 was 100 days old, with a total oocyst count of 660,000 per ml. and sporulation count of 85%. The culture of E. tenella used for the infection of the replicate groups five weeks later was 11 days old, with a total oocyst count of 528,250 per ml. and sporulation count of 90%. Serial dilutions were made to give the appropriate number of oocysts per ml. for each experimental group, by the addition of distilled water to an aliquot of the culture, which was suspended in 2% potassium dichromate solution.

Administration of Inoculum.

The infecting dose was given in 1 ml. of water to each experimental bird. It was administered directly into the crop using an automatic dosing syringe. During inoculation the stock of oocysts from which the syringe was filled was kept in suspension by decanting the solution between two beakers.

All the experimental birds were inoculated in the morning. Food was withdrawn from E.1 and E.4; E.2 and E.5; E.3 and E.6; and their appropriate replicate groups at 0, 3 and 12 hours before infection respectively (Tables S. 18 and 19).

Experimental Design.

Ten birds were selected at random for each experimental group and transferred to the experimental unit on the day before inoculation, with the exception of the non-infected control group which were left in the isolation unit. The birds were 14 days old on day 0.

Birds of the same age were selected at random from a later consignment of chicks for the replicate groups E.1/2 - E.6/2 and the appropriate non-infected control group.

The experimental groups designated E.1 - E.3 received a total of 4,000 and E.4 - E.6 a total of 32,000 sporulated oocysts per bird respectively on Day 0. Food was withdrawn from E.1 and E.4; E.2 and E.5; and E.3 and E.6 at 0, 3 and 12 hours before inoculation respectively.

Similar numbers of oocysts were given to the birds in each appropriate replicate group five weeks later. These birds were infected to observe whether the same results, with particular reference to changes in haemoglobin concentration, mortality and total oocyst production, could be reproduced in each group when the food had been withdrawn for the same period of time, 0, 3 and 12 hours before inoculation.

Haemoglobin estimations were made on all the experimental birds on the day prior to inoculation, day 0-1, and after infection on day 6.

The birds of control and E.1 - E.6 experimental groups were weighed two days before day 0 on day 0-2 and after infection on day 10 and day 19. The birds of control/2 and the replicate groups E.1/2 - E.6/2 were weighed on the day before day 0, on day 0-1, and after infection on day 10 and day 20.

The total oocyst production of each group was recorded each day during the patent period of the disease from day 7 to day 20 inclusively.

Post mortem examination was carried out on day 6 on all the birds which died on day 6. Survivors were killed on day 20.

Clinical signs and mortality were also recorded.

RESULTS.

(1) Haematology.

The results (Tables 3. 18 and 19) of the haemoglobin estimations in the control birds did not show any significant variation in the haemoglobin concentration of those birds during the course of the experiment.

On day 0-1 the results did not indicate any difference between the experimental groups and their corresponding control groups, with the exception of E.4.

On day 6 there was a significant decrease in the haemoglobin level of the experimental groups, E.1 - 3g., E.4 and E.6 - 4.4g. and in E.5 - 2g. There was no decrease in the haemoglobin concentration of E.2. A small decrease of 1.6g. was recorded in E.3.

The results (Table 3. 19) of the replicate were very similar to those recorded in E.1 - E.6, with the exception of E.4/2 and E.6/2, where a greater decrease in the concentration of haemoglobin occurred on day 6.

(2) Mortality.

After inoculation on day 0, deaths occurred on day 5 in E.1 (10%); E.4 (60%); E.4/2 (40%); E.5 (10%); E.5/2 (30%); E.6 (20%) and E.6/2 (20%); (Table 3. 24).

Table 3. 18.

The Mean Haemoglobin Concentration of the Experimental Chickens E.1-E.3 and E.4-E.6, inoculated with 4,000 and 32,000 Sporulated Oocysts of *Eimeria tenella*, per Chicken respectively, on day 0; Food being withdrawn 0, 3 and 12 Hours respectively before Inoculation.

Group	Dose of Oocysts	Time Food Withdrawn	Day 0-1	Day 0+6	Haemoglobin* Decrease
Control	nil	0 hours	9.1 ±0.2	10.2 ±0.5	nil
E.1.	4,000	0 hours	9.4 ±0.8	6.3 ±2.0	3.1
E.2.	4,000	3 hours	9.5 ±0.5	9.5 ±0.7	nil
E.3.	4,000	12 hours	9.5 ±1.3	8.0 ±2.0	1.5
E.4.	32,000	0 hours	8.3 ±0.9	3.9 ±0.5	4.4
E.5.	32,000	3 hours	8.9 ±0.6	6.7 ±1.5	2.2
E.6.	32,000	12 hours	9.0 ±0.8	4.6 ±1.6	4.4

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

Table 3. 19.

The Mean Haemoglobin Concentration of the Replicate Groups E.1/2 - E.3/2 and E.4/2 - E.6/2, inoculated with 4,000 and 32,000 Sporulated Oocysts of *Eimeria tenella*, per Chicken respectively, on Day 0; Food being withdrawn 0, 3 and 12 Hours before Inoculation respectively.
12 Hours

Group	Dose of Oocysts	Time Food Withdrawn	Day 0-1	Day 0+6	Haemoglobin* Decrease
Control	nil	0 hours	9.8 ±0.6	9.7 ±0.6	nil
E.1/2	4,000	0 hours	9.8 ±1.0	6.4 ±2.0	3.4
E.2/2	4,000	3 hours	9.3 ±0.6	9.2 ±1.2	nil
E.3/2	4,000	12 hours	9.5 ±0.7	8.4 ±1.5	1.1
E.4/2	32,000	0 hours	9.5 ±0.7	3.9 ±1.0	5.6
E.5/2	32,000	3 hours	9.4 ±0.8	6.5 ±2.0	2.9
E.6/2	32,000	12 hours	9.7 ±0.8	4.6 ±4.4	5.1

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

(3) Clinical Findings.

Clinical symptoms were first seen on day 4, when a little blood was found in the faeces of groups E.4, E.5 and E.6. Haemorrhage was most marked in group E.6.

On day 5 the first evidence of haemorrhage was recorded in the groups E.1 - E.3, where it was most pronounced in group E.1. The birds in these groups appeared slightly depressed. Morbidity was far greater in E.4 and E.6, and was associated with severe haemorrhage. Both morbidity and haemorrhage appeared slightly less severe in E.5 than in E.4 and E.6.

On day 6 no evidence of depression was seen in the birds of groups E.2 or E.3. The birds in E.1 and E.5 appeared slightly depressed, while morbidity was quite marked in E.4 and E.6. A little blood was found on the trays from each group except E.3.

On day 7 there was no indication of morbidity in any of the experimental groups. A little blood was present on the trays of each group. No evidence of haemorrhage was seen after day 7.

Similar changes were recorded in the replicate groups.

(4) Growth Rate.

The results (Tables 3.20 and 21) showed no significant variation in weight between the non-infected control groups and their respective groups E.1 - E.6 and E.1/2 - E.6/2 before inoculation.

On day 10 the weight gain in groups E.1 and E.3 was slightly less than that recorded in E.2, which was similar to the gain attained by the non-infected control group. On day 19 there was no significant difference between the total weight gain of the birds in E.1 - E.3 and the control group.

Table 3. 20.

The Mean Weight Gains of the Experimental Chickens on Day 10 and Day 19 to Illustrate the Effect on the growth Rate Associated with Low and High Levels of Infection with Sporulated Oocysts of *Eimeria tenella*, Food being Withdrawn 0, 6 and 12 Hours Before Inoculation Respectively.

Group	Dose of Oocysts	Time Food Withdrawn	Weight Day0-2	Gain on Day 10	% Gain	Total Gain on Day 19	% Gain
Control	nil	0 Hours	153*	237	100**	466	100
E.1.	4,000	0 hours	167	226	96	456	98
E.2.	4,000	6 hours	157	247	104	476	102
E.3.	4,000	12 hours	146	227	96	484	104
E.4.	32,000	0 hours	142	132	56	336	72
E.5.	32,000	6 hours	143	215	91	460	99
E.6.	32,000	12 hours	152	183	77	446	96

* Weight in grammes.

**- Weight gain as % of the value recorded in the non-infected control group.

There was a marked depression of growth rate in E.4 and E.6., where the gain in weight equalled only 56% and 77% respectively of that recorded in the non-infected control group on Day 10. The effect was less marked in group E.5 (91%) compared with that in E.4 and E.6. On day 19 there was no significant difference in the total weight gains of E.5 and E.6 compared with that recorded in the control group. Retardation of growth was still evident in group E.4, where the total gain in weight equalled only 72% of that attained by the control group.

Table 3. 21.

The Mean Weight Gains of the Replicate Groups of Experimental Chickens on Day 10 and Day 20 to Illustrate the Effect on the Growth Rate Associated with Low and High Levels of Infection with Sporulated Oocysts of *Eimeria tenella*, Food being Withdrawn 0, 3 and 12 Hours Before Inoculation Respectively.

Group	Dose of Oocysts	Time Food Withdrawn	Weight Day0-1	Gain on Day 10	% Gain	Total Gain on Day 20.	% Gain
Control	nil	0 hours	144*	184	100**	416	100
E.1/2	4,000	0 hours	152	171	93	417	100
E.2/2	4,000	3 hours	148	216	117	457	110
E.3/2	4,000	12 hours	143	183	100	423	102
E.4/2	32,000	0 hours	158	162	89	387	93
E.5/2	32,000	3 hours	155	176	96	428	103
E.6/2	32,000	12 hours	147	158	86	378	91

* Weight in grammes.

** Weight gain as % of the value recorded in the non-infected control group.

The effect on the growth rate was less marked in the replicate groups. There was no significant difference in the weight gains between the control group and E.1/2 - E.3/2 after infection on Day 0, although the results suggested that there was some resemblance to those recorded in E.1 - E.3 on day 10. There was evidence of slight retardation of growth in E.4/2 and E.6/2 on both day 10 and day 20. The effect was less marked in group E.5/2, where the growth rate was equal to 96% of that in the control group on day 10, and showed no variation on day 20.

(5) Oocyst Production.

The results (Table 3. 22) showed that the maximum oocyst production occurred in all groups on day 7 and 8. There was a marked decrease in the oocyst output of each group, except E.1, on day 9. There was little variation in the oocyst production between day 9 and day 12 when a further reduction in output occurred in E.2, followed by a similar decrease in E.4 on day 13, and in the remaining groups on day 14 with the exception of E.1 where the decrease occurred on day 17. Very small numbers of oocysts were present in the samples from E.1 - E.5 until day 19 and in E.6 until day 20, when the final samples were examined.

The total oocyst production per bird was 58 million in E.1, being nearly double that recorded in E.2 where production averaged 30 million per bird, compared with 45 million per bird in E.3. Oocyst production was 37 million per bird in E.4, which was approximately half that recorded in E.5 and E.6 of 75 and 70 million per bird respectively.

The oocyst production of the replicate groups (Table 3. 23) was slightly greater in the majority of groups, compared with that from E.1 - E.6. However, the daily output and the total production per bird followed a similar pattern in both E.1 - E.6 and E.1/2 - E.6/2.

(6) Pathology.

Post mortem examination of the birds which died on day 5 revealed typical lesions of acute caecal coccidiosis. There was no variation in the severity of the lesions in the birds from the different groups.

Table 3. 22.

The Average Total Daily Oocyst Production of the Experimental Groups E.1 - E.3 and E.4 - E.6, Expressed in Millions of Oocysts per Bird, After Inoculation with 4,000 and 32,000 Sporulated Oocysts of E. tenella per Chicken Respectively, Food being Withdrawn 0, 3 and 12 Hours Before Inoculation Respectively.

Day	E.1.	E.2.	E.3.	E.4.	E.5.	E.6.
7	24.3	18.0	21.5	20.7	37.4	42.5
8	1.9	4.3	6.3	4.1	15.6	9.3
9	3.6	1.8	3.4	2.1	6.2	2.9
10	3.8	3.6	4.8	2.2	2.5	4.1
11	7.4	1.6	4.0	3.3	3.7	2.6
12	4.1	.2	1.9	1.6	5.2	1.8
13	3.0	.1	2.0	.7	3.0	1.1
14	5.0	.1	.6	.8	.8	2.6
15	2.2	.1	.2	1.0	1.0	.9
16	2.0	*	.1	.3	.4	.5
17	.4	*	*	.1	.1	.4
18	.2	*	*	.3	*	.7
19	*	*	*	.2	*	.1
20	-	*	-	-	-	.1
Total** per Bird	58	30	45	37	75	70

* Indicates that oocyst production was less than 50,000 per bird.

** The total production of oocysts per bird calculated to the nearest million oocysts.

Table 3. 23.

The Average Total Daily Oocyst Production of the Replicate Groups of E.1/2 - E.3/2 and E.4/2 - E.6/2, Expressed in Millions of Oocysts per Chicken, After Inoculation with 4,000 and 32,000 Sporulated Oocysts of *E. tenella* per Chicken Respectively on Day 0, Food being Withdrawn 0, 3 and 12 Hours Respectively Before Inoculation.

Day	E.1/2	E.2/2	E.3/2	E.4/2	E.5/2	E.6/2.
7	52.8	18.3	26.6	31.4	36.9	48.5
8	11.4	11.1	8.8	7.6	15.7	18.1
9	5.7	6.0	5.9	4.8	6.6	4.8
10	2.4	1.5	2.8	.7	1.5	1.2
11	2.1	.7	2.1	1.0	.8	.8
12	3.1	.2	1.3	.6	.9	.7
13	1.5	.1	.3	1.3	.4	.8
14	.8	.2	.4	2.0	.8	.9
15	.4	.8	.2	1.9	.7	.7
16	.3	1.5	.6	.5	.2	2.2
17	.2	.7	1.3	.1	.2	1.1
18	*	.4	.4	.1	.3	.3
19	*	.1	.3	*	.1	.1
20	*	*	.1	.1	.3	*
Total** per Bird	81	42	51	52	64	90

* Indicates that oocyst production was less than 30,000 per bird.

** The total production of oocysts per bird, calculated to the nearest million oocysts.

Table 3. 24.

The Pathogenic Effects of Low and High Levels of Infection of E. tenella in 14 Day Old Chickens When Food was Withdrawn at Different Intervals of Time Before Inoculation.

Group	Dose of Oocysts	Time Food Withdrawn	Haemoglobin Decrease (1)	Haemoglobin Decrease (2)	Mortality (1)	Mortality (2)	% Weight Gain (1)	% Weight Gain (2)	Oocyst Output (1)	Oocyst Output (2)
Control	nil	0 hours	-	-	-	-	100	100**	-	-
E.1 E.1/2	4,000	0 hours	3.1*	3.4	1	-	96	93	58	80***
E.2 E.2/2	4,000	3 hours	-	-	-	-	104	117	30	42
E.3 E.3/2	4,000	12 hours	1.5	1.1	-	-	96	100	45	51
E.4 E.4/2	32,000	0 hours	4.4	5.6	5	4	56	89	37	52
E.5 E.5/2	32,000	3 hours	2.2	2.9	1	3	91	96	75	64
E.6 E.6/2	32,000	12 hours	4.4	5.1	2	2	77	86	70	90

* Haemoglobin expressed as grammes per 100 ml. of blood. (1) and (2) represent the initial and replicate groups respectively.

** The gain in weight expressed as a percentage of the gain recorded in the non-infected control group, calculated on the difference between the values recorded before inoculation on day 0-1 or 0-2 and the value attained on day 10 after infection.

*** The average total oocyst production per bird on day 7 to day 20 inclusive, calculated to the nearest million oocysts.

Table 3. 25

The Mean Haemoglobin Concentration of the Broiler Type Hybrid Groups E.1 and E.3 and the Leghorn Type Hybrid Groups E.2 and E.4, Inoculated with 4,000 and 32,000 Sporulated Oocysts of E. tenella, per Bird Respectively on day 0.

Group	Dose of Oocysts	Breed	Day 0-1	Day 0+6	Haemoglobin Decrease
Control	nil	Broiler Hybrid	9.1 ±0.2	10.2 ±0.5	nil
Control/2	nil	"	9.8 ±0.6	9.7 ±0.6	nil
Control	nil	Leghorn Hybrid	9.6 ±1.1	10.7 ±0.8	nil
Control/2	nil	"	9.8 ±0.6	10.2 ±0.4	nil
E.1.	4,000	Broiler Hybrid	9.5 ±0.5	9.5 ±0.7	nil
E.1/2**	4,000	"	9.3 ±0.6	9.2 ±1.2	nil
E.2.	4,000	Leghorn Hybrid	8.9 ±1.0	7.7 ±1.8	1.2
E.2/2	4,000	"	9.5 ±0.2	7.3 ±1.7	2.2
E.3.	32,000	Broiler Hybrid	8.9 ±0.6	6.7 ±1.5	2.2
E.3/2	32,000	"	9.4 ±0.6	6.5 ±2.0	2.9
E.4.	32,000	Leghorn Hybrid	9.8 ±0.9	5.6 ±1.6	4.2
E.4/2	32,000	"	9.8 ±0.5	5.0 ±1.2	4.8

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

** E.1/2, E.2/2, E.3/2 and E.4/2 represent replicate groups of E.1 - E.4 respectively.

DISCUSSION.

The results (Table 3. 18 and 19) showed no significant variation in the concentration of haemoglobin in the blood of the experimental groups on the day before inoculation with the exception of group E.4 where the mean value was slightly lower than the values recorded in the other groups. However, the individual results of the birds in E.4 were within accepted normal limits. This difference was probably due to chance. The close similarity of the results after infection between group E.4 and the corresponding replicate group E.4/2, in which no difference was recorded in the mean haemoglobin value on day 0-1 indicates that the difference seen in E.4 was not significant.

The changes which were recorded in the concentration of haemoglobin of the experimental groups on day 6 (Tables 3. 18, 19 and 24) followed a similar pattern in each series of birds, E.1 - E.3 and E.1/2 - E.3/2 on the one hand, and E.4 - E.6 and E.4/2 - E.6/2 on the other. When a low dose of 4,000 oocysts per bird was administered, the greatest fall in the level of haemoglobin occurred in groups E.1 and E1/2, in contrast to E.2 and E.2/2 where no change was seen in the concentration of haemoglobin. A small decrease of approximately 1 g. of haemoglobin was recorded in E.3 and E.3/2. These results clearly suggested a definite variation in the pathogenic effect of the parasite in each respective group which was emphasised by the close similarity of the results in the corresponding replicate groups.

The greater pathogenicity of E. tenella in E.1 and E.1/2 was reflected in the clinical findings and by mortality. It was also clearly indicated by the higher total oocyst production of these groups compared with that recorded in the other groups which received 4,000 oocysts per bird (Tables 3. 22 and 23).

The results (Tables 3. 18, 19 and 20) showed a very marked decrease in the level of haemoglobin in both groups E.4 and E.6, the fall being significantly less in group E.6. The changes in the concentration of haemoglobin in the replicate groups were also most pronounced in E.4/2 and E.6/2, where the decrease was slightly greater than that recorded in E.4 and E.6. This may have been associated with a variation in pathogenicity of the culture used to inoculate the replicate groups; this was 11 days old, compared with 100 days in the case of the culture administered to the birds in groups E.1 - E.6.

Mortality (Table 3. 24) was higher in E.4 and E.4/2 than in E.6 and E.6/2, which suggested that the pathogenic effect of the parasite was greater in the former groups, although there was no difference in the haemoglobin concentration in the blood of these groups on day 6. The total oocyst production was significantly less in E.4 and E.4/2 compared with the other groups which had received 32,000 oocysts per bird. This observation also indicated that the pathogenic effects were more severe in E.4 and E.4/2, as earlier investigations had shown that oocyst production decreased when the severity of infection increased following the administration of high doses of oocysts in fully susceptible chickens.

The gain in weight (Tables 3. 20 and 21), recorded on day 10, varied slightly in the birds which had received the low dose of oocysts, being lowest in E.1 and E.1/2 and greatest in E.2 and E.2/2. The effect was slightly more pronounced in the birds which received 32,000 oocysts, the greatest retardation of growth being seen in group E.4 where it was still evident on day 20 when the total weight gain was calculated. The effect on the growth rate was less marked in the replicate groups.

These results (Table 3. 24) clearly indicated that there was a significant relationship between the feeding regime before inoculation and the subsequent pathogenicity of the parasite in 14 day old chickens after administration of either 4,000 or 32,000 sporulated oocysts per bird.

The experiment demonstrated conclusively that the most severe pathogenic effects occur in chickens which were not starved before administration of the infective dose, the variation in pathogenicity being very marked when the chickens received low levels of infection. The pathogenic effects of the parasite were significantly reduced following either dose of oocysts when the birds were starved for three hours prior to inoculation. However, the detrimental effects of the disease were slightly more pronounced after the low dose of oocysts when food was withdrawn for a period of twelve hours before infection, compared with the effect when food was removed for only three hours. In contrast, birds receiving the high dose of oocysts appeared only slightly less severely affected than the birds which were not starved before inoculation.

The high oocyst production recorded in the birds which were allowed free access to food before infection with a low dose of oocysts illustrated the importance of low levels of infection in relation to the epidemiology of the parasite.

These experimental results fail to confirm the observations of Edgar and Herrick (1944) who concluded that birds allowed access to food at all times were more resistant to infection with oocysts of E. tenella, although the effect was less when the infective dose was increased from 50,000 to 200,000 oocysts per bird. Their conclusions were based on the difference in mortality and weight gains between the chickens, aged 3 or 4 weeks on day 0, which had their food withdrawn the evening before infection and similar chickens which had access to food before inoculation. No differences were recorded in the death rate of 14 day old chickens after infection with a high dose of oocysts.

The explanation of the relationship between the feeding regime before inoculation and the subsequent pathogenic effects of E. tenella in chickens is not clearly defined.

Edgar and Herrick (1944) suggest that the time taken for the oocysts and sporozoites to reach the caecum may be significant. Experimental evidence has shown that the caecum is reached in approximately one or three hours when the crop is empty and full of food respectively. These workers also question the importance of the action of the digestive secretions on the parasite and suggest that the oocysts and sporozoites may be adversely affected by a delay in passage through the upper part of the digestive tract.

The motility of the alimentary tract could also be a significant factor contributing to the difference in the pathogenicity of the parasite seen under each feeding regime. The motility of the tract is high in the normal chicken, being responsible for the rapid passage of food down the alimentary canal. Therefore it is possible that the presence of food in the tract slows the passage of the parsite in the intestine, giving a longer period of time for successful infection before the parasite is passed out in the faeces. This factor could explain the lower pathogenicity of a standard infective dose given after a three hour period of starvation due to their quicker passage through the alimentary canal and subsequent loss in the faeces. It is suggested that the motility of the intestine is reduced after a period of twelve hours starvation, which would then explain the increasing pathogenicity observed in chickens subjected to this regime.

EXPERIMENT FIVE.

THE PATHOGENIC EFFECTS OF 4,000 AND 32,000 SPORULATED OOCYSTS OF EIMERIA TENELLA IN 14 DAY OLD BROILER AND LEGHORN TYPE HYBRID CHICKENS.

EXPERIMENTAL AIMS.

The experiment was planned to study the pathogenic effect of *Eimeria tenella* in different breeds of chicken. It was important to establish the significance of this factor in relation to possible variations in the severity of the disease, which might be associated with the breed of chicken and not directly due to differences in the pathogenicity of the culture used for inoculation or the magnitude of the infective dose. The pathogenic effects of low and high levels of infection were compared in two completely different types of bird, selected to represent a typical broiler hybrid, bred for meat production on the one hand, and a typical leghorn hybrid bred for commercial egg production on the other hand.

The severity of the disease in the experimental chickens was determined from observations made on the changes in the concentration of haemoglobin, on mortality, on clinical signs, on growth rate and on oocyst production during the patent phase of the infection.

Earlier work suggested that slight differences in the pathogenicity of *E. tenella* were not indicated clearly by a marked variation in the severity of the lesions in the caeca; therefore birds were not killed for post mortem examination after inoculation.

MATERIALS AND METHODS.

Experimental Birds.

Broiler type hybrid "as hatched" cockerel and pullet chicks (Chunky Chicks, Nicols Ltd.) and Leghorn type hybrid cockerel chicks (Sterling Poultry Products, Ltd.) were used in the experiment. They were reared in complete isolation and were transferred to the experimental unit on the day before inoculation, where they were kept in metal cages with wire floors. A chick mash, prepared from a formula used by Joyner and Davies (1960), was available ad lib. to the chickens.

Parasitology.

The culture of E. tenella used for the infection of the experimental birds on day 0 was 100 days old, with a total oocyst count of 660,000 per ml. and sporulation count of 85%. The culture of E. tenella used to inoculate the replicate groups five weeks later was 11 days old, with a total oocyst count of 628,250 per ml. and sporulation count of 90%. Serial dilutions were made to give the appropriate number of oocysts per ml. for each experimental group, by the addition of distilled water to an aliquot of the culture, which was suspended in 2% potassium dichromate solution.

Administration of Inoculum.

The infecting dose was given in 1 ml. of water to each chicken. It was administered directly into the crop using an automatic dosing syringe. During inoculation the stock of oocysts from which the syringe was filled was kept in suspension by decanting the solution between two beakers.

All the experimental birds were inoculated in the morning. Food was withdrawn from all the experimental birds three hours before infection and was returned to the birds two hours after the birds had been inoculated.

Experimental Design.

Ten birds were selected at random for each experimental group and transferred to the experimental unit the day before inoculation, with the exception of the non-infected control chickens which were left in the isolation unit. The birds were 14 days old on day 0.

Birds of the same age were selected at random from a later consignment of chicks for the replicate groups E.1/2 - E.4/2 and the appropriate non-infected control groups.

The broiler hybrid and leghorn hybrid chickens were placed in groups E.1 and E.3, and E.2 and E.4 respectively.

The experimental groups E.1 and E.2 received a total of 4,000 and E.3 and E.4 a total of 32,000 sporulated oocysts per bird respectively on day 0. Similar numbers of oocysts were given to the birds in each appropriate replicate group five weeks later.

Haemoglobin estimations were made on all experimental birds on the day before inoculation, day 0-1, and after infection on day 6.

The birds of the control and E.1 - E.4 experimental groups were weighed two days before day 0, day 0-2, and after infection on day 10 and day 19. The birds of the replicate groups E.1/2 - E.4/2 and their control groups were weighed on the day before inoculation, day 0-1, and after infection on day 10 and day 20.

The total oocyst production of each group was recorded each day during the patent period of the disease from day 7 to day 20.

Post mortem examination was carried out on day 6 on all the birds which died on day 5. Survivors were killed on day 20.

Clinical signs and mortality were also recorded.

RESULTS.

(1) Haematology.

The results (Tables 3. 25 and 28) of the haemoglobin estimations in the control birds did not show any significant variation in the haemoglobin concentration of these birds during the course of the experiment.

On day 0-1 the results did not indicate any difference between the experimental groups and their corresponding control groups.

On day 6 there was no variation in concentration of haemoglobin in group E.1 or group E.1/2. There was a slight decrease of 1.2g. in the level of haemoglobin in E.2. This was more marked in the replicate group E.2/2 when the decrease was 2.2 g. of haemoglobin. There was a significant fall in the level of haemoglobin of 2.2g. in E.3 and of 4.2g. in E.4. The decrease was slightly greater in the replicate groups, being 2.9g. and 4.8g. respectively.

(2) Mortality.

After inoculation on day 0, deaths occurred on day 5 in E.2 and E.2/2 (20%), E.3 (10%), E.3/2 (30%), E.4 (40%), and E.4/2 (30%). No birds died in groups E.1 or E.1/2 (Table 3. 28).

(3) Clinical Findings.

Clinical symptoms were first evident on day 4 when a little blood was found on the trays from the experimental groups E.3 and E.4.

On day 5 haemorrhage was quite severe in groups E.1 and E.3, being most pronounced in the latter group. The birds in these groups appeared only slightly depressed. Morbidity was very marked in both groups E.2 and E.4 where it was associated with very severe haemorrhage.

On day 6 the birds in group E.1 showed no evidence of morbidity, while those in group E.3 still appeared slightly depressed. Morbidity was marked in groups E.2 and E.4, being very severe in the latter group. Slight evidence of haemorrhage was found in each group except E.1.

On day 7 there was no indication of morbidity in any of the experimental birds. A little blood was seen on the trays of each group. No sign of haemorrhage was found after day 7.

Similar changes were recorded in the replicate groups, with the exception of day 4, when haemorrhage was also seen in group E.2/2.

(4) Growth Rate.

The results (Table 3. 26) showed no significant variation in weight between the non-infected control groups and their respective experimental groups before infection. However, the weight of the broiler hybrids was slightly greater than that of the leghorn hybrid chickens. The weight in each replicate group was slightly less than that of the chickens which were infected initially.

On day 10 the weight gain was slightly less in group E.2 compared with E.1, which was similar to the gain attained by the non-infected control group. However, there was no difference between the growth rate of the replicate groups E.1/2 and E.2/2 and their respective control groups. although the gain in weight was slightly greater in the broiler hybrid birds compared with the leghorn hybrid chickens. On day 19 the total weight gain appeared slightly less in the replicate groups E.2/2 compared with the other groups which also received the low dose of oocysts.

Slight growth retardation was evident in each group of birds on day 10, which had been inoculated with 32,000 oocysts on day 0, compared with the non-infected control groups. This did not appear to be associated with the breed of the experimental birds. On day 19 the total weight gain of the broiler hybrid chickens equalled that attained by the non-infected control group. Retardation of growth was still evident in the leghorn type hybrid chickens, the total weight gain being approximately 10% less than that in the corresponding control groups.

(5) Oocyst Production.

The results (Table 5. 27) showed that the maximum oocyst production occurred in each group between day 7 and 9 after infection. There was a marked decrease in the daily output of each group after day 9 which was followed by a further reduction in output in all groups by day 14. Very small numbers of oocysts were present in the majority of the samples until day 20, when the final samples were collected.

The average total oocyst production per bird was slightly less in the broiler hybrid groups than in the leghorn hybrid groups after infection with 4,000 oocysts per bird, the output being from 30 - 41 and 46 - 64 million oocysts per bird in the broiler and leghorn hybrid groups respectively.

When the infective dose was increased to 32,000 oocysts per bird, the oocyst production was significantly higher in the broiler type hybrid chickens, ranging from 64 - 75 million oocysts, compared with an average total output of 28 - 58 million oocysts per bird in the leghorn hybrid chickens.

There was a marked variation in the oocyst production of the broiler type hybrid chickens which appeared to be related to the different levels of infection. The variation was not apparent in the oocyst output from the leghorn hybrid chickens.

Table 3. 26.

The Mean Weight Gains of the Broiler Type Hybrid Chickens E.1 and E.3 and the Leghorn Type Hybrid Chickens E.2 and E.4, Inoculated with 4,000 and 32,000 Sporulated Oocysts of *E. tenella*, per bird respectively, on Day 0.

Group	Dose of Oocysts	Breed	Weight Day0-2	Gain on Day 10	% Gain	Total Gain on Day 19	% Gain
Control	nil	Broiler Hybrid	153*	237	100	466	100
Control/2	nil	"	144**	184	100	416	100
Control	nil	Leghorn Hybrid	116	160	100	317	100
Control/2	nil	"	113	119	100	296	100
E.1.	4,000	Broiler Hybrid	157	247	104	476	102
E.1/2.	4,000	"	148	216	117	457	110
E.2.	4,000	Leghorn Hybrid	110	147	92	312	98
E.2/2	4,000	"	118	120	101	276	93
E.3.	32,000	Broiler Hybrid	143	125	91	460	99
E.3/2	32,000	"	156	176	96	428	103
E.4.	32,000	Leghorn Hybrid	115	146	91	286	90
E.4/2	32,000	"	123	106	88	256	86

* Weight in grammes.

** The initial weights of the replicate groups were recorded on day 0-1 and the final weight on day 20.

Table 3. 27.

The Average Total Daily Oocyst Production of the Broiler Type Hybrid Chickens, Group E.1 and E.3, and the Leghorn Hybrid Chickens, Group E.2 and E.4, Inoculated with 4,000 and 32,000 Sporulated Oocysts of Eimeria tenella, per Bird respectively, on Day 0.

Day	E.1.	E.1/2	E.2	E.2/2	E.3	E.3/2	E.4	E.4
7	18.0	18.3	26.5	30.0	37.4	35.9	37.1	5.0
8	4.3	11.1	11.8	6.6	15.6	15.7	6.5	7.9
9	1.8	6.0	5.0	5.8	5.2	6.6	4.1	5.6
10	3.6	1.5	2.4	1.3	2.5	1.5	.6	1.6
11	1.6	.7	4.2	1.1	3.7	.8	1.8	1.6
12	.2	.2	2.2	1.1	5.2	.8	2.6	.6
13	.1	.1	.9	1.6	3.0	.4	1.6	1.3
14	.1	.2	.4	.8	.7	1.1	1.1	1.0
15	.1	.8	.2	.4	1.0	.7	.7	2.6
16	*	1.5	.3	.1	.4	.2	.8	.6
17	*	.7	*	.1	.6	.4	.6	2.6
18	*	.4	*	.1	.2	.1	.4	.3
19	*	.1	*	*	*	*	.1	*
20	*	*	-	*	*	*	*	-
Total** per Bird	30	41	54	46	75	64	58	28

* Indicates that oocyst production was less than 50,000 per bird.

** The total production of oocysts per bird calculated to the nearest million oocysts.

Table 3. 28.

The Pathogenic Effects of Low and High Levels of Infection with E. tenella in 74 Broiler Type Hybrid Chickens and Leghorn Hybrid Chickens.

Group	Dose of Oocysts	Breed	Haemoglobin* Decrease	Mortality	Weight** Gain Day 10	%	Oocyst Output***
Control	-	Broiler Hybrid	-	-	237	100	-
Control/2	-	"	-	-	184	100	-
Control	-	Leghorn Hybrid	-	-	160	100	-
Control/2	-	"	-	-	119	100	-
E. 1.	4,000	Broiler Hybrid	-	-	247	104	30
E. 1/2	"	"	-	-	216	117	42
E. 2.	"	Leghorn Hybrid	1.2	2	147	92	54
E. 2/2	"	"	2.2	2	120	101	46
E. 3.	32,000	Broiler Hybrid	2.2	1	215	91	75
E. 3/2	"	"	2.9	3	176	96	64
E. 4.	"	Leghorn Hybrid	4.2	4	146	91	59
E. 4/2	"	"	4.8	3	123	88	28

* Haemoglobin expressed as grammes per 100 ml. of blood. The decrease is calculated on the difference between the values recorded on day 0-1 and day 6.

** The weight gain measured in grammes and representing the difference between the values recorded on day 0-2 and day 10, with the exception of the replicate groups where the initial weight was recorded on day 0-1.

*** The average total oocyst production per bird on day 7 to day 20 inclusive, calculated to the nearest million oocysts.

(6) Pathology.

Post mortem examination of the birds which died on day 6 revealed typical lesions of acute caecal coccidiosis. There was no variation in the severity of the lesions in the birds from the different groups.

DISCUSSION.

The results (Table 3. 25) showed no significant variation in the concentratio of haemoglobin in the blood of the experimental groups before infection on day 0. No difference was demonstrated in the level of haemoglobin between the broiler or leghorn type hybrid birds.

After administration of the low dose of 4,000 oocysts per bird, a decrease occurred in the concentration of haemoglobin in the leghorn hybrid chickens, which was most marked in the replicate group. No change was seen in the level of haemoglobin on day 6 in either of the groups of broiler type hybrid chickens, E.1 and E.1/2. The greater pathogenicity of the parasite in the leghorn hybrid chickens was confirmed by the clinical findings and by mortality (Table 3. 28). The difference was also reflected in the oocyst production during the patent phase of the disease. Following the initial infection of4,000 oocysts per bird, the average total output of oocysts was 30 and 54 million per bird in the broiler and leghorn hybrid groups, E.1 and E.2 respectively. In the replicate groups the variation was less marked, being 42 and 46 million per bird respectively. The lower oocyst production recorded in the leghorn hybrid replicate groups may have been due to an increase in the severity of the lesions in the caeca, as the pathogenic effects of the parasite appeared greater in the replicate group. The weight gains were slightly lower in the leghorn hybrid birds on comparison with the corresponding broiler hybrid group. This suggested a possible depression of the growth rate in the former birds.

although the variation in the results was not significant (Table 3. 26). The results (Table 3. 28) also clearly demonstrated a variation in the pathogenic effects of the parasite in the leghorn and broiler type hybrid chickens following the administration of 32,000 oocysts per bird. A significant decrease was recorded in the concentration of haemoglobin of approximately 4g. and 2g. in the leghorn and broiler hybrid chickens respectively. Morbidity was also greater in the former birds, although there was no significant variation in the number of deaths which occurred in each group on day 6. The growth rate was slightly depressed in each group (Table 3. 28) on day 10. However, this was not reflected in the total weight gain of the broiler type hybrid groups on day 19 in contrast to the less satisfactory weight gain recorded in the corresponding non-infected control group. The greater pathogenicity of the parasite in the leghorn type hybrid chickens was also indicated by the lower oocyst production of these birds (Table 3. 27). Preliminary investigations had shown that oocyst production decreased when the severity of the infection increased following the administration of high doses of oocysts in fully susceptible chickens.

These experimental results demonstrated conclusively that the pathogenic effect of a standard dose of 4,000 or 32,000 sporulated oocysts of E. tenella varied in severity when administered to 14 day old leghorn and broiler type hybrid chickens. The results clearly indicated that the variation in the pathogenicity of the parasite was directly associated with the difference in susceptibility of each breed to infection. The most significant variation between the pathogenic effect of E. tenella in each breed occurred at the low level of infection. The difference was less marked at the high level of infection. However, in each instance the severity of disease was greater in the leghorn type hybrid chickens than in the corresponding group of broiler type hybrid birds.

The pathogenicity of the 10 day old culture appeared slightly greater than that of the 100 day old culture used for the inoculation of groups E.1/2 - E.4/2 and E.1 - E.4 respectively.

EXPERIMENT SIX.

THE INFLUENCE OF THE RATION ON THE PATHOGENIC EFFECTS OF 4,000 AND 32,000 SPORULATED OOCYSTS OF EIMERIA TENELLA IN 14 DAY OLD BROILER AND LEGHORN TYPE HYBRID CHICKENS.

EXPERIMENTAL AIMS.

The experiment was designed to study the influence of the diet on the pathogenic effect of *Eimeria tenella* in 14 day old chickens. It was important to establish the significance of this factor in relation to possible variations in the severity of the disease, which might be associated with the ration fed to the birds and not directly due to differences in the susceptibility of the chicken selected for infection, the magnitude of the infective dose or the pathogenicity of the culture used for inoculation. Two chick rations were chosen for comparison, one representing a simple mixture used in the field on the general farm, and the second prepared from a formula used by Joyner and Davies (1960) in their experimental work with *E. tenella*. The pathogenic effects of low and high levels of infection were compared in broiler and leghorn hybrid chickens which were reared on each ration.

The severity of the disease in each group was determined from observations made on the changes in the concentration of haemoglobin, on mortality, on clinical signs, on growth rate and oocyst production during the patent phase of the infection. Preliminary investigations suggested that slight differences in the pathogenicity of *E. tenella* were not indicated clearly by a marked variation in the severity of the lesions in the caeca; therefore birds were not killed for post mortem examination during the experiment.

MATERIALS AND METHODS.

Experimental Birds.

Broiler hybrid "as hatched" cockerel and pullet chicks (Chunky Chicks, (Nichols Ltd.) and Leghorn hybrid cockerel chicks (White Link Chicks, Sterling Poultry Products Ltd.) were used in the experiment. They were reared in complete isolation and were transferred to the experimental unit on the day before inoculation, where they were kept in metal cages with wire floors. Each consignment of chicks was divided into two groups on arrival and were placed on Ration 1 and Ration 2 respectively. Ration 1 was a chick mash prepared from a formula used by Joyner and Davies (1960). Ration 2 represented a simple mixture used in the field on the general farm. (Appendix). The appropriate chick mash and water were available ad lib. to the chickens during the experiment, except before inoculation when food was withdrawn from all the experimental birds for three hours before infection and returned two hours after administration of the infective dose.

<u>Parasitology.</u>

The culture of E. tenella used for the infection of the experimental birds on day 0 was 11 days old, with a total oocyst count of 528,000 per ml. and sporulation count of 90%. Serial dilutions were made to give the appropriate number of oocysts per ml. for each experimental group, by the addition of distilled water to an aliquot of the culture, which was suspended in 2½% potassium dichromate solution.

<u>Administration of Inoculum.</u>

The infecting dose was given in 1 ml. of water to each chicken. It was administered directly into the crop using an automatic dosing syringe. During inoculation the stock of oocysts from which the syringe was filled was kept in suspension by decanting the solution between two beakers.

All the experimental birds were inoculated in the morning.

<u>Experimental Design.</u>

The birds were selected at random for each experimental group from the broiler and leghorn hybrid chicks, which had been reared on each ration in separate brooders in the isolation unit. The birds were 14 days old on day 0. The broiler and leghorn hybrid chicks were placed in groups E.1, E.3, E.5, E.7 and E.2, E.4, E.6 and E.8 respectively, and transferred to the experimental unit the day before inoculation, day 0-1. The birds in each non-infected control broup were left in the isolation unit.

Ration 1 and Ration 2 were fed to groups E.1, E.2, E.5, E.6, and E.3, E.4, E.7, E.8, and the appropriate control birds respectively.

The experimental groups E.1 - E.4 received a total of 4,000 and E.5 - E.8 a total of 32,000 sporulated oocysts per bird respectively, on day 0.

Haemoglobin estimations were made on all experimental birds on the day before infection, day 0-1, and after inoculation on day 6.

The birds were weighed on the day before inoculation, day 0-1, and after infection on day 10 and day 20.

The total oocyst production of each group was recorded each day during the patent period of the disease from day 7 to day 20.

Post mortem examination was carried out on day 6 on all the birds which died on day 5 and day 6. Survivors were killed on day 20.

Clinical signs and mortality were also recorded.

RESULTS.

(1) Haematology.

The results (Tables 3. 29 and 32) of the haemoglobin estimations in the control birds did not show any significant variation in the haemoglobin concentration of these birds during the course of the experiment.

On day 0-1 the results did not indicate any difference betweenthe experimental groups and their corresponding control groups, with the exception of group E.3 and E.7.

On day 6 there was no variation in the concentration of haemoglobin in group E.1. There was a slight decrease of approximately 2g. of haemoglobin in groups E.2 and E.4. A more marked decrease of 2.7 g. of haemoglobin was recorded in E.3. Haemorrhage was severe in E.5 and E.6 where a fall of 3 and 4g. of haemoglobin was seen in each group respectively. The greatest change occurred in groups E.7 and E.8 where the decrease was 5.7g. and 5.3g. of haemoglobin respectively.

(2) Mortality.

Deaths occurred in E.2 (20%), in E.5, E.6 and E.7 (30%), on day 5 and in E.8 (10%) on day 6 (Table 3.32). No birds died in groups E.1, E.3 or E.4.

(3) Clinical Findings.

Clinical symptoms were first evident on day 4 when a little blood was found on the trays of the experimental groups E.2, E.5, E.6, E.7 and E.8.

On day 5, haemorrhage was quite severe in groups E.1 - E.4, being most marked in E.2 where the birds were depressed, in contrast to the chickens in E.1, E.3 and E.4 which appeared only slightly depressed. Haemorrhage was very severe in E.6, E.7 and E.8 and was associated with high morbidity. Clinical signs were less severe in group E.5.

On day 6 no indication of morbidity was present in E.1 and E.3, while the birds in E.2 and E.4 appeared slightly depressed. Morbidity was still pronounced in E.6, E.7 and E.8. No sign of depression was seen in E.5. Slight haemorrhage was evident in each group.

After day 7 no evidence of haemorrhage or morbidity was seen in any of the experimental groups.

(4) Growth Rate.

The results (Table 3. 30) showed no significant variation in the weight of the non-infected control groups and their respective experimental groups before infection. The weight of the broiler birds was slightly greater than that of the leghorn hybrid chickens. Growth appeared slightly better in the birds on Ration 1, compared with those on Ration 2.

On day 10 and day 20, the weight gain of group E.1 and E.3 was similar to that recorded in the non-infected control groups. The growth rate appeared less satisfactory in E.2 on day 20 and in E.4 on both day 10 and day 20. Slight retardation of growth was seen in E.5 - E.8 on day 10. This was still evident in groups E.6 and E.8 on day 20, in contrast to E.5 and E.7 where the total weight gain was similar to that attained by the non-infected control chickens.

(5) Pathology.

Post mortem examination of the birds which died on day 5 and 6 revealed typical lesions of acute caecal coccidiosis. There was no variation in the severity of the lesions in the birds from the different groups.

Table 3. 29.

The Mean Haemoglobin Concentration of Broiler Type Hybrid and Leghorn Hybrid Chickens Reared on Two Different Rations and Inoculated with 4,000 or 32,000 Sporulated Oocysts of E. tenella per Bird on Day 0.

Group	Dose of Oocysts	Breed	Ration	Day 0-1	Day 0+6	Haemoglobin Decrease
Control	nil	Broiler Hybrid	No. 1	9.8* ±0.6	9.7 ±0.6	nil
Control	nil	Leghorn Hybrid	"	9.8 ±0.6	10.2 ±0.4	nil
Control	nil	Broiler Hybrid	No. 2	10.6 ±0.8	10.4 ±0.5	nil
Control	nil	Leghorn Hybrid	"	9.9 ±0.4	9.9 ±0.5	nil
E. 1.	4,000	Broiler Hybrid	No. 1	9.3 ±0.6	9.2 ±1.2	nil
E. 2.	4,000	Leghorn Hybrid	"	9.5 ±0.2	7.3 ±1.7	2.2
E. 3.	4,000	Broiler Hybrid	No. 2 "	9.6 ±0.7	6.9 ±4.5	2.7
E. 4.	4,000	Leghorn Hybrid	"	10.2 ±0.6	8.3 ±2.3	1.9
E. 5.	32,000	Broiler Hybrid	No. 1	9.4 ±0.6	6.5 ±2.0	2.9
E. 6.	32,000	Leghorn Hybrid	"	9.8 ±0.9	5.6 ±1.5	4.2
E. 7.	32,000	Broiler Hybrid	No. 2	9.4 ±0.9	3.7 ±5.2	5.7
E. 8.	32,000	Leghorn Hybrid	"	10.1 ±0.4	4.8 ±1.7	5.3

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

Table 3. 30.

The Mean Weight Gains of the Broiler Type Hybrid and Leghorn Hybrid Chickens Reared on Two Different Rations and Inoculated with 4,000 or 32,000 Sporulated Oocyte of *E. tenella*, per Bird, on Day 0.

Group	Dose of Oocysts	Breed	Ration	Weight Day0-1	Gain on Day 10	%Gain	Gain on Day 20	%Gain
Control	nil	Broiler Hybrid	No.1	144*	184	100	416	100
Control	nil	Leghorn Hybrid	"	113	119	100	296	100
Control	nil	Broiler Hybrid	No.2	127	153	100	342	100
Control	nil	Leghorn Hybrid	"	111	119	100	277	100
E.1.	4,000	Broiler Hybrid	No.1	148	216	117	457	110
E.2.	4,000	Leghorn Hybrid	"	118	120	101	276	93
E.3.	4,000	Broiler Hybrid	No.2	136	163	107	367	107
E.4.	4,000	Leghorn Hybrid	"	110	105	88	256	92
E.5.	32,000	Broiler Hybrid	No.1	155	176	96	428	103
E.6.	32,000	Leghorn Hybrid	"	123	105	88	255	86
E.7.	32,000	Broiler Hybrid	No.2	136	136	89	366	107
E.8.	32,000	Leghorn Hybrid	"	108	86	72	256	92

* Weight in grammes.

Table 3. 31.

The Average Total Daily Oocyst Production of the Broiler Type Hybrid Groups E.1, E.3, E.5 and E.7, and the Leghorn Type Hybrid Groups E.2, E.4, E.6 and E.8, Reared on Two Different Rations. E.1 - E.4 Inoculated with 4,000 Oocysts and E.5 - E.8 Inoculated with 32,000 Oocysts of E. tenella, per Bird, on Day 0.

Day	E.1.	E.2.	E.3.	E.4.	E.5.	E.6.	E.7.	E.8.
7	18.3	30.0	11.4	12.9	36.9	3.0	22.6	14.4
8	11.1	6.6	3.0	5.4	15.7	7.9	11.4	4.8
9	6. 0	3.8	2.2	4.9	6.6	5.5	5.3	4.8
10	1.6	1.3	3.5	.8	1.5	1.8	.4	1.1
11	.7	1.1	1.0	1.9	.8	1.5	.7	.1
12	.2	1.1	1.1	1.6	.8	.5	1.5	1.1
13	.1	1.6	1.1	.5	.4	1.3	2.2	1.6
14	.2	.4	.5	1.0	.7	1.0	.4	1.6
15	.8	.4	.7	2.8	.7	2.6	.8	.3
16	1.5	.1	.3	2.3	.2	.5	.6	.4
17	.7	.1	.1	.3	.4	2.6	.2	.2
18	.4	.1	.5	.4	.1	.3	*	.1
19	.1	*	1.0	.1	*	*	*	*
20	*	*	.3	.3	*	-	*	.1
Total** per Bird	41	46	27	36	64	28	46	31

* Indicates that oocyst production was less than 50,000 per bird.

** The total production of oocysts per bird, calculated tothe nearest million oocysts.

Note. E.1, E.2, E.5 and E.6 given Ration 1.

E.3, E.4, E.7 and E.8 given Ration 2.

Table 3. 32.

The Pathogenic Effect of Low and High Levels of Infection with *E. tenella* in Broiler Type Hybrid Chickens and Leghorn Hybrid Chickens Reared on Two Different Rations.

Group	Dose of Oocysts	Breed	Ration	Haemoglobin Decrease	Mortality	Weight Gain Day 10	%	Oocyst Output
Control	-	Broiler Hybrid	No.1	-	-	184	100**	-
Control	-	Leghorn Hybrid	"	-	-	119	100	-
Control	-	Broiler Hybrid	No.2	-	-	153	100	-
Control	-	Leghorn Hybrid	"	-	-	119	100	-
E.1.	4,000	Broiler Hybrid	No.1	-	-	216	117	42***
E.2.	"	Leghorn Hybrid	"	2.2*	2	120	101	46
E.3.	"	Broiler Hybrid	No.2	2.7	-	163	107	27
E.4.	"	Leghorn Hybrid	"	1.9	-	106	88	36
E.5.	32,000	Broiler Hybrid	No.1	2.9	3	176	96	64
E.6.	"	Leghorn Hybrid	"	4.2	3	105	88	28
E.7.	"	Broiler Hybrid	No.2	5.7	3	136	89	40
E.8.	"	Leghorn Hybrid	"	5.3	1	86	72	31

* Haemoglobin expressed as grammes per 100 ml. of blood. The decrease represents the difference between the values recorded on day 0-1 and day 6.

** The weight gain measured in grammes and calculated from the values recorded on day 0-1 and day 10. The % gain was calculated on the gain recorded in the appropriate non-infected control group.

*** The average total oocyst production per bird from day 7 to day 20 inclusive, calculated to the nearest million oocysts.

(6) Oocyst Production.

The results (Table 3. 31) showed that the maximum oocyst production occurred in each group on days 7 - 9 after infection. There was a marked reduction in oocyst output in every group except E.3 on day 10. Small numbers of oocysts were present in the majority of samples until day 20, when the final samples were examined.

The average total oocyst production per bird was slightly greater in the birds on Ration 1 compared with that of the chickens on Ration 2, after administration of 4,000 oocysts per bird. There was also a slight variation in the oocyst output of the broiler and leghorn hybrid birds, being greater in the latter breed. This difference was most pronounced in the chickens which were fed on Ration 2.

Following administration of 32,000 oocysts per bird, the oocyst production was also greater in the broiler birds fed on Ration 1, compared with those on Ration 2, but this difference was not reflected in the oocyst output of the leghorn hybrid groups where there was no significant variation between the birds fed on each ration. In contrast to the variation shown in production associated with the breed of the chickens after a low level of infection, the oocyst output appeared significantly greater in the broiler groups compared with the leghorn hybrid chickens at the high level of infection.

DISCUSSION.

The results (Table 3. 29) of the haemoglobin estimation on the day before inoculation demonstrated a slight difference in the mean value of the haemoglobin concentration between the control group and the corresponding experimental groups E.3 and E.7. However, this difference could be explained by the variation which occurs in the value of the haemoglobin concentration in normal chickens. The individual results were within accepted normal limits.

The results (Table 3. 32) clearly indicated a variation in the pathogenic effects of Eimeria tenella between the experimental groups reared on Ration 1 and Ration 2 respectively.

After administration of 4,000 oocysts per bird, there was a decrease in the concentration of haemoglobin in only the leghorn hybrid birds on Ration 1, in contrast to the chickens on Ration 2 where a fall occurred in the level of haemoglobin in both the broiler and the leghorn hybrid groups. The clinical findings and the mortality (Table 3. 32) indicated that the pathogenic effects of the parasite were greater in the leghorn hybrid birds on Ration 1, although the decrease in the level of haemoglobin was similar in each group, E.2 and E.4. The difference was also reflected in the growth rate and the total oocyst production. Detrimental effects were slight in the corresponding broiler hybrid birds reared on Ration 1, the most significant evidence of disease being the high total oocyst production of 42 million oocysts per bird. However, the most pronounced decrease in the level of haemoglobin was recorded in the broiler hybrid chickens reared on Ration 2. The severity of the infection in these birds was emphasised by the clinical findings and by the lower oocyst production. These results clearly indicated that the broiler hybrid chickens which had been reared on Ration 2 were more susceptible to infection with E. tenella than those which had been reared on Ration 1. The pathogenic effect of the low level of infection also appeared greater in the broiler hybrid group compared with that in the leghorn hybrid group which was reared on Ration 2, although the growth rate was less satisfactory in the latter group. The total oocyst production was higher in the birds of each group which were reared on Ration 1.

Following the administration of 32,000 oocysts per bird, the severest haemorrhage occurred in the chickens which were reared on Ration 2, being slightly greater in the broiler hybrid group E.7 than in the corresponding leghorn hybrid group E.8. The changes in the level of haemoglobin were significantly less in the chickens on Ration 1, the decrease being most marked in the leghorn hybrid group E.6. Morbidity was high in only the leghorn hybrid birds on Ration 1, in contrast to the birds on Ration 2 where it was marked in both groups E.7 and E.8. However, mortality was similar in each group of birds on Ration 1 and in the broiler hybrid chickens on Ration 2, being lower in the leghorn hybrid group E.8. These results indicated that the pathogenic effects of the parasite were more marked in the birds on Ration 2, the significance of the lower mortality in the leghorn hybrid group on this ration being doubtful. The weight gains were also less satisfactory on day 10 in these chickens, being lowest in the leghorn hybrid group E.8.

There was a marked difference in the total oocyst production of the broiler hybrid chickens. This emphasised the severity of the infection in the group which were reared on Ration 2, in contrast to the corresponding group on Ration 1 in which the pathogenic effects of the parasite were less pronounced. No significant variation in susceptibility to infection was demonstrated between the birds on Ration 2, although the results of the haemoglobin estimation appeared to suggest that infection was more severe in the broiler hybrid group E.7. There was a marked difference between the birds which were reared on Ration 1. The clinical findings and the reduction in the level of haemoglobin clearly indicated that the pathogenicity of the parasite was greater in the leghorn hybrid chickens compared with that in the corresponding broiler chickens of E.5. The difference was also reflected in the total oocyst production of the birds, the lower output of the leghorn hybrid chickens being associated with severe lesions in the caeca.

These observations demonstrated conclusively that the pathogenic effects of a standard dose of oocysts of Eimeria tenella were influenced by the ration. The experimental results also indicated that the influence of the ration on the pathogenicity of the parasite varied significantly in the broiler and leghorn hybrid chickens.

The variation in the pathogenic effects of E. tenella in chickens reared on different rations has been recognised by several workers. Early observations suggested that a beneficial effect could be attributed to the use of milk products in the diet as a means of limiting the mortality from coccidiosis in the field (Fatham 1915; Beach and O'Orl 1925; Beach and Davies 1925). However, later findings were considered to demonstrate that the pathogenicity of the parasite was enhanced when dried buttermilk was included in the ration (Becker and Water 1938, 1939; Becker and Wilke 1938). Other factors believed to be useful in alleviating the disease include rations with a high protein and Vitamin A and B content (Allen 1932); reduced mortality has also been recorded in birds reared on a ration with a high carbohydrate content and low protein and fibre levels (Mann 1947). Recent experiments have confirmed the beneficial effect of Vitamin A in the ration, showing a significant decrease in mortality and higher weight gains when supplementary Vitamin A was included in the ration or in the drinking water (Gerriots 1961). Observations also indicated that carotene was less efficient in promoting growth than Vitamin A in both infected and control birds (Erasmus, Scott and Levine 1960). The detrimental effects of E. tenella have also been shown to be enhanced when the diet is low in Vitamin K. The experimental studies suggest that the higher mortality recorded in this instance is associated with severe haemorrhage, resulting from the prolonged clotting and prothrombin times seen in chickens deficient in Vitamin K (Harmer and Tugwell 1956; Tugwell, Stephens and Harmer 1957; and Otto, Jerko, Frost and Perdue 1958).

Analysis of each diet in the present experiment indicated that Ration 1 was very low in Vitamin K and contained lower levels of Vitamin A, E and D_3 than Ration 2, although of these Vitamin A was probably only significantly less, being 1,000 i.u. per lb. greater in the latter diet. However, the production energy was higher in Ration 1, being 861 calories per lb. compared with 811 calories per lb. in Ration 2. The fibre content was also slightly lower in the former diet. No marked difference was present in the levels of protein, oil or minerals in each ration.

The experimental results clearly demonstrated evidence of more severe haemorrhage in the chickens given Ration 2, which leads to the conclusion that factors other than the level of Vitamin K must be significant in relation to the degree of haemorrhage which occurs during the disease after a standard dose of oocysts of E. tenella. The analysis of each ration also suggests that the factors concerned may not be necessarily related to Vitamin A, since this was significantly higher in Ration 2, or protein levels, two dietetic factors mentioned in earlier studies.

The importance of the differences in the productive energy and fibre content of each ration is not clear, although the variation in energy levels was reflected in the growth rate, being most pronounced in the weight gains of the broiler type hybrid control chickens. The significance of these factors in relation to susceptibility to disease has been indicated by the experimental studies of Mann (1945 and 1947).

The variation in susceptibility to infection demonstrated in each type of chicken on the different diets may be correlated with the individual nutritional requirement of each breed of bird.

Table 3. 33.

RATION 1.

Maize	28 lb.
Barley	25.2 lb.
Wheat feed	33.6 lb.
Decort. Ground nut meal	2.8 lb.
Soya bean meal	8.4 lb.
Meat meal	5.6 lb.
White fish meal	5.6 lb.
Lime	1 lb/cwt.
$CaPO_4$	1 lb/cwt.
Vitamealo Superconcentrate Special	0.25 lb/cwt.

RATION 2.

Maize	22.4 lb.
Barley	22.4 lb.
Wheat	22.4 lb.
Fine Middlings	22.4 lb.
Vitamealo Chick con	22.4 lb.

Calculated Analysis.

	Protein	Oil	Fibre	Productive Energy	CaO	P_2O_5
No. 1	19%	3.8%	3.7%	861 cal/lb.	1.7%	1.5%
No. 2	19.8%	4.0%	4.5%	811 cal/lb.	1.9%	1.[illegible]%

	Vitamin A	Vitamin K	Vitamin D_3	Riboflavin	B_{12}
No. 1	2566 i.u/lb.	nil	654 i.u/lb.	3.08mgm/lb.	.006mgm/lb.
No. 2	3571 i.u/lb.		893 i.u/lb.	4.4 mgm/lb.	3.36 mgm/lb.

	Manganese	Iron	Copper	Cobalt	Iodine
No. 1	50g./ton	20g./ton	2g./ton	1g./ton	2g./ton
No. 2	50g./ton	20g./ton	30g./ton	5g./ton	10g./ton

SUMMARY OF PART I.

SUMMARY OF PART ONE.

A series of six experiments were performed to investigate the pathogenic effects of the parasite Eimeria tenella in the domestic fowl. The initial experiments were designed to establish criteria for the evaluation of the severity of the disease. Replicate groups were included in these experiments to ensure that the disease could be reproduced with consistent pathogenicity by the administration of a standard dose of sporulated oocysts. The relationship between the size of the infecting dose and the subsequent pathogenic effects of the infection was studied, together with the degree of resistance conferred by a single infection against reinfection with a standard challenge dose of sporulated oocysts. Observations were also made to determine the significance of the age, breed, diet and feeding habits of the chickens on the pathogenicity of the parasite.

The findings of experiments 1, 2 and 3 clearly demonstrated that several aspects of the disease should be considered in the accurate assessment of the pathogenic effects of the parasite. The severity of an infection can be determined satisfactorily by consideration of the haemoglobin concentration, the clinical signs and the mortality during the acute phase of the disease, together with the results of the weight gains, the post mortem findings and the total oocyst production of the chickens during the patent period of the infection. The relative importance of each criterion was shown by the experimental observations.

The pathogenic effects of the parasite are directly associated with the development of the second stage schizont when the migration of the parasitised cells leads to a marked disorganisation of the tissues in the submucosa. This culminates in the erosion of the mucosa and severe haemorrhage on the fifth day after infection. Therefore, estimations were made of the changes in the haemoglobin concentration of the blood during the course of the disease and the results related to the clinical signs, the mortality and the post mortem findings. The results showed that no detrimental effects were associated with the daily collection of blood samples. They also demonstrated that the maximum decrease in the concentration of haemoglobin could be measured from the difference in the values of an estimation made between day 0-1 and day 4 and one made on day 6, after both the initial and challenge infections respectively. Following the administration of doses ranging from 1,000 to 500,000 sporulated oocysts per bird in 4 week old chickens, the pathogenic effects of 4,000 to 8,000, 16,000 and 32,000 to 256,000 oocysts were illustrated by a decrease of 2g., 3g. and 4. - 5g. of haemoglobin respectively. No significant difference was recorded in the decrease of haemoglobin between doses ranging from 32,000 to 256,000 oocysts per bird, although the maximum dose of 500,000 oocysts was followed by the most pronounced fall of 5.5g. of haemoglobin. The value of this parameter as one criterion for the determination of the pathogenicity of E. tenella was emphasised by the close similarity of the results in the replicate groups. The consistent changes in the haemoglobin concentration following administration of the same dose of oocysts were in complete contrast to the observations made on the death rate after infection when there was a wide variation in the number of birds which died in the corresponding groups of infected chickens. The results of the haemoglobin estimation in the individual birds failed to show any direct relationship between mortality and the initial haemoglobin level or the subsequent decrease after infection. There was also no difference in mortality between birds which had haemoglobin levels at either limit of the range of values recorded before the effects of haemorrhage became apparent. Similarly, the actual decrease in the haemoglobin did not indicate any definite difference between survivors and the birds which died, as some of the lowest values were recorded among survivors; some of the blood samples were taken immediately before death. It was not possible to establish a level of dosage with oocysts which would consistently lead to a death rate of 50% after inoculation. Sporadic deaths occurred after infection with doses ranging from 4,000 to 16,000 oocysts per bird. Levels of infection ranging from 32,000 to 500,000 oocysts per bird were followed by a death rate of 10% to 70%. There was no direct relationship between the size of the infective dose and the number of birds which died during the acute phase of the disease. Similarly, there was little correlation between mortality and the clinical signs; birds died suddenly while others, which appeared equally depressed, survived. Therefore, it is concluded that mortality alone is of very doubtful significance as a reliable parameter for the evaluation of differences in the pathogenic effect of the parasite.

There was a definite association between the level of infection and the incidence and severity of the clinical signs of disease, the degree and duration of the haemorrhage and morbidity increasing as the birds received larger doses of oocysts. Evidence of haemorrhage was recorded in the absence of any change in the level of haemoglobin on the fifth day after infection with a relatively low number of oocysts. Signs of morbidity were usually absent if the degree of haemorrhage was slight. Anorexia and depression were present on day 3 or day 4 after infection, when a decrease was recorded in the concentration of the haemoglobin during the acute phase of the disease. Following the administration of a very high dose of oocysts, the marked pathogenicity of the infection was demonstrated both by the earlier onset of haemorrhage seen on day 3 or day 4 and by the longer duration of haemorrhage recorded in the survivors. The severity of the haemorrhage was also reflected in the very marked increase in the water consumption of the survivors on day 5 and day 6, together with evidence of pronounced morbidity. Clinical symptoms were rarely apparent after day 8, although the adverse effects of the parsite were illustrated by the poor weight gains of the more severely affected birds after the acute phase of the disease. The variation in the weight gains of certain groups of birds indicated a significant difference between the pathogenicity of the appropriate levels of infection. These differences were not shown by the estimation of haemoglobin on day 6 which emphasised the importance of observations made on the weight gains of the chickens after inoculation. Therefore the value was confirmed of both the clinical findings and the weight gains as criteria for use in the determination of the pathogenic effects of E. tenella.

The results of serial post mortem examinations showed a definite relationship between the level of infection and the severity and duration of lesions in the caeca. There was close agreement between the clinical observations and the post mortem findings during the acute phase of the disease. Post mortem observations emphasised the differences indicated by the other criteria and also suggested further variations between the pathogenicity of certain levels of infection, confirming the value of this diagnostic procedure as another criterion in the evaluation of the severity of the disease. The importance of post mortem examination was also demonstrated in the determination of the minimum interval of time between inoculation and reinfection with a challenge dose of oocysts. The persistent lesions found in the caeca of some of the birds after the administration of a high dose of oocysts showed that reinfection was contraindicated for at least a fortnight to avoid an incorrect assessment of the immunity to E. tenella.

These results confirmed the earlier findings of Waletzky and Hughes (1949).

The total oocyst counts made after the administration of doses ranging from 1,000 to 500,000 oocysts per bird in 4 week old chickens indicated a correlation between the total oocyst production of the chickens during the patent period of the disease and the level of infection. The results showed that oocyst production was relatively high even after low doses of oocysts. The total number of oocysts increased significantly after quite a severe infection, while it decreased markedly when the birds received a very high dose of oocysts. The daily oocyst counts showed a wide variation from day to day which was not associated with the level of infection, emphasising the importance of total oocyst counts throughout the patent period in contrast to counts made only on selected days. These results illustrated the significance of the total oocyst production as a useful criterion to confirm variations in the severity of the infection suggested by other observations. However, it is of limited value alone. The total oocyst production of the birds is of particular importance after either the administration of a very low dose of oocysts or following reinfection of resistant birds to establish both the presence and degree of infection as in those circumstances no other evidence of infection may be seen.

These observations demonstrate conclusively that the pathogenic effects of Eimeria tenella cannot be assessed satisfactorily from the consideration of only one aspect of the infection.

The close similarity of the detrimental effects of the parasite recorded between the infected chickens and the corresponding replicate groups confirmed that the disease could be reproduced with consistent pathogenicity by the administration of a standard dose of sporulated oocysts. This was emphasised most clearly when the results of the haemoglobin estimations were compared between appropriate groups which received the same dose of oocysts.

The experimental observations indicate a definite relationship between the age of the bird, the number of oocysts contained in the inoculum and the severity of the subsequent disease. Slight differences were noted in the pathogenicity of the doses of oocysts ranging from 1,000 to 64,000 oocysts per bird between appropriate groups of chickens aged 2, 4 and 6 weeks old. The variation became less as the dose of oocysts increased until it became negligible at high levels of infection. The observations show that the pathogenic effect of comparatively low doses of oocysts were most pronounced in the youngest chickens. This could be attributed to the smaller size of the bird rather than to any specific age resistance to the parasite in the older birds. It would appear reasonable to suggest that the degree of haemorrhage and tissue damage resulting from a specified dose of oocysts might constitute an overwhelming infection for a young bird while proving non-fatal for an older bird. This explanation is supported by the higher reproductive potential of the parasite recorded in the older birds after the administration of a standard infection of 4,000 or 32,000 oocysts to 2 and 4 week old chickens.

Studies made on the pathogenicity of a standard dose of 128,000 oocysts per bird failed to demonstrate any significant difference in the susceptibility to infection between chickens aged, 3, 4, 5 and 6 weeks old which could be directly attributed to the age of the birds. The highest death rate was recorded in 4 and 5 week old chickens during the acute phase of the disease. The lesion score bore some resemblance to the mortality but the results of the haemoglobin estimations failed to confirm any definite variation between each group of birds. However, the retardation of growth was less severe in 4 week old birds than in birds aged 6 weeks old after administration of doses of oocysts ranging from 16,000 to 256,000 per bird. This observation was in agreement with the results of Edgar (1955) who found that retardation of growth increased in severity with the age of the chicken between two and six weeks of age.

Quite marked differences were recorded in the severity of the disease after inoculation with doses of oocysts ranging from 1,000 to 500,000 oocysts in four week old chickens. The detrimental effects of relatively small doses of 1,000 to 2,000 oocysts per bird were illustrated by the slightly less satisfactory weight gains attained by these chickens compared with the non-infected control chickens and by the high total oocyst production of the birds during the patent period of the disease. The pathogenic effects of the parasite became a little more pronounced when the dose was raised to 4,000 or 8,000 oocysts per bird. The first significant decrease in the level of haemoglobin occurred at these levels of infection, although no sign of morbidity was seen in the birds during the acute phase of the disease. There was a marked difference in the severity of infection with these doses of oocysts and one of 16,000 oocysts per bird, which was shown both by the greater decrease in the concentration of haemoglobin recorded on day 6 and by the mortality which ranged from 10% to 40%. The higher pathogenicity of the latter dose of 16,000 oocysts was also reflected in the total oocyst production by an increase from approximately 100 to 150 million oocysts per bird. There was a significant increase in the deleterious effects of the parasite when the dose was raised to 32,000 oocysts. This was demonstrated by the results of the haemoglobin estimation on day 6 and by the death rate of 10% to 70%. However, as the level of infection was raised from 32,000 to 256,000 oocysts per bird, no further decrease occurred in the concentration of haemoglobin in the blood and there was no significant difference in mortality between birds given doses of oocysts ranging from 32,000 to 500,000 per bird, the severity of the disease being shown by the higher morbidity, the poor mean weight gains and the marked fall in oocyst production. No definite variation was demonstrated between the pathogenic effects of an infection with 32,000 or 64,000 oocysts per bird, although morbidity appeared greater in the group which received 64,000 oocysts. The pathogenicity of the parasite was significantly higher when the inoculum contained either 128,000 or 256,000 oocysts per bird, no difference being indicated between these levels of infection. The detrimental effects were illustrated by the high morbidity observed during the acute phase of the disease and by the marked decrease in the mean weight gains of the chickens which was far more pronounced than that recorded in the group given 64,000 oocysts per bird. There was also a marked fall in the total oocyst production of these chickens, which fell from approximately 160 to 90 million oocysts per bird.

Observations on the growth rate and on the concentration of haemoglobin indicated a marked difference in the pathogenicity of 256,000 and 500,000 oocysts per bird. The lowest mean value of haemoglobin in the blood and the most severe retardation of growth were recorded in the chickens which received 500,000 oocysts. The highest morbidity was also seen in those birds, while the recovery period appeared very protracted after the acute phase of the infection.

There was little difference between the oocyst production of chickens infected with 1,000 to 8,000 oocysts per bird on the one hand, and birds receiving 128,000 to 500,000 oocysts per bird on the other. It would appear reasonable to suggest that the comparatively low oocyst production recorded in the latter birds was directly related to the greater pathogenicity of the higher levels of infection as the oocyst production was significantly greater in birds given doses of oocysts ranging from 16,000 to 64,000 oocysts per bird in which the detrimental effects of the parasite were less severe. It is possible that the tremendous disorganisation of the mucosa and the severe haemorrhage associated with schizogony could interfere with oocyst production in two ways, (i) due to a severe decrease in the number of epithelial cells after widespread erosion of the mucosa, and (ii) a loss of second generation merozoites when haemorrhage is severe. Another significant factor could be the "crowding effect" described by Brackett and Bliznick (1952).

The fall in the reproductive potential of E. tenella as the infective dose increased was shown clearly by the comparison of the infective dose and the total oocyst production per bird, giving the number of oocysts produced per oocyst ingested. This was in agreement with the observations of Brackett and Bliznick (1952).

Following the administration of doses ranging from 4,000 to 256,000 oocysts per bird, a marked variation was recorded in the pathogenic effects of 32,000 and 64,000 oocysts between corresponding groups of four and six week old chickens. The detrimental effects of these levels of infection appeared similar in the younger birds, in contrast to the variation in the pathogenic effect of these doses of oocysts in the older chickens. The variation was suggested by the results of the haemoglobin estimation on day 6 and it was confirmed by the clinical findings and by the marked difference in the mean weight gains of the six week old chickens recorded after the acute phase of the disease. The pathogenicity of the other levels of infection appeared very similar in the corresponding groups of four and six week old birds.

The findings of Experiment 4 indicated that there was a significant relationship between the feeding regime before inoculation and the subsequent pathogenicity of the parasite in 14 day old chickens after administration of either 4,000 or 32,000 oocysts per bird.

The results demonstrated conclusively that the most severe pathogenic effects occur in chickens which were not starved before administration of the infective dose, the variation in pathogenicity being very marked when the chickens received low doses of oocysts. The pathogenic effects of the parasite were significantly reduced following either dose of oocysts if the birds were starved for three hours prior to inoculation. However, the detrimental effects of the disease were slightly more pronounced after the low dose of 4,000 oocysts when the food was withdrawn for a period of twelve hours before infection, compared with the effect when food was removed for only three hours; in contrast, birds receiving the high dose of 32,000 oocysts appeared only slightly less severely affected than the birds which were not starved before inoculation. The higher pathogenicity of the parasite was demonstrated by the results of the haemoglobin estimations and by the death rate during the acute phase of the disease when the food was not withdrawn from the birds before infection. These differences were confirmed by observations made on the total daily oocyst production of the chickens during the patent period of the disease. Following administration of the low dose of oocysts the total oocyst output of the birds allowed free access to food was approximately double that recorded in the birds which were starved for three hours before inoculation. This emphasised the significance of low levels of infection in relation to the epidemiology of the disease.

The results of Experiment 6 demonstrated conclusively that the pathogenic effect of a standard dose of 4,000 or 32,000 sporulated oocysts of E. tenella varied in severity when administered to 14 day old leghorn and broiler type hybrid chickens. The findings indicated that the variation in the pathogenicity of the parasite was directly associated with the difference in the susceptibility of each breed to infection. The most significant variation between the pathogenic effect of E. tenella in each breed occurred at the low level of infection, the difference being less marked at the high level of infection.

However, in each instance the severity of the disease was greater in the leghorn type hybrid chickens than in the corresponding group of broiler type hybrid birds. The greater pathogenicity of the parasite in the leghorn hybrid chickens was demonstrated by the results of the haemoglobin estimations on day 6, the clinical findings and the total oocyst production during the patent phase of the disease. It was also reflected by the death of two leghorn hybrid chickens in each appropriate group after infection with the low dose of oocysts, no deaths being recorded in the corresponding groups of broiler hybrid chickens.

The findings of Experiment 6 demonstrated conclusively that the pathogenic effects of a standard dose of oocysts of Eimeria tenella were influenced by the ration. The results also indicated that the influence of the ration on the pathogenicity of the parasite varied significantly in the broiler and leghorn hybrid chickens.

The variation in susceptibility to infection demonstrated in each type of chicken on the different diets may be correlated with the individual nutritional requirements of each breed of bird.

The influence of the diet on the pathogenicity of the parasite was evident at both low and high levels of infection. The variation in the pathogenic effects of the parasite in the birds reared on different diets was shown by the clinical findings, the death rate and the results of the haemoglobin estimations. Small differences were recorded between the mean weight gains of the birds, although the importance of these results was doubtful due to the variation in the productive energy levels of each ration. There was a marked difference in oocyst production of the chickens which suggested that the diet could be a significant factor in relation to the epidemiology of the disease.

Analysis of each diet indicated differences in the levels of Vitamin A, B, D_3 and E, the productive energy level and the fibre content. No marked difference was present in the levels of protein, oil or minerals of the rations.

The results demonstrated evidence of more severe haemorrhage in chickens given a ration believed to contain adequate levels of Vitamin K. This suggests that factors other than the level of Vitamin K must be significant in relation to the degree of haemorrhage which occurs during the acute phase of the disease after inoculation with a standard dose of oocysts of E. tenella. The analysis also indicates that the factors concerned may not necessarily be related to Vitamin A, since this was significantly higher in the diet on which the maximum decrease of haemoglobin occurred on day 6.

The importance of the differences in the productive energy and fibre content of each ration is not clear, although the variation in energy levels was reflected in the growth rate, being most pronounced in the weight gains of the non-infected broiler hybrid birds.

The protein levels were similar in each ration so that protein would not appear to be a significant factor in the present study.

Observations were also made on the level of immunity conferred by a single infection against reinfection with a heavy challenge dose of oocysts. The results indicated that a significant degree of resistance was conferred by doses ranging from 1,000 to 500,000 oocysts per bird against a standard challenge dose of 64,000 oocysts given 21 days after the initial infection. However, the birds inoculated with a dose of 1,000 to 4,000 oocysts on day 0 appeared slightly susceptible to reinfection. This was reflected by the changes in the concentration of haemoglobin after challenge and by the presence of slight lesions in the caeca of the birds killed for examination from the group vaccinated with 4,000 oocysts. No significant detrimental effects were seen after the challenge infection in the chickens inoculated with doses ranging from 8,000 to 500,000 oocysts on day 0, although slight evidence of haemorrhage in certain groups indicated that these birds were not totally resistant to reinfection.

The results suggested that the most successful immunising dose of oocysts from the series investigated was a dose of 8,000 oocysts per bird. This level of infection did not cause mortality or severe morbidity after inoculation and was not associated with any signs of reinfection after challenge. However, the high oocyst production and the retardation of growth which were recorded in this group after inoculation demonstrated conclusively the disadvantages associated with vaccination with low numbers of normal oocysts. The high total oocyst production of the chickens after inoculation is of particular significance with reference to the epidemiology of the disease under intensive methods of poultry management in the field, while the less satisfactory weight gains could be important when the chickens are graded for marketing.

SECTION 4

EXPERIMENTAL WORK PART 2

STUDIES ON THE EFFECT OF X-IRRADIATION ON THE PATHOGENICITY AND IMMUNOGENIC POTENTIAL OF SPORULATED OOCYSTS OF THE PROTOZOAN PARASITE EIMERIA TENELLA IN THE DOMESTIC FOWL.

INTRODUCTION.

Early immunological studies in the rat demonstrated that resistance was conferred by infection with irradiated larva of the helminth parasite Trichinella spiralis. No detrimental effects were associated with immunisation, the irradiated larvae developing into sterile adults, giving an intestinal infection without any subsequent muscle invasion by the parasite (Levin and Evans, 1942). These observations were confirmed by Gould and Goberg (1958). It was suggested by Levin and Evans (1942) that an irradiated larval vaccine might have practical implications in the control of the disease.

Subsequent experimental work has shown that certain parasitic helminth infectionscan be controlled successfully by the administration of an irradiated larval vaccine. Satisfactory immunisation has been achieved against Dictyocaulus viviparus in cattle (Jarret et al., 1958a, 1958b, 1959a, 1959b, 1960b and 1961a), Haemonchus contortus in sheep (Jarret et al., 1959c, 1961b, Trichostrongylus colubriformis in sheep (Jarret et al., 1960d; Gordon et al., 1960; Mulligan et al., in press), Uncinaria stenocephala in the dog (Dow et al., 1959, 1961), Ancylostoma caninum in the dog (Miller, T., in preparation), Ascaris lumbricoides in the guinea pig (Dow et al., unpublished) and Cysticercus fasciolaris in the mouse (Dow et al., 1962). This work has been reviewed fully by Urquhart, Jarret and Mulligan (1962).

The initiation of the present investigations on the effects of X-irradiation on the protozoan parasite Eimeria tenella was directly influenced by the promising results in the field of the irradiated larval vaccine against infection with Dictyocaulus vivparcus.

The first observations recorded on the effects of X-irradiation on the pathogenicity of E. tenella were made by Albanese and Smetana (1937).

Chickens aged 2 to 3 weeks old were inoculated with approximately 15,000 to 20,000 oocysts which had been exposed to Rontgen doses ranging from 300R to 27,000R. The results indicated that a minimum exposure of 9,000R was necessary to prevent mortality. Studies were also made on the oocyst production of chickens after infection with doses ranging from 125 to 365 irradiated oocysts per bird. The results suggested that the pre-patent period of the infection was increased from six to seven days,when the oocysts had been exposed to 4,500R to 13,500R, while the duration of the patent period decreased progressively as the dose of X-irradiation increased from 4,500R to 13,500R. The total oocyst production decreased significantly when the inoculum had been exposed to 6,750R, becoming negligible at 13,500R. Comparison between the effect of high and low intensity X-irradiation on the reproductive potential of the parasite suggested that oocyst production was reduced to a greater degree by the high intensity X-irradiation. The experimental findings demonstrated that sporulation was significantly reduced when oocysts were exposed to 4,500R before incubation. The results indicated that unsporulated oocysts were more susceptible to X-irradiation compared with sporulated oocysts. Observations on the excystation of sporozoites "in vitrio" showed that excystation was 32% and 18% for normal and irradiated oocysts respectively. Albanese and Smetana considered that irradiation must produce some effect other than a simple reduction in the size of the infective dose of oocysts. Following inoculation with oocysts exposed to 3,120R, the oocyst production was lower than the estimated value suggested by the results of the excystation experiment. These findings were interpreated as evidence in support of the hypothesis that the effects of X-irradiation on the parasite were manifest after a latent period. They suggested that although some sporozoites excyst they may fail to complete their life cycle, development being inhibited subsequently during either schizogony or gametogony.

These workers concluded that the reproductive potential of the parasite was enhanced when sporulated oocysts were exposed to 2,250R. However, the number of birds observed in each group was small. It was possible that the difference in the oocyst production between the appropriate groups was due, either to the accepted variation which can occur between two groups of chickens given the same dose of oocysts, or to the variation in oocyst production between individual birds. It was also concluded that there was no decrease in the reproductive potential of the parasite following exposure to 4,500R. The results did not show any difference between the reproductive potential of the parasite following administration of 365 and 125 normal and irradiated oocysts respectively. This could indicate that the total oocyst production was reduced after exposure to 4,500R, since it would be anticipated that the reproductive potential should have been greater after the lower dose of oocysts, if the observations made on the reproductive potential of the parasite following inoculation with doses ranging from 1,000 to 8,000 oocysts remain true for very low levels of infection. It was possible, however, that the difference in the number of oocysts contained in the inoculum for each group was not significant, due to the inherent inaccuracies of oocyst counting techniques.

Further studies by Waxler (1941) confirmed that the pathogenicity of the parasite was significantly reduced after exposure to X-irradiation. No mortality occurred in 35 day old chickens after infection with a standard dose of 200,000 oocysts exposed to either 4,500R, 9,000R or 13,000R before inoculation. However, the results of the haemoglobin estimation on day 7 demonstrated a decrease of approximately 5g., 2g., and 1g., of haemoglobin in each group respectively. Following administration of oocysts exposed to 9,000R, mortality was approximately 4% in 5 and 24 day old chicks, in contrast to a death rate of 50% in corresponding birds given a similar dose of normal oocysts. No deaths occurred when 5 day old chicks were infected with oocysts exposed to 18,500R.

Waxler demonstrated that immunity was conferred by infection with irradiated oocysts against reinfection with a high challenge dose of normal oocysts. Thirty-five day old chicks inoculated with 200,000 oocysts exposed to 9,000R were reinfected with a similar dose of normal oocysts 35 days after the initial infection. No deaths occurred in these birds, although a slight decrease of 2.5g. and 1.5g. of haemoglobin was recorded on the seventh day after the immunising and challenge infections respectively. The pathogenicity of the oocysts before exposure to X-irradiation was confirmed in fully susceptible chickens by the death rate of 50% and the marked decrease of 3g. to 4g. in the level of haemoglobin during the acute phase of the disease.

The second series of experiments, therefore, was designed to study the effects of X-irradiation on Eimeria tenella, with special reference to (i) the pathogenicity of the parasite and (ii) the resistance to reinfection conferred by irradiated oocysts.

The selection of the range of Rontgen doses for investigation was made on a purely arbitrary basis as it was felt that there was unlikely to be any correlation with the method of X-irradiation carried out by Albanese and Smetana (1937) or by Waxler (1941).

Preliminary investigations were made over a wide range of Rontgen doses in order to establish the levels of X-irradiation where changes in the pathogenicity of the parasite are first recognised and where the parasite is completely inactivated. It was then important to determine the most satisfactory Rontgen dose which reduced the detrimental effects of the parasite to a minimum without inhibiting the development of resistance to reinfection with normal oocysts.

Observations were necessary to establish both the optimum number of irradiated oocysts in the inoculum and the number of doses of vaccine required to confer satisfactory immunity against a high challenge dose of oocysts. It was also necessary to determine the significance of the oocyst production following vaccination, with reference to the introduction of infection, which might subsequently be responsible for a cycle of continuous infection, leading to the build up of a heavy challenge infection under intensive methods of husbandry.

Finally it was essential to ascertain (i) if a high level of immunity could be conferred by vaccination with irradiated oocysts in young chicks to ensure that a satisfactory degree of resistance was developed before a challenge infection was established under intensive methods of husbandry, and (ii) if the protection was adequate against reinfection throughout the life of the broiler chicken.

EXPERIMENT SEVEN.

THE PATHOGENIC EFFECTS OF A STANDARD DOSE OF SPORULATED OOCYSTS OF *EIMERIA TENELLA*, AFTER EXPOSURE TO LEVELS OF X-IRRADIATION RANGING FROM 5,000 TO 80,000 RONTGENS IN 3 WEEK OLD CHICKENS AND THE IMMUNITY TO REINFECTION WITH A STANDARD CHALLENGE DOSE OF 32,000 NORMAL SPORULATED OOCYSTS 21 DAYS AFTER INOCULATION.

EXPERIMENTAL AIMS.

The experiment was designed to study the effects of X-irradiation on sporulated oocysts of *E. tenella*, with particular reference to the severity of the disease in chickens after inoculation with a standard dose of oocysts, and also the degree of immunity conferred on survivors to reinfection. Observations were made over a wide range of Rontgen doses with the object of establishing the levels of X-irradiation where changes in the pathogenicity of the parasite are first detectable and where the parasite is completely inactivated. The selection of the range of Rontgen doses (hereafter referred to as R) was made on a purely arbitrary basis as it was felt that there was unlikely to be any accurate correlation with the method of X-irradiation carried out by Albanese and Smetana (1937) or by Waxler (1941).

In order to assess the attenuating effects of X-irradiation, two main factors had to be borne in mind, (a) the dose administered must be of the same magnitude as that of normal oocysts which would consistently produce clinical disease in susceptible chickens, and (b) this dose should not be such as would produce an overwhelming infection, and so mask the effects of the ionising radiation. These factors were also significant in the selection of an appropriate challenge dose of oocysts for reinfection of the birds when it was essential to avoid overchallenge of the birds during the initial evaluation of the immunogenic effect of irradiated oocysts. The standard dose of 32,000 sporulated oocysts administered on both Day 0 and Day 21, for the initial and challenge infections respectively, fulfilled these conditions, being evaluated on the results of earlier investigations when the pathogenicity of this level of infection was clearly demonstrated in chickens of 3 and 6 weeks of age.

The pathogenic effects of the parasite were compared between chickens given irradiated oocysts exposed to doses ranging from 5,000R to 80,000R and chickens given normal oocysts. The severity of the disease in each group was determined from observations made on the changes in the concentration of haemoglobin, on mortality, on clinical signs and on growth rate. Oocyst production was also measured during the patent period of the disease after the initial infection. Birds selected at random from each group were killed for post mortem examination during the acute phase of the disease to determine whether there was any variation in the severity of the caecal lesions between chickens given normal oocysts and those receiving irradiated oocysts.

The degree of immunity conferred on the chickens from the initial infection with irradiated oocysts was compared with that in the survivors from the group given normal oocysts following reinfection on day 21. The pathogenicity of the challenge dose of oocysts was also studied in fully susceptible chickens to establish the pathogenic effects of the inoculum. Observations were made on the changes in the haemoglobin concentration, on mortality, on clinical signs and on growth rate. The surviving birds were all killed on the seventh day after reinfection for post mortem examination, to determine if significant differences were present between the lesions in the caeca of the chickens from each group

MATERIALS AND METHODS.

Experimental Birds.

Broiler hybrid cockerel chicks were used in the experiment. They were reared in complete isolation and were transferred to the experimental units the day before inoculation, where they were kept in metal cages with wire floors. The chicks given irradiated oocysts were kept in a separate room from those receiving normal oocysts. The chicks were fed on a proprietary chick food

(British Oil and Cake Mills, Ltd. - Baby Chick Crumbs) which was available ad lib. to the birds, except on day 0 and day 21 when it was withdrawn for approximately five and four hours respectively before administration of the infective dose. Food was not withheld from the chickens in the fully susceptible challenge group before inoculation.

Parasitology.

The culture of E. tenella used for the infection of the experimental birds on day 0 was 28 days old, with a total oocyst count of 109,000 per ml. and sporulation count of 80%.

Weight 15 ml. aliquots of the culture were each exposed to the appropriate dose of X-irradiation two days before inoculation on day 0-2. Total oocyst counts were carried out on each aliquot of culture on day 0-1. Dilutions were made to give 16,000 sporulated oocysts per ml. on day 0, by the addition of distilled water to an aliquot of the culture which was suspended in 2% potassium dichromate solution.

The culture administered on day 21 was eleven days old, with a total oocyst count of 900,000 per ml. and sporulation count of 90%. Dilutions were made to give 16,000 sporulated oocysts per ml. by the addition of distilled water to an aliquot of culture, which was suspended in 2% potassium dichromate solution.

Administration of Inoculum.

The infecting dose was given in 2 ml. of water on day 0 and on day 21. It was administered directly into the crop of each chicken using an automatic dosing syringe. During inoculation the stock of oocysts from which the syringe was filled was kept in suspension by decanting the solution between two beakers.

The birds were inoculated in the afternoon on day 0. The birds receiving irradiated oocysts were infected before those receiving normal oocysts. The challenge dose of oocysts was administered to the chickens in the morning on day 21.

Experimental Design.

Ten birds were selected at random for each experimental group. The birds were 24 days old on day 0. The non-infected control chickens remained in isolation during the experiment. The other groups were transferred to the experimental units on day 0-1 with the exception of the chickens of the fully susceptible challenge group Ch. which were kept in isolation until day 20.

The chickens in groups R.1 to R.8 received 32,000 sporulated oocysts on day 0 which had been exposed to 5,000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000 and 80,000 Rontgen respectively on day 0-2. The chickens in groups N.1 and N.1/2 each received 32,000 normal sporulated oocysts.

All the surviving chickens, together with group Ch., received a challenge dose of 32,000 sporulated oocysts on day 21.

Haemoglobin estimations were made on all the experimental chickens after inoculation on day 0, on day 1, 4, 5, 6, 7, 8, 10 and 15. Estimations were also made the day before reinfection on day 20, i.e. C-1, and after challenge on day 4, 5,6 and 7.

The birds were weighed on the day before the initial infection on day 0-1 and on day 11 and 21. The birds were also weighed after slaughter on day 28.

The total oocyst production of each group was recorded daily during the patent period of the disease from day 7 to day 21.

Birds were also infected on day 0 for post mortem examination on day 4, 5 and 6 in groups N.1 and R.2 - R.7; birds from R.1 and R.8 were examined on day 6 only.

Post mortem examination was carried out on day 6 on the birds which died on day 5 and day 6, after the initial infection, and on day 28 on the birds which died on day 26, after the challenge infection. Survivors were killed for examination on day 28.

Clinical signs and mortality were also recorded

RESULTS.

(1) Haematology.

The results (Tables 4. 1 and 2) of the haemoglobin estimations in the non-infected control group did not show any significant variation in the haemoglobin concentration of these chickens during the course of the experiment.

On day 1 the results did not indicate any difference between the level of haemoglobin in either the chickens given irradiated oocysts, the chickens given normal oocysts or the non-infected control chickens. On day 5 a slight decrease occurred in the haemoglobin concentration of group N.1 and N.1/2. This was very marked on day 6 when there was a decrease of 5g. of haemoglobin. The level increased a little on day 7, although it remained low until day 13 when values began to approach those recorded in the non-infected control group. The only change recorded in the concentration of haemoglobin in the chickens given irradiated oocysts after the initial infection occurred in group R.1 where there there was a decrease of approximately 2g. of haemoglobin on day 6. The level remained low on day 7 and 1, increasing on day 10, and returning to normal values on day 13. No significant variation was recorded in the level of haemoglobin in the birds of Groups R.2 - R.8 during this period.

On day 20, C-1, the results (Table 4. 2) did not demonstrate any difference between the experimental groups. The first decrease in the level of haemoglobin after reinfection occurred on day 5 in the group Ch. and also in groups

R.5, R.6, R.7 and R.8. The decrease was slightly greater on day 6 and 7 when there was a fall of 2g. of haemoglobin in groups Ch., R.5 and R.6, and of approximately 1.5g. in R.7 and R.8. No decrease occurred in the concentration of haemoglobin in the birds of Groups E.1, R.1, R.2, R.3 or R.4 after reinfection.

(2) Mortality.

Deaths occurred in both E.1 and E.1/2 on day 5 and 6 when mortality totalled 50% and 40% in each group respectively. No birds died in Groups R.1 to R.8 at this time. Following reinfection on day 21, one bird died in group R.8, although none died in the fully susceptible group Ch.

Table 4. 1.

The mean Haemoglobin Concentration in the Blood of the Experimental Chickens after Infection with a Standard Dose of Oocysts of E. tenella, which had been subjected to Different Doses of X-Irradiation before Administration, the R Groups or Normal Oocysts the E Groups.

Group	Day After Inoculation.							
	(+1)	(4)	(5)	(6)	(7)	(8)	(10)	(13)
Con:	7.8* ±0.8**	7.0 ±0.8	8.3 ±0.5	8.1 ±0.5	8.2 ±0.6	7.2 ±0.7	8.0 ±1.0	7.5 ±0.6
E.1	8.1 ±0.4	6.6 ±0.4	6.4 ±1.1	2.9 ±0.4	3.7 ±0.5	3.9 ±0.7	5.0 ±1.3	7.0 ±1.0
E.1/2	8.6 ±0.7	6.8 ±0.9	6.1 ±1.3	3.5 ±1.7	4.5 ±1.0	4.4 ±1.3	5.4 ±1.1	7.4 ±0.2
R.1	8.0 ±0.5	7.5 ±0.5	7.2 ±0.4	5.8 ±0.9	6.1 ±0.8	5.9 ±0.7	7.0 ±0.8	7.5 ±0.9
R.2	7.8 ±0.6	7.4 ±0.5	8.3 ±0.9	6.8 ±0.6	7.5 ±0.5	6.7 ±1.1	7.5 ±0.9	7.2 ±0.6
R.3	8.0 ±0.6	7.5 ±0.7	7.6 ±0.4	7.3 ±0.5	7.6 ±0.5	8.1 ±0.6	7.2 ±0.6	7.2 ±0.8
R.4	8.1 ±0.7	7.3 ±0.6	7.8 ±0.8	7.0 ±0.8	7.5 ±0.7	8.1 ±0.6	7.3 ±0.7	7.3 ±0.6
R.5	8.3 ±0.5	7.1 ±0.6	7.5 ±0.4	7.5 ±0.6	7.3 ±0.4	7.4 ±0.9	7.5 ±0.6	7.8 ±0.7
R.6	8.4 ±0.7	6.7 ±0.9	7.4 ±0.9	7.1 ±0.9	7.0 ±0.6	6.8 ±1.0	7.7 ±1.0	7.6 ±0.9
R.7.	8.6 ±0.6	7.4 ±0.6	7.8 ±0.5	7.4 ±0.5	8.0 ±0.5	7.2 ±1.1	8.1 ±0.7	7.9 ±0.2
R.8	8.2 ±0.5	7.1 ±0.7	7.3 ±0.6	6.9 ±1.0	7.8 ±0.6	7.5 ±1.0	7.9 ±0.6	7.4 ±1.0

* The mean haemoglobin concentration expressed in grammes per 100 ml. of blood.

** The standard deviation.

Table 4. 2.

The Mean Haemoglobin Concentration in the Blood of the Experimental Chickens after Reinfection with a Standard Challenge Dose of 32,000 Sporulated Oocysts of E. tenella on Day 21.

Group	Day After Inoculation.					
	(-1)	(+4)	(5)	(6)	(7)	+4 - +7
Con:	7.8 * ±0.8 **	7.0 ±0.6	7.4 ±0.8	7.6 ±1.0	7.0 ±0.9	nil
Chall:	7.2 ±1.0	7.5 ±0.9	6.5 ±1.3	6.1 ±1.2	5.3 ±1.4	2.0
E.1a.	7.8 ±0.1	7.6 ±0.5	7.6 ±0.2	8.2 ±0.4	7.2 ±0.8	0.5
E.1/2	7.7 ±0.7	7.7 ±0.5	7.6 ±1.0	7.9 ±0.4	7.1 ±0.9	0.5
R.1.	7.3 ±0.7	7.3 ±0.2	7.3 ±0.8	8.1 ±1.2	6.9 ±1.1	0.4
R.2.	7.1 ±0.3	6.8 ±0.7	7.5 ±0.5	7.4 ±0.8	6.5 ±0.4	0.2
R.3.	6.9 ±0.7	6.9 ±0.5	6.8 ±0.6	7.2 ±1.3	6.5 ±0.7	0.3
R.4.	7.3 ±0.7	7.0 ±1.0	7.0 ±1.1	7.0 ±1.3	6.5 ±1.3	0.5
R.5.	7.6 ±0.2	7.4 ±0.7	5.9 ±1.3	5.7 ±1.5	5.6 ±0.8	2.0
R.6.	7.4 ±0.9	7.3 ±0.8	6.0 ±1.1	5.9 ±1.2	5.5 ±0.6	2.0
R.7.	7.7 ±0.2	7.8 ±0.9	7.1 ±1.2	6.7 ±1.2	6.2 ±0.3	1.6
R.8.	7.4 ±0.6	7.4 ±0.9	6.5 ±1.5	6.4 ±1.7	5.9 ±0.8	1.5

* The mean haemoglobin concentration expressed in grammes per 100 ml. of blood.

** The standard deviation.

(3) Clinical Findings.

Clinical signs were first seen on the evening of day 4 when a little blood was found on the trays from the groups N.1 and N.1/2 given normal oocysts on day 0. No evidence of haemorrhage was present in groups R.1 to R.8.

On day 5 haemorrhage was quite severe in N.1 and N.1/2 where the surviving chickens appeared very depressed, in contrast to the birds of Groups R.1 - R.8 where no evidence of morbidity was seen. However, a little blood was present on the trays from group R.1. No sign of haemorrhage was found in R.2 - R.8. On day 6 and 7 morbidity was still evident in N.1 and N.1/2, being very severe on day 6 when haemorrhage was pronounced. Slight haemorrhage was also seen in R.1.

Following reinfection on day 21, the first clinical symptoms were seen on C+5, i.e., day 26. Morbidity was quite severe in group Ch. where the birds appeared rather weak and depressed, although haemorrhage did not seem very marked. Morbidity was still evident in these birds on both day 6 and 7. no sign of morbidity was present in the chickens of R.1 to R.8, although one bird was found dead on day 26 in R.8. Slight evidence of haemorrhage was present in groups R.2, R.3 and R.4. Haemorrhage was most severe in R.5 and R.6, being slightly less pronounced in groups R.7 and R.8. A little blood was still present in the cages of these groups on day 27, in contrast to day 28 when haemorrhage was seen in only R.5 and R.6. No indication of haemorrhage was found in group N.1 or R.1 after challenge.

(4) Growth Rate.

The results (Table 4. 3) showed no significant variation in the weight of the non-infected control group and the birds of N.1, N.1/2 and R.1 - R.8 before inoculation on Day 0.

On day 11 there was no significant difference between the growth rate of the non-infected control birds and the chickens which received the irradiated inoculum on Day 0. The growth rate was quite severely affected in the chickens receiving normal oocysts, being equal to only 50% and 60% of that attained by the non-infected control group. This was still clearly seen on day 21 when the birds were weighed before reinfection. At this time the weight gains of groups R.1 - R.8 were also less than those recorded in the non-infected control group, ranging from 70% to 80% of the value recorded in the control group. The results (Table 4. 5) demonstrated no significant variation in the dead weight of the non-infected control chickens and the birds of group N.1, N.1/2 and R.1 to R.8 on day 28.

(5) Pathology.

(i) Birds Selected at Random for Examination on Day 4, 5 and 6.

Typical lesions of acute caecal coccidiosis were found in the chickens representing group N.1 which received 32,000 normal sporulated oocysts. On day 4, the mucosa was severely thickened and haemorrhage was quite marked, being associated with widespread erosion of the epithelium. The lumen contained either blood-stained exudate or extravasated blood and cellular debris which was tar-like in appearance. On day 5 the birds died before examination. The caeca were distended by the presence of blood in the lumen. Widespread haemorrhages were evident, associated with severe erosion of the caecal mucosa. Lesions were still very severe in the survivors of this group on day 6, examination demonstrating the presence of extravasated blood and cellular debris in the lumen of the caeca, together with thickening of the mucosa and erosion of the epithelium. Quite marked lesions were also found in the chickens which received oocysts exposed to 5,000R before inoculation.

Table 4. 3.

The Mean Weight Gains of The Experimental Chickens on Day 11 and Day 21 to Illustrate the Effect on the Growth Rate Associated With Infection with a Standard Dose of 32,000 Sporulated Oocysts of E. tenella per Bird on Day 0, the Inoculum Given to Groups R.1 to R.8 being Exposed to Different Levels of X-Irradiation on the Day Before Administration of the Infective Dose.

Group	Day 0-1	Gain on Day 11	Weight Gain As % Control	Gain on Day 21	Weight Gain As % Control
Control	360*	273	100	739**	100
E.1.	344	163	60	436	59
E.1/2	340	137	50	400	54
R.1.	336	282	103	528	71
R.2.	338	300	110	555	75
R.3	326	285	104	535	71
R.4.	329	297	109	519	70
R.5.	343	270	99	554	75
R.6.	330	303	111	582	79
R.7.	338	282	103	552	75
R.8.	335	264	97	513	70

* Weight in grammes.

** The value on day 21 represents the total weight gain, i.e., day 21 - day 0-1

Birds from this group were examined on day 6 only. The mucosa appeared thickened and was raised into ridges, presenting a corrugated appearance. The lumen contained varying amounts of extravasated blood and cellular debris which appeared uncoagulated in some caeca, while in others the debris had a tar-like consistency. In contrast to R.1, no significant lesions were found in the caeca of the chickens of group R.2 which received oocysts exposed to 10,000R before inoculation. One or two small pin-point haemorrhages were seen on the mucosa, which also appeared very slightly thickened in the caeca of the chicken examined on day 4. However, no lesions were found in the chickens of this group on either day 5 or day 6. No lesions were demonstrated in the caeca of the chickens of groups R.3 - R.8 after the initial infection, which received oocysts exposed to doses of X-irradiation ranging from 30,000 to 80,000 Rontgens before inoculation.

(ii) Post mortem examination of the Birds Killed on Day 28 after Reinfection on Day 21.

Typical lesions of caecal coccidiosis were present in the caeca of the fully susceptible chickens of group Ch. The mucosa was severely thickened and was raised into longitudinal ridges, giving a corrugated appearance. Erosion of the epithelium was marked, being associated with numerous small haemorrhages on the mucosa. The caecal lumen contained cores of cellular debris and extravasated in eight of the ten survivors from this group.

No evidence of recent infection was found after the challenge inoculation in any of the eleven survivors from groups N.1 and N.1/2 which received normal oocysts on day 0. However, examination demonstrated the presence of slight fibrosis in the caecal wall, indicating residual lesions from the initial infection.

No significant lesions were present in the ten chickens of group R.1 which received oocysts exposed to 5,000R on day 0. The only sign of reinfection was shown in the slight thickening of the longitudinal folds of the mucosa in the caeca which appeared more pronounced when compared with those in the caeca of the non-infected control birds. Slight lesions were present in six of the ten birds examined from group R.2 which were given oocysts exposed to 10,000R on day 0. The mucosa was a little thickened with evidence of small haemorrhages, together with slight erosion of the epithelium. A small amount of cellular debris and extravasated blood was present in the caecal contents of these birds. Similar lesions were present in only three of the ten birds from group R.3, which received oocysts exposed to 20,000R before inoculation on day 0. In the remaining seven chickens from this group the caecal wall appeared slightly thickened. However, no evidence of haemorrhage was seen in the contents of the caeca which appeared quite normal. In contrast to R.3, the lesions were more severe in the birds of groups R.4 and R.5, which received oocysts exposed to 30,000R and 40,000R respectively on day 0. The caecal mucosa was slightly thickened in each bird. Cellular debris and extravasated blood was present in the lumen of the caeca in the majority, although erosion of the mucosa appeared slightly greater on the chickens of R.5. Lesions were very marked in the birds from R.6 and R.7 which received oocysts exposed to 50,000R and 60,000R before infection on day 0, although they were less severe than those in group R.8. Examination showed thickening of the mucosa and erosion of the epithelium in the caeca which contained varying amounts of cellular debris and extravasated blood in the lumen. The caeca appeared slightly contracted in the birds of group R.7. No difference was demonstrated in the severity of the lesions between the chickens of group R.8, which received oocysts exposed to 80,000R before infection on day 0, and the fully susceptible chickens of group Ch.

(iii) Post mortem Examination of the Birds which Died after Inoculation on Day 0 and after Reinfection on Day 21.

Examination of the birds which died on day 5 and 6 from group E.1 and E.1/2, and the bird from R.8 which died on day 26, showed typical lesions of acute caecal coccidiosis.

(6) Oocyst Production.

The results (Table 4. 4) showed no significant variation in the total oocyst production between the chickens of groups E.1 and R.1 in which the average output was 158 and 152 million per bird respectively. There was a significant difference between these birds and group R.2 in which the total production was only 8 million per bird. There was also a marked variation in the duration of the patent period; oocysts were demonstrated in the faeces of E.1 and R.1 between day 7 and day 21 when the birds were reinfected, in contrast to R.2 where oocysts were recorded in samples between day 7 and day 14 only. The maximum oocyst production occurred on day 7 and 8 in each group. A small number of oocysts were present in the faeces of group R.3 between day 16 and day 21. No oocysts were demonstrated in the faeces from the chickens in groups R.4 to R.8.

Table 4. 4.

The Average Total Daily Oocyst Production of the Groups E.1 and R.1 to R.8, Expressed in Millions of Oocysts per Bird, After Infection with a Standard Dose of 32,000 Sporulated Oocysts of E. tenella per Chicken, the Inoculum Given to Groups R.1 to R.8 being Exposed to Different Levels of X-Irradiation on the Day Before Administration of the Infective Dose.

Day	E.1.	R.1.	R.2.	R.3.	R.4	R.5	R.6	R.7	R.8
7	66.8	82.0	2.0	-	-	-	-	-	-
8	63.0	41.4	3.5	-	-	-	-	-	-
9	8.2	6.2	0.9	-	-	-	-	-	-
10	4.1	2.5	0.8	-	-	-	-	-	-
11	2.4	6.4	0.4	-	-	-	-	-	-
12	3.9	2.2	0.1	-	-	-	-	-	-
13	2.8	3.6	*	-	-	-	-	-	-
14	1.9	2.9	*	-	-	-	-	-	-
15	0.9	1.0	-	-	-	-	-	-	
16	1.9	1.2	-	0.7	-	-	-	-	-
17	0.8	1.2	-	1.5	-	-	-	-	-
18	0.7	0.7	-	1.4	-	-	-	-	-
19	0.4	1.3	-	0.8	-	-	-	-	-
20	0.2	0.1	-	0.2	-	-	-	-	-
21	0.2	0.3	-	0.1	-	-	-	-	-
Total** per Bird	158	152	8	5	0	0	0	0	0

* Indicates that oocyst production was less than 50,000 per bird on that day

** The total production of oocysts per bird, calculated to the nearest million oocysts.

Table 4. 5.

The Pathogenic Effects of E. tenella in Chickens Following Inoculation with a Standard Dose of 32,000 Sporulated Oocysts on Day 0, the Inoculum Given to Groups R.1 to R.8 being Exposed to Different Levels of X-irradiation on Day 0-1, and Subsequently after Reinfection on Day 21 with a Standard Challenge Dose of 32,000 Oocysts per Bird.

Group	Rontgen Dose	Haemoglobin* Decrease (1)	(2)	Mortality (1)	(2)	Wt.Gain Day 11	Dead Wt. Day 28	Oocyst Output
Con:	-	-	-	-	-	100**	1100	-
Ch.	-	-	2.0	-	-	-	1155	-
E.1.	-	5.2	-	5	-	60	1089	158***
E.1/2	-	5.0	-	4	-	60	1052	-
R.1.	5,000	2.2	-	-	-	105	1142	152
R.2.	10,000	-	-	-	-	110	1155	8
R.3.	20,000	-	-	-	-	104	1132	5****
R.4.	30,000	-	-	-	-	109	1103	-
R.5.	40,000	-	2.0	-	-	99	1121	-
R.6.	50,000	-	2.0	-	-	111	1144	-
R.7.	60,000	-	1.6	-	-	103	1160	-
R.8.	80,000	-	1.5	-	1	97	1124	-

* Haemoglobin expressed as grammes per 100 ml. of blood. (1) and (2) represent the decrease recorded following initial and challenge infections on day 0 and day 21 respectively.

** The gain in weight expressed as percentage of the gain recorded in the non-infected control group which was attained between day 0-1 and day 11 after the initial infection on day 0.

*** The average total oocyst production per bird from day 7 to day 21 inclusive, calculated to the nearest million oocysts. No counts were made on group E.1/2.

**** This oocyst production was recorded between day 16 to day 21 and probably represents an extraneous infection contracted during the early part of the patent phase.

DISCUSSION.

The experimental results (Table 4. 5) clearly demonstrated a significant difference between the pathogenic effects of normal sporulated oocysts of E. tenella and those which had been exposed to X-irradiation. The pathogenicity of the culture used in the experiment was confirmed by the severity of the disease in the chickens which received normal sporulated oocysts on Day 0 (groups E.1 and E.1/2). (Fig. 12)

The results of the haemoglobin estimation (Table 4. 1), after the initial infection, indicated a marked variation in the degree of haemorrhage between control group E.1 and the bird in group R.1 which received sporulated oocysts exposed to 5,000R. The maximum decreases in the concentration of haemoglobin were 5g. and 2g. respectively, the values approaching normal levels on day 13 and 10. The lower pathogenicity of the parasite in group R.1 was also reflected in the clinical signs, in the growth rate and by the absence of mortality. No evidence of morbidity or depression of growth rate was observed during the acute phase of the disease in the chickens which received irradiated oocysts, in contrast to group E.1 and E.1/2 where morbidity and mortality were severe, with marked depression of growth in the survivors. However, quite severe lesions were found in the caeca of the chickens of group R.1 on day 6. The detrimental effect of the parasite after exposure to 5,000R were also illustrated by the oocyst production during the patent period of approximately 152 million per bird (Table 4. 4), which closely approached that of the survivors in group E.1 of 158 million per bird.

The results of the haemoglobin estimation (Table 4. 1) also showed a marked difference between the pathogenic effects of E. tenella in group R.1, which received oocysts exposed to 5,000R, and group R.2 which received oocysts exposed to 10,000R before inoculation. No change was seen in the concentration

FIGURE 12.

THE EFFECT OF X-IRRADIATION ON THE PATHOGENICITY OF EIMERIA TENELLA - DAY 0 + 6

Note:-

(1) Typical lesions of acute caecal coccidiosis in bird (left) given normal oocysts.

(2) The less severe lesions in caeca of bird given the same dose of 32,000 oocysts after exposure to 5,000 Roentgens.

(3) The normal appearances of caeca from bird (R.2) given 32,000 oocysts after exposure to 10,000R.

of haemoglobin in R.2, in contrast to group R.1 where a decrease of 2g. of haemoglobin occurred on day 6. This difference was confirmed by post mortem examination on day 6 when no lesions were found in the caeca from the chickens of group R.2. The sole evidence of infection in these birds was the presence of a relatively small number of oocysts in the faeces between day 7 and day 14; the patent period was shorter than that in E.1 and R.1 (Table 4. 4). The average total oocyst production of 8 million per bird in group R.2 was significantly less than that in E.1 and R.1 which was 168 and 162 million per bird respectively.

No sign of infection was observed in the chickens of groups R.3 to R.8 after inoculation with sporulated oocysts which had been exposed to doses of X-irradiation ranging from 20,000R to 80,000R. A small number of oocysts were present in the faeces from group R.5 between day 16 and day 21. No oocysts were seen in the samples from this group between day 7 and day 15. This observation would appear to suggest evidence of extraneous infection contracted at the beginning of the patent period. It would seem to be very unlikely that the prepatent period of the parasite would be increased from seven to sixteen days due to irradiation of the inoculum.

There was no indication of any adverse effect on the growth rate of the chickens in groups R.1 to R.8 following inoculation on day 11, in contrast to the survivors of group E.1 and E.1/2 whose weight gains equalled only 50% to 60% of the values recorded in the non-infected control group (Table 4. 5). However, the weight gains were less satisfactory in the chickens of groups R.1 to R.8 on day 21 when compared with those in the control group, although no difference was recorded between the dead weight of these groups and the control group on day 28. Other observations failed to demonstrate any pathogenic effects related to infection with E. tenella in the chickens of groups R.3 to R.8. Therefore it is possible that the difference between the growth rate of these chickens and the non-infected control chickens on day 21 could have been associated with some factor related to a variation in the management of the birds in the experimental and isolation units respectively, rather than directly attributable to any detrimental effect from the administration of irradiated oocysts on day 0.

The pathogenicity of the challenge dose of oocysts was demonstrated by the decrease in the level of haemoglobin (Table 4. 2) which occurred in the fully susceptible chickens of group Ch. following inoculation on day 2. The severity of the infection was emphasised by the high morbidity, shown by the marked depression of the birds, although no deaths occurred during the acute phase of the disease in this group. The pathogenic effects of the parasite were confirmed at post mortem examination on day 28 when typical lesions of severe caecal coccidiosis were found in the caeca. (Figures 13 and 14).

The results of the estimation of haemoglobin (Table 4. 2) showed no change in the concentration of haemoglobin in the chickens of group R.1 to R.4, although slight evidence of haemorrhage was demonstrated by the presence of a little blood in the faeces of the birds in groups R.2, R.3 and R.4. However, a significant decrease of approximately 2g. of haemoglobin was recorded in the chickens of groups R.5 to R.8 after reinfection on day 21. The decrease was similar to that recorded in the fully susceptible chickens of group Ch. in which morbidity was also very marked, in contrast to the birds of R.5 to R.8 which showed no sign of depression during the acute stage of the disease.

The variation in the degree of resistance conferred by the administration of sporulated oocysts exposed to different levels of X-irradiation was emphasised further by the results of post mortem examination on day 28. No evidence of recent infection was found in the survivors of R.1 and R.1/2, indicating good resistance to reinfection. However, the marked pathogenicity of the parasite, after inoculation with normal sporulated oocysts on day 0, illustrated by the mortality, the decrease in haemoglobin, the depression of growth and the high oocyst output of the survivors in these groups clearly demonstrated the disadvantages and limitations of this method of immunisation. No significant lesions were present in the birds of group R.1 which suggested that a high degree of resistance is conferred on birds after inoculation with sporulated oocysts exposed to 5,000R. Slight lesions were demonstrated in the caeca of some of the chickens of groups R.2 and R.3, although there was no similarity with the lesions demonstrated in the fully susceptible chickens, indicating that a significant degree of immunity to reinfection was also stimulated by inoculation with sporulated oocysts exposed to 10,000R and 20,000R. Evidence of infection was found in fewer birds in group R.3 than in group R.2. This observation could represent further evidence confirming the extraneous infection in group R.3 suggested by the presence of oocysts in the faeces between day 16 and day 21, rather than indicating the development of greater immunity associated with some difference in the inoculum on day 0. (Figures 13 and 14).

Quite marked lesions were present in the birds of groups R.4 and R.5 and also in the chickens from R.6 and R.7, where the changes were more pronounced. However, the lesions in these groups were less severe than those in R.8 which resembled those in the fully susceptible birds of group Ch. These findings showed that a small degree of immunity was conferred by the administration of sporulated oocysts exposed to 30,000R, 40,000R, 50,000R and 60,000R. The susceptibility of the chickens in group R.8 was emphasised by the death of one bird after reinfection which indicated that the oocysts had been completely inactivated by a dose of 80,000R. (Figures 15 and 16).

FIGURE 13.

IMMUNITY CONFERRED BY OOCYSTS EXPOSED TO 5,000R - 30,000R AGAINST INFECTION WITH 32,000 NORMAL OOCYSTS.

Note:-

(1) Typical lesions of caecal coccidiosis in the fully susceptible bird (extreme left - Ch.).

(2) Normal appearance of caeca in bird immunised with oocysts exposed to 5,000R (R.1).

(3) The very slight lesions in caeca of birds immunised with oocysts exposed to 10,000R or 20,000R (R.2 and R.3), compared with the slightly more pronounced evidence of infection in the bird immunised with oocysts exposed to 30,000R (R.4).

FIGURE 14.

IMMUNITY CONFERRED BY OOCYSTS EXPOSED TO 5,000R - 30,000R AGAINST INFECTION WITH 32,000 NORMAL OOCYSTS.

Note:-

(1) Marked evidence of widespread erosion of caecal mucosa and small haemorrhages on caecal wall of fully susceptible bird (Ch.)

(2) Absence of marked lesions of the caecal mucosa in birds immunised with oocysts exposed to 5,000R, 10,000R or 20,000R (R.1, R.2 and R.3).

(3) The presence of slight lesions in the caecal mucosa of bird immunised with oocysts exposed to 30,000R (R.4).

FIGURE 15.

IMMUNITY CONFERRED BY OOCYSTS EXPOSED TO 40,000R, 50,000R, 60,000R OR 80,000R AGAINST REINFECTION WITH 32,000 NORMAL OOCYSTS.

Note:-

(1) Typical lesions of acute caecal coccidiosis in fully susceptible bird (Ch.).

(2) Quite pronounced evidence of reinfection in birds immunised with oocysts exposed to 40,000R, 50,000R or 60,000R (R.5, R.6, R.7) although lesions appear less severe compared with those in fully susceptible bird or the bird immunised with oocysts exposed to 80,000R (R.8).

FIGURE 18.

IMMUNITY CONFERRED BY OOCYSTS EXPOSED TO 40,000R, 50,000R, 60,000R or 80,000R AGAINST REINFECTION WITH 32,000 NORMAL OOCYSTS.

Note:-

(1) Widespread erosion mucosa and small haemorrhages on caecal walls of fully susceptible bird (Ch.).

(2) The thickening of caecal mucosa in bird immunised with oocysts exposed to 40,000R (R.5).

(3) The quite pronounced erosion of the caecal mucosa in bird immunised with oocysts exposed to 50,000R (R.6) and the marked thickening of caecal wall and small haemorrhages in the caeca of bird immunised with oocysts exposed to 60,000R or 80,000R (R.7 and R.8).

The experimental observations lead to the following conclusions:-

(1) The pathogenic effects of sporulated oocysts are significantly reduced after exposure to 5,000R, the lowest level of X-irradiation studied in the experiment. The oocysts confer a high degree of resistance to reinfection with a challenge dose of 32,000 sporulated oocysts 21 days later. The resistance is only slightly less than the immunity demonstrated in the survivors from an infection with a similar dose of normal oocysts. The total oocyst production of the birds after inoculation shows no significant variation from that of survivors given normal oocysts. This constitutes a serious disadvantage to the practical immunisation with oocysts exposed to 5,000R, as oocyst production represents an important factor in the epidemiology of the disease, a high production favouring the dissemination of the parasite under intensive methods of management in the field.

(2) The pathogenic effects of sporulated oocysts are negligible after exposure to 10,000R. The total oocyst production per bird after inoculation is very significantly reduced compared with that of similar chickens given either normal oocysts or oocysts exposed to 5,000R before inoculation. The oocysts confer a significant degree of resistance to reinfection with 32,000 sporulated oocysts 21 days later. No evidence of clinical disease occurs after challenge, although slight lesions are found in the caeca on post mortem examination.

(3) No evidence of infection is seen in chickens after administration of sporulated oocysts exposed to 20,000R. The oocysts confer a significant degree of resistance to reinfection with 32,000 sporulated oocysts 21 days later. No evidence of clinical disease occurs after challenge, although slight lesions are found in the caeca of some birds on post mortem examination.

(4) The immunisation of chickens against infection with E. tenella by the administration of oocysts exposed to 10,000R or 20,000R may have practical

implications in the field without the disadvantages associated with the pathogenic effects of the parasite after further experimental studies.

(5) No evidence of infection is seen in chickens after infection with oocysts exposed to 30,000R, 40,000R, 50,000R, 60,000R and 80,000R. The oocysts exposed to 30,000R confer a significant degree of immunityto reinfection with 32,000 sporulated oocysts, although this is less than the immunity developed after administration of oocysts exposed to 20,000R. There is no decrease in the concentration of haemoglobin after challenge, but lesions are more pronounced in the caeca on post mortem examination when compared with those found in the birds inoculated with oocysts exposed to 20,000R on day 0.

(6) The oocysts exposed to 40,000R, 50,000R and 60,000R appear to stimulate the development of a slight degree of resistance to reinfection with 32,000 sporulated oocysts. There is no significant variation in the decrease of haemoglobin between the fully susceptible birds and the birds given irradiated oocysts exposed to 40,000R, 50,000R and 60,000R. There is a marked difference between the lesions in the caeca, which are more severe in the susceptible chickens after challenge.

(7) The development of resistance to reinfection after inoculation with irradiated oocysts without evidence of oocyst production during the immunising infection indicates that certain levels of X-irradiation only partially inhibit the development of the parasite. The absence of lesions in the caeca after inoculation suggest that the life cycle is interrupted before completion of second stage schizogony, the phase responsible for the pathogenic effects of the parasite associated with the disruption of the mucosa and the related haemorrhage. The development of immunity in the birds inoculated with oocysts exposed to 30,000R confirms that the decrease in the pathogenicity of the parasite following X-irradiation is not due to a simple reduction in the number

of viable oocysts in the inoculum alone, since no oocysts were found in the faeces of this group after the initial infection.

(8) No evidence of infection is seen in chickens after administration of sporulated oocysts exposed to 80,000R. The birds appear fully susceptible to reinfection 21 days after inoculation. These results indicate that sporulated oocysts of E. tenella are completely inactivated by exposure to 80,000R.

Albanese and Smetana (1937) studied the effect of doses of X-irradiation ranging from 300R to 27,000R on the pathogenicity of a standard dose of approximately 20,000 sporulated oocysts of E. tenella in two to three week old Rhode Island Red chickens. Deaths occurred in birds which received oocysts exposed to 4,500R and to 6,750R; mortality ceased when the oocysts were exposed to 9,000R. These observations indicated that the pathogenicity of the parasite was not reduced by exposure to 6,750R, in contrast to the results in the present experiment when the detrimental effects of the parasite were significantly less after exposure to 5,000R, the lowest level of X-irradiation investigated. It is possible that technical differences in the method of X-irradiation could be a significant factor in the variation between the experimental observations. These include the dose rate per minute of 75R and 568R respectively and the use of a filter in the present experiment. The difference in the age of the experimental chickens could also be important, although the slightly higher standard dose of oocysts in the present study might tend to compensate for this variation.

These workers also investigated the oocyst production in chickens after inoculation with small numbers of oocysts exposed to doses of X-irradiation ranging from 2,250R to 13,500R. The results demonstrated that the prepatent period was increased from six to seven days when the oocysts were exposed to doses of 4,500 or more Rontgens, while the patent period was significantly reduced when the chickens received oocysts exposed to 11,250R and 13,500R, the highest levels of X-irradiation studied. The reproductive potential of the parasite was compared between chickens receiving normal and irradiated oocysts. No significant difference was recorded when the oocysts were subjected to 4,500R, while the value progressively decreased as the dose of X-irradiation increased from 6,750R to 13,500R when the production of oocysts was practically negligible. Close agreement on the effect of X-irradiation on the oocyst production of the parasite, expressed in terms of numbers of oocysts produced per oocyst administered, is shown by comparison of the reproductive potential after exposure to 4,500R, 9,000R, 13,500R and 5,000R, 10,000R in the work of Albanese and Smotana and in the present experiment respectively. The patent period of the parasite was also significantly reduced after irradiation with 10,000R, although no change was recorded in the duration of the prepatent period at this Rontgen level in the present study.

Experiments were designed by Albanese and Smotana (1937) to investigate the effect of X-irradiation on the excystation of the sporozoites from the oocyst in vitro. These observations, together with those from their initial experiments on the effect of X-rays on oocyst production, led them to believe that X-irradiation must produce some effects other than a simple reduction in the size of the infective dose. They concluded that the results indicated a latent period phenomenon, suggesting the the effect of X-irradiation was manifest after a period of time. These workers suspected that the development of the parasite might proceed to schizogony, but fail to pass through gametogony successfully. This theory is fully supported in the present experiment when resistance was demonstrated in chickens which passed no oocysts during the patent period after inoculation with oocysts exposed to 30,000R, as it is generally accepted that the development of resistance is closely associated with the phase of schizogony in the life cycle of E. tenella. The results of chemotherapy trials suggested that the very early phases in the life cycle were not the highly significant factors in the production of resistance. However, this work indicated that complete suppression of schizogony resulted in a susceptible bird (Kendall and McCullough, 1952). Therefore some of the sporozoites must have proceeded to schizogony after exposure to 30,000R, although no oocysts were recovered from the birds after inoculation during the patent phase of the infection because resistance was demonstrated against reinfection.

Waxler (1941) observed a marked reduction in the detrimental effect of a dose of 200,000 oocysts in 5 week old chickens after exposure to 9,000R and 13,500R, but no significant variation between the pathogenicity of oocysts exposed to 4,500R and normal oocysts. He compared the effects of oocysts exposed to 9,000R and 13,500R in chickens aged 5, 24 and 35 days old and found that slight mortality still occurred in 5 and 24 day old chickens given oocysts exposed to 9,000R. Resistance was also demonstrated to reinfection with 200,000 oocysts five weeks after the initial infection with 200,000 oocysts exposed to 9,000R in 5 week old chickens. The results are not in complete agreement with the present findings as there are some quite marked differences between the changes in the concentration of haemoglobin following administration of the irradiated oocysts and the challenge dose of oocysts. There was no indication of any variation between the pathogenicity of the culture of E. tenella administered to the experimental birds in each study respectively. Equally severe changes were recorded in the control chickens after the administration of normal oocysts, demonstrated by the fall in the level of haemoglobin and by mortality. This appears to indicate that the effect of X-irradiation was less marked on the pathogenicity of the parasite in the work of Waxler compared with the effect of similar levels of X-irradiation in the present study. However, the experiments were not strictly comparable as there were differences in the number of irradiated oocysts in the standard infective dose and in the challenge dose of oocysts in the time between the administration of the initial and challenge infections and in the age of the chickens.

Waxler (1941) also concluded that the effect of X-irradiation on the pathogenicity of sporulated oocysts of E. tenella was not alone due to a simple reduction in the number of oocysts in the inoculum. He believed this fact was supported by the findings of his own studies, together with those of Albanese and Smetana (1937). These results demonstrated that mortality failed to occur in chickens when the size of the inoculum was increased tenfold from 20,000 to 200,000 oocysts in the respective experiments, the exposure to X-irradiation being similar.

EXPERIMENT EIGHT.

THE PATHOGENIC EFFECTS OF A STANDARD DOSE OF SPORULATED OOCYSTS OF EIMERIA TENELLA AFTER EXPOSURE TO LEVELS OF X-IRRADIATION, RANGING FROM 5,000 TO 40,000 RONTGENS IN 3 WEEK OLD CHICKENS, AND THE IMMUNITY TO REINFECTION WITH A STANDARD CHALLENGE DOSE OF 64,000 NORMAL SPORULATED OOCYSTS 21 DAYS AFTER INOCULATION.

EXPERIMENTAL AIMS.

The experiment was designed to confirm the observations made earlier on the effects of X-irradiation on the pathogenicity of sporulated oocysts of E. tenella and on the degree of resistance conferred by infection with irradiated oocysts to reinfection. The preliminary investigation indicated that the pathogenicity of the oocysts was significantly reduced after exposure to 5,000R, although no difference was recorded in the total oocyst production during the patent period. However, when the oocysts were exposed to 10,000R oocyst production was negligible compared with that of birds given a similar number of normal oocysts, while no evidence of infection was seen after inoculation following exposure to doses of 20,000R to 80,000R. Immunity to reinfection was clearly demonstrated in the birds which received oocysts exposed to doses of X-irradiation ranging from 5,000R to 30,000R; resistance was significantly less when the oocysts were exposed to higher Rontgen doses, the parasite appearing completely inactivated by exposure to 80,000R. These results determined the selection of the levels of X-irradiation ranging from 5,000R to 40,000 R for further investigation. The interval between each Rontgen dose was halved so that the effect of X-irradiation on the parasite could be studied in greater detail while confirming the initial experimental findings. The challenge dose of oocysts was increased to 64,000 as it was important to establish the degree of immunity to a high level of infection and also show if there was a significant variation in the resistance correlated with the level of irradiation of the initial inoculum.

The severity of the disease in each group of chickens was determined from observations made on the changes in the concentration of haemoglobin, on mortality, on clinical signs, and on growth rate after each infection. Oöcyst production was also recorded during the patent period of the disease after the initial inoculation. Birds were killed for examination during the acute phase of the disease to confirm the difference in the pathogenic effects of the parasite following X-irradiation.

MATERIALS AND METHODS.

Experimental Birds.

Broiler type hybrid cockerel chickens were used in the experiment. They were reared in complete isolation and were transferred to the experimental units the day before inoculation where they were kept in metal cages with wire floors. The chickens given irradiated oocysts were housed in a separate room from those receiving normal oocysts. The chicks were fed on a proprietary chick food (British Oil and Cake Mills, Ltd. - Baby Chick Crumbs) which was available ad lib. to the birds, except on day 0 and day 21 when it was withdrawn for approximately five and four hours respectively before administration of the infective dose.

Parasitology.

The culture of E. tenella used for the infection of the experimental birds on day 0 was 24 days old, with a total oocyst count of 329,000 per ml. and sporulation count of 86%.

Ten 10 ml. aliquots of the culture were each exposed to the appropriate dose of X-irradiation two days before inoculation, i.e., day 0-2. Total oocyst counts were carried out on each aliquot of culture on day 0-1. Dilutions were made to give 16,000 sporulated oocysts per ml. on day 0 by the addition of distille water to the culture which was suspended in 2% potassium dichromate solution.

The culture administered on day 21 was 45 days old and contained 926,500 oocysts per ml. with a sporulation count of 75%. Dilutions were made to give 32,000 sporulated oocysts per ml. by the addition of distilled water to an aliquot of the culture.

Administration of the Inoculum.

The infecting dose of oocysts was given in 2 ml. of water on day 0 and on day 21. It was administered directly into the crop using an automatic dosing syringe. During inoculation the stock of oocysts from which the syringe was filled was kept in suspension by decanting the solution between two beakers.

The birds were inoculated in the afternoon on day 0. The birds receiving irradiated oocysts were infected before those receiving normal oocysts. The challenge dose of oocysts was administered to the chickens in the morning on day 21.

Experimental Design.

Ten birds were selected at random for each experimental group. The chickens were 24 days old on day 0. The non-infected control chickens remained in isolation during the experiment. The other groups were transferred to the experimental units on day 0-1, with the exception of the chickens from the fully susceptible challenge group which remained in the isolation unit until day 21.

The chickens in groups R.1 to R.9 received 32,000 sporulated oocysts on day 0 which had been exposed to 5,000, 7,500, 10,000, 15,000, 20,000, 25,000, 30,000, 35,000 and 40,000 Rontgens respectively on day 0-2. The chickens in groups N.1 and N.1/2 each received 32,000 normal sporulated oocysts on day 0.

All the surviving chickens, together with group Ch., received a challenge dose of 64,000 sporulated oocysts on day 21.

Haemoglobin estimations were made on all the chickens after inoculation on day 0 and on day 1, 4, 5, 6, 7, 8, 11 and 15. Estimations were also made on the day before reinfection on day 20, i.e., C-1 and after challenge on day 4, 5, 6 and 7.

The birds were weighed before infection on day 0-1 and on day 12 and day 19. The birds were also weighed on day 28 after slaughter.

The total oocyst production of each group was recorded daily during the patent period of the disease from day 7 to day 21.

Birds were also infected on day 0 for post mortem examination on day 1, 2, 3, 4, 5 and 6 in groups E.1, R.3, R.5, R.7 and R.9.

Post mortem examination was carried out on day 6 on the birds which died on day 5 and 6 after the initial infection, and on day 28 on the chickens which died after the challenge infection. Survivors were killed for examination on day 28.

Clinical signs and mortality were also recorded.

RESULTS.

(1) Haematology.

The results (Tables 4. 6 and 4. 7) of the haemoglobin estimations in the non-infected control group did not show any significant variation in the haemoglobin concentration of these chickens during the course of the experiment.

On day 1 and day 4 the results did not indicate any difference between the level of haemoglobin in the chickens given irradiated oocysts, the chickens given normal oocysts or the non-infected control chickens. On day 5 there was a marked decrease in the concentration of haemoglobin of approximately 4g. in group E.1 and E.1/2, and also a small decrease of 1g. of haemoglobin in group R.1 and R.2. On day 6 the level of haemoglobin was slightly lower in group

E.1/2 and R.1, no further decrease being recorded in the values of groups R.1 or R.2. On day 7 the concentration of haemoglobin began to increase, normal values being recorded in group R.2, in contrast to groups E.1, E.1/2 and R.1 where levels did not return to normal limits until day 11. No decrease in the level of haemoglobin occurred during this period in groups R.3 to R.9.

On day 20, C-1, the results (Table 4. 7) did not demonstrate any difference in the level of haemoglobin between the experimental chickens, with the exception of the non-infected control group where the value was slightly greater than that of the other groups. However, on day 25, C+4, this discrepancy was not present. The first decrease in the concentration of haemoglobin after reinfection was recorded on day 26, C+5, when there was a marked fall in the level of haemoglobin of groups Ch. and R.4 to R.9. The lowest values of haemoglobin were recorded on day 27 or day 28 in groups Ch., R.4 and R.5, when there was also a significant decrease of 1.8g. of haemoglobin in group R.3. The maximum decrease observed in the other birds ranged from 2.3g. to 3.3g. of haemoglobin. No change was seen in groups E.1, E.1/2, R.1 and R.2 after reinfection.

(2) Mortality.

Deaths occurred in both group E.1 and E.1/2 on day 5 and 6 when mortality equalled 70% and 80% respectively. One death was also recorded in group R.1 on day 5.

Following the administration of the challenge dose of 64,000 sporulated oocysts on day 21, deaths occurred in the group of fully susceptible birds of group Ch. and in the chickens of groups R.3 to R.9. The death rate was 10%

Table 4. 6.

The Mean Haemoglobin Concentration in the Blood of the Chickens after Infection with a Standard Dose of 32,000 Sporulated Oocysts of *E. tenella*, the Inoculum Administered to Groups R.1 to R.9 being Exposed to X-irradiation on the Day Before Inoculation, Day 0-1.

Group	Day After Inoculation.							
	(+1)	(4)	(5)	(6)	(7)	(8)	(11)	(15)
Con:	7.2* ±0.7**	7.2 ±0.6	7.3 ±0.4	7.2 ±0.7	7.0 ±0.4	7.1 ±0.5	8.2 ±0.7	8.2 ±0.7
E.1	7.1 ±0.8	7.5 ±0.8	2.9 ±0.7	3.0 ±1.4	4.6 ±0	5.4 ±1.4	7.6 ±1.4	6.8 ±0.4
E.1/2	7.1 ±0.6	6.9 ±0.4	3.3 ±1.3	2.6 ±0.8	4.2 ±1.1	6.1 ±1.7	7.4 ±1.7	6.5 ±0
R.1.	6.9 ±0.3	6.9 ±1.1	5.9 ±1.0	4.5 ±0.8	5.6 ±0.9	5.6 ±0.8	7.7 ±0.8	7.4 ±0.3
R.2.	7.2 ±0.6	7.4 ±0.6	6.2 ±0.4	6.2 ±0.9	7.5 ±0.7	6.6 ±0.7	7.6 ±0.4	7.6 ±0.9
R.3.	7.4 ±0.7	6.5 ±2.4	6.1 ±1.4	6.8 ±0.8	7.1 ±0.5	7.4 ±0.4	8.1 ±0.1	7.7 ±0.5
R.4.	7.9 ±0.6	6.9 ±0.3	6.6 ±0.5	6.9 ±0.3	7.5 ±0.5	6.6 ±0.8	7.9 ±0.7	7.6 ±1.1
R.5.	7.2 ±0.4	7.0 ±0.3	6.2 ±0.9	7.1 ±0.3	7.4 ±0.8	7.5 ±0.8	8.0 ±0.4	7.5 ±0.6
R.6.	7.1 ±1.7	7.5 ±0.6	6.1 ±0.3	7.0 ±1.0	7.3 ±0.6	7.3 ±0.6	7.8 ±0.4	7.5 ±0.5
R.7.	7.5 ±1.8	7.6 ±0.7	6.6 ±0.5	7.3 ±1.3	7.1 ±0.6	7.3 ±1.6	7.6 ±0.9	7.6 ±0.7
R.8.	7.3 ±0.6	7.4 ±0.3	6.5 ±0.6	6.9 ±0.5	7.3 ±0.7	7.1 ±0.8	8.2 ±0.4	7.5 ±0.8
R.9.	7.1 ±1.2	7.3 ±0.5	6.9 ±0.6	7.0 ±0.7	7.8 ±0.5	7.3 ±0.6	7.6 ±0.6	7.5 ±0.3

* Mean haemoglobin concentration, expressed in grammes per 100 ml. of blood.

** The standard deviation.

Table 4. 7.

The Mean Haemoglobin Concentration in the Blood of the Chickens after Reinfection with a Standard Challenge Dose of 64,000 Sporulated Oocysts of *Eimeria tenella* per Bird on Day 21.

Group.	Day After Inoculation.					
	(-1)	(+4)	(5)	(6)	(7)	+4 - +6
Con:	7.9* ±1.2**	7.2	7.4	7.2	7.0 ±1.3	nil
Ch.	6.8 ±0.6	7.2 ±0.5	4.5 ±0.7	3.9 ±0.8	3.4 ±0.7	3.3
E.1.	6.3 ±0.5	7.4 ±0.7	6.8 ±0.6	7.1 ±1.1	7.0 ±1.3	0.3
E.1/2	6.4 ±0.3	6.7 ±0.3	7.1 ±0.6	7.9 ±0.3	8.0 ±0.0	nil
R.1.	6.6 ±0.2	7.4 ±0.4	7.0 ±0.6	6.8 ±0.2	6.8 ±0.7	0.6
R.2.	6.9 ±1.5	7.0 ±0.6	7.1 ±0.8	6.9 ±0.9	6.6 ±0.5	0.4
R.3.	7.0 ±0.5	7.0 ±0.6	6.3 ±1.3	5.2 ±1.7	5.5 ±1.1	1.8
R.4.	6.9 ±0.8	7.1 ±0.7	5.4 ±1.6	4.6 ±1.9	5.1 ±0.6	2.5
R.5.	6.9 ±0.4	6.6 ±0.4	4.6 ±0.5	3.6 ±0.4	3.8 ±0.7	3.0
R.6.	6.6 ±0.6	6.6 ±0.6	3.2 ±0.9	3.9 ±0.9	4.3 ±1.3	2.7
R.7.	6.9 ±0.6	7.0 ±0.3	4.0 ±1.0	4.5 ±1.0	4.6 ±1.4	2.5
R.8.	6.9 ±1.0	6.8 ±0.7	3.8 ±0.5	4.5 ±0.5	4.5 ±1.1	2.3
R.9.	7.1 ±0.6	7.2 ±0.5	3.7 ±0.6	3.9 ±0.7	4.1 ±0.5	3.3

* Mean haemoglobin concentration expressed in grammes per 100 ml. of blood.

** The standard deviation.

in group R.3 and R.5, 20% in R.9, 30% in R.7, 40% in Ch., R.4 and R.6 and 60% in R.8 (Table 4. 10).

(3) Clinical Findings.

Clinical signs were observed first on the evening of day 4 when a little blood was present on the trays of the birds in group E.1 and E.1/2. No evidence of haemorrhage was seen in groups R.1 to R.9. On day 5 haemorrhage was very severe in groups E.1 and E.1/2 and the survivors were very depressed. Haemorrhage was also very marked in the chickens of group R.1 although morbidity was very slight. A little blood was found on the trays of the birds in group R.2. but no sign of depression was observed in these chickens. On day 6 haemorrhage was still severe in group E.1 and E.1/2, in contrast to R.1. where haemorrhage appeared less marked. Very slight evidence of haemorrhage was present in group R.2. Morbidity was still high in the survivors of groups E.1 and E.1/2, unlike the chickens in the groups which received irradiated oocysts where no indication of depression was seen. Blood was not found in the faeces of groups E.1, E.1/2, R.1 or R.2 after day 6. No evidence of clinical disease was recorded in the groups R.3 to R.9 after inoculation with irradiated oocysts on day 0.

Following reinfection on day 21, the first clinical symptoms were present on day 24, i.e., C+4, when a little blood was found in the faeces from the fully susceptible chickens of group Ch. and from the birds of groups R.3 to R.9. On day 26, i.e., C+5, morbidity was very marked in birds of groups Ch. and R.4 to R.9 and was associated with severe haemorrhage. Morbidity was slightly lower in the chickens of group R.3, although evidence of haemorrhage was pronounced. No sign of depression occurred in groups E.1, E.1/2, R.1 and R.2. but a little blood was present in the faeces from the chickens of groups

R.1 and R.2. On day 27, i.e., C+6, slight evidence of haemorrhage was present in groups Ch., R.4, R.5, R.6, R.7 and R.8. Blood was no longer seen in the faeces from groups R.1, R.2, R.3 and R.9. Evidence of morbidity was still apparent in chickens of groups R.4 to R.9 and also in group Ch.

(4) Weight Gains.

The results (Table 4. 8) showed no significant variation in the weight of the non-infected control group and the birds of groups E.1, E.1/2 and R.1 to R.9 before inoculation on day 0.

On day 12 there was no significant difference between the mean weight gains of the non-infected control chickens and the birds which received irradiated oocysts in groups R.1 to R.9. The growth rate was severely retarded in birds which received an equal number of normal oocysts in group E.1 and E.1/2, where the mean weight gains of the survivors equalled only 64% of the value attained by the non-infected control chickens. This difference was still present on day 19 when the mean weight gain of the chickens in E.1 and E.1/2 represented only 70% of the value recorded in the control chickens.

The results (Table 4. 10) indicated little difference between the dead weight of the birds of groups R.1 to R.4 and the non-infected chickens of the control group on day 28, i.e., C+7. The weights of the birds in groups Ch., E.1 and R.5 to R.9 appeared slightly less satisfactory, being approximately 20% less than the value recorded in the control chickens. The dead weight of the chickens of group E.1/2 was a little higher than that of the survivors from group E.1, being 86% of the mean value in the non-infected chickens.

(5) Pathology.

(i) Birds Representing Groups R.1, R.3, R.5, R.7 and R.9, Selected at Random for Examination 24, 48, 60, 72, 84, 96, 108, 120 and 132 Hours after Inoculation.

Table 4. 8.

The Mean Weight Gains of the Chickens on Day 12 and Day 19 Showing the Effect on the Growth Rate Associated with Infection with a Standard Dose of 32,000 Sporulated Oocysts of E. tenella per Bird on Day 0. The Inoculum Administered to Groups R.1 to R.9 was Exposed to Different Levels of X-Irradiation on the Day before Administration of the Infective Dose.

Group	Rontgen Dose	Wt. Day 0-1	Wt.Gain Day 12	%Wt.Gain Control	Wt.Gain Day 19	%Wt.Gain Control
Control	-	337*	368	100	595**	100
E.1.	-	313	238	64	436	73
E.1/2	-	310	238	64	419	70
R.1.	5,000	331	314	84	553	93
R.2.	7,500	340	367	97	593	100
R.3.	10,000	338	334	91	573	96
R.4.	15,000	334	350	95	573	96
R.5.	20,000	328	315	86	572	96
R.6.	25,000	329	349	95	588	99
R.7.	30,000	356	322	88	541	91
R.8.	35,000	320	344	93	567	94
R.9.	40,000	335	340	92	547	92

* Weight expressed in grammes

** The value on Day 19 represents the total weight gain, i.e., Day 19 - Day 0-1

No evidence of infection was seen in the caeca of the birds examined 24, 48 and 60 hours after inoculation. The first indication was infection was recognised at 72 hours when quite marked changes were present in the caeca of the birds from group R.1. The mucosa was thickened with numerous small pin-point haemorrhages on the surface, associated with erosion of the epithelium. The contents of the caeca appeared quite normal in these birds. The mucosa was only very slightly thickened in the caeca of the chickens from group R.3. No sign of haemorrhage was seen in the caeca of this group. Severe lesions were present in one bird from group R.1 at 84 hours after infection. The caecal mucosa was severely thickened, with small haemorrhages on the surface which were reflected in the blood-stained caecal contents. Lesions were less severe in the second bird of this group, the only indication of infection being shown by the thickening of the caecal mucosa. Very slight thickening of the mucosa was present in the birds from group R.3 in which no evidence of haemorrhage was seen. Severe lesions were present in both birds from group R.1 at 96 hours. The caecal mucosa was markedly thickened with numerous small haemorrhages on the surface and the caecal contents contained blood, which was more pronounced in one bird. The mucosa was only slightly thickened in the chickens examined from group R.3. There was no indication of haemorrhage in these birds. Typical lesions of acute coccidiosis were found in the birds from group R.1 at 108 and 120 hours. Evidence of haemorrhage was marked and there was distension of the caecal lumen with blood following wide-spread erosion of the caecal mucosa. The caeca were very friable and were easily ruptured on examination. Only very slight lesions were found in the caeca of the chickens from group R.3. The mucosa appeared a little thickened, with one or two small pin-point haemorrhages present on the surface. However, there was no indication of erosion of the mucosa in these birds, in contrast to the findings in group R.1. The caecal contents also appeared quite normal in this group. There were no survivors available for examination at 132 hours from group R.1. Very slight lesions were seen in the birds from group R.3. The caecal mucosa was slightly thickened with evidence of small haemorrhages on the surface, although there was no indication of haemorrhage found in the contentsof the caeca. Similar changes were present in the birds of this group examined 154 hours on the seventh day after infection.

No evidence of infection was found on post-mortem examination of chickens from groups R.5, R.7 and R.9 after inoculation.

(ii) Post-mortem Examination of the Birds Killed on Day 28, after Reinfection on Day 21.

Typical lesions of acute caecal coccidiosis were demonstrated in the surviving birds from the fully susceptible chickens of group Ch. The caeca were very friable, being easily ruptured on removal from the abdominal cavity. Haemorrhage was very severe and was associated with widespread erosion of the caecal mucosa. The caecal lumena were distended by extravasated blood and cellular debris. Similar changes were present in the caeca of the chickens from groups R.5 to R.9.

No evidence of recent infection was found in the caeca of the five survivors from group E.1 and E.1/2, although the caeca appeared smaller than normal with evidence of fibrosis in the caecal walls, suggesting the presence of residual lesions from the initial infection on day 0.

Very slight lesions were seen in the chickens from groups R.1 and R.2, where the sole indication of recent infection was reflected in the slight thickening of the caecal mucosa. Quite severe changes were present in the caeca of the birds from group R.3 and R.4, although they were less marked than those found in the fully susceptible birds of group Ch. and the birds of R.5 to R.9. The mucosa was thickened and the caecal lumen contained a little cellular debris and extravasated blood.

(iii) Post-mortem Examination of the Birds which Died after Inoculation on Day 0 and after Re-infection on Day 21.

Examination of the birds which died on day 5 or 6 from groups E.1, E.1/2 and R.1, and the birds which died on day 26 from groups Ch., R.3, R.4 R.5, R.6, R.7, R.8 and R.9, showed typical lesions of acute caecal coccidiosis.

(6) Oocyst Production.

The results (Table 4. 9) showed no significant variation in the total oocyst production between the chickens of groups E.1 and R.1, although the oocyst production was slightly higher in group R.1, the total average oocyst output per bird being 87 and 96 million respectively. There was a marked reduction in the oocyst production of groups R.2 and R.3, their output per bird being 53 and 7 million compared with 87 million per bird in group E.1. Negligible numbers of oocysts were recovered from the faeces of groups R.4 and R.5, while none were found in the samples from groups R.6 to R.9 during the patent period of the idsease after inoculation on day 0.

Table 4. 9.

The Average Total Daily Oocyst Production of the Chickens Expressed in Millions of Oocysts per Bird, After Infection with a Standard Dose of 32,000 Sporulated Oocysts per Bird on Day 0; the Inoculum Administered to Groups R.1 to R.9 was Exposed to Different Levels of X-Irradiation on the Day before Inoculation, Day 0-1.

Day	E.1.	R.1	R.2	R.3	R.4	R.5	R.6	R.7	R.8	R.9.
7	69.0	50. 0	14.0	2.1	-	*	-	-	-	-
8	5.5	12.0	11.6	3.0	*	.2	-	-	-	-
9	2.2	10.6	10.0	1.2	0.1	*	-	-	-	-
10	1.2	1.9	8.7	0.5	*	*	-	-	-	-
11	0.9	2.6	4.2	0.3	*	*	-	-	-	-
12	3.8	4.4	1.8	0.1	-	-	-	-	-	-
13	0.3	7.5	1.2	*	-	*	-	-	-	-
14	0.9	3.3	0.4	-	-	*	-	-	-	-
15	0.8	1.8	0.2	-	-	*	-	-	-	-
16	1.5	1.1	0.1	*	-	*	-	-	-	-
17	0.1	0.4	*	-	*	-	-	-	-	-
18	0.2	0.1	*	*	*	-	-	-	-	-
19	*	*	*	-	0.1	-	-	-	-	-
20	*	*	*	*	0.1	-	-	-	-	-
21	*	*	*	-	*	-	-	-	-	-
Total** per Bird	87	96	63	7	0.4	0.4	-	-	-	-

* Indicates that oocyst production was less than 50,000 per bird on that day.

** The total production of oocysts per bird, calculated to the nearest million oocysts.

Table 4. 10.

The Pathogenic Effects of E. tenella in Chickens Following Inoculation with a standard Dose of 32,000 Sporulated Oocysts on Day 0, the Inoculum Given to Groups R.1 to R.9 being Exposed to Different Levels of X-irradiation on Day 0-1, and Subsequently after Reinfection on Day 21 with a Standard Challenge Dose of 64,000 Sporulated Oocysts per bird.

Group	Rontgen Dose	Haemoglobin* Decrease		Mortality		%Wt.Gain Day 12	%Dead Wt. Day 28	Oocyst Output
		(1)	(2)	(1)	(2)			
Control	-	-	-	-	-	100**	100**	-
Ch.	-	-	3.3	-	4	-	76	-
E.1.	-	4.2	-	7	-	64	78	87***
E.1/2	-	4.3	-	8	-	64	86	
R.1.	5,000	2.5	-	1	-	84	93	96
R.2.	7,500	1.0	-	-	-	97	97	53
R.3.	10,000	-	1.8	-	1	91	90	7
R.4.	15,000	-	2.5	-	4	95	86	0.4
R.5.	20,000	-	3.0	-	1	86	78	0.4
R.6.	25,000	-	3.0	-	4	96	78	-
R.7.	30,000	-	3.0	-	3	88	79	-
R.8.	35,000	-	3.0	-	6	93	78	-
R.9.	40,000	-	3.0	-	2	92	75	-

* Haemoglobin expressed as grammes per 100 ml. of blood. (1) and (2) represent the decrease recorded following the initial and challenge infections on day 0 and day 21 respectively.

** The gain in weight expressed as a percentage of the gain recorded in the non-infected control group which was attained between day 0-1 and day 12. Similarly dead weight expressed as percentage of the dead weight recorded on day 28 in the non-infected control chickens.

*** The average total oocyst production per bird from day 7 to day 21 inclusive, calculated to the nearest million oocysts. No counts made on group E.1/2.

DISCUSSION.

The experimental results (Table 4. 10) clearly demonstrated a significant difference in the pathogenic effects of the sporulated oocysts of E. tenella after exposure to X-irradiation. The pathogenicity of the culture used in the experiment was confirmed by the severity of the disease in the chickens which received normal oocysts on day 0 in group E.1 and E.1/2.

The results of the haemoglobin estimation (Table 4. 6) after the initial infection on day 0 indicated a marked variation in the degree of haemorrhage between group E.1 and Groups R.1 and R.2, the decrease in haemoglobin being approximately 4g., 2.5g., and 1g. respectively. The difference in the severity of the infection between these groups was also reflected in the clinical findings, in mortality and by the respective weight gains. These observations clearly demonstrated that the pathogenicity of the oocysts was reduced by exposure to 5,000R of X-irradiation, although detrimental effects were still evident after inoculation. These were indicated by the death of one chicken during the acute phase of the disease and also by the total oocyst production per bird which was slightly greater than that of the chickens receiving normal oocysts. There was quite a marked variation between the effects of 5,000R and 7,500R on the subsequent pathogenicity of the parasite. No deaths were recorded in group R.2 which received oocysts exposed to 7,500R, while the decrease in the level of haemoglobin and the total oocyst production were both significantly less in these birds after infection.

When oocysts were exposed to 10,000R before inoculation, the beneficial effects of X-irradiation were clearly emphasised by the absence of any significant pathogenic effects after administration of the infective dose. No indication of clinical disease was seen, while serial post mortem examination after inoculation failed to show any significant lesions, in contrast to the very severe changes demonstrated in the caeca of the birds which received normal oocysts. The only evidence of infection was shown by the presence of oocysts in the faeces during the patent phase of the disease, the average total oocyst production being 7 million per bird, compared with 87 million per bird in the group given non-irradiated oocysts. The growth rate of the chickens equalled that of the control group and showed no sign of less satisfactory weight gains, unlike group N.1, where the depression of growth was marked during the acute stage of the disease.

The results demonstrated a slight difference between the effect of 10,000R on the one hand and 15,000R and 20,000R on the other, illustrated by the oocyst production after inoculation when the total output per bird was only 0.4 million in the latter group. Similarly a small difference was indicated between the effect of 20,000R and 25,000R and examination of the faeces failed to demonstrate the presence of any oocysts during the patent period of the disease in samples from the birds which received oocysts exposed to 25,000R.

The results did not suggest any variation in the effect of levels of X-irradiation ranging from 25,000R to 40,000R on the pathogenicity of the parasite and no evidence of infection was recorded in the birds which received oocysts exposed to 25,000R, 30,000R, 35,000R and 40,000R.

The pathogenicity of the challenge dose of oocysts was confirmed by the marked fall of 4g. of haemoglobin, by the mortality and by the high morbidity recorded in the fully susceptible chickens of group Ch.

The results of the haemoglobin estimation (Table 4. 7) demonstrated a marked difference in the resistance to reinfection between the chickens given oocysts exposed to 5,000R or 7,500R and those receiving oocysts exposed to 10,000R before inoculation. No change occurred in the concentration of haemoglobin in the chickens of group R.1 or R.2, in contrast to a decrease of 1.8g. of haemoglobin in the birds of the group R.3. The difference was confirmed by the death of one bird in R.3 and by post mortem examination made on day 28 when only slight lesions were found in the caeca of the chickens from R.1 and R.2, compared with quite severe lesions in the survivors from R.3. These observations indicated that good immunity is developed after inoculation with oocysts exposed to either 5,000R or 7,500R, while resistance to a high challenge dose of oocysts is significantly lower in chickens inoculated with oocysts exposed to 10,000R. Resistance was also good in the survivors receiving normal oocysts on day 0, but the disadvantages and limitations of this method of immunisation were clearly illustrated by the mortality, the decrease in haemoglobin, the depression of weight gains and the high total oocyst production which was recorded in these chickens after inoculation. Unfortunately administration of oocysts exposed to 5,000R and 7,500R is also contraindicated as detrimental effects were observed after inoculation, although the pathogenicity of the parasite was less.

The results of the haemoglobin estimation after reinfection, together with the less satisfactory weight gains and the high morbidity and mortality in the chickens of groups R.4 to R.9, showed that a single standard dose of 52,000 oocysts exposed to levels of X-irradiation ranging from 15,000R to 40,000R does not confer a singificant degree of immunity to reinfection with a high challenge dose of oocysts 21 days later.

These observations show conclusively that sporulated oocysts of *E. tenella* must be exposed to a minimum dose of 10,000R before administration to fully susceptible chickens to ensure that no pathogenic effects occur after inoculation. The results also show that the administration of irradiated oocysts of *E. tenella* confers resistance to reinfection without deleterious effects during immunisation. However, the standard infective dose of 32,000 oocysts appears to be too low when the chickens are exposed to a high challenge infection 21 days later.

The results confirm the observations made in the previous experiment on the beneficial effect of X-irradiation on the pathogenicity of sporulated oocysts of *E. tenella*. There was very close agreement between the results of the corresponding groups after inoculation with oocysts exposed to 5,000R, 10,000R, 20,000R, 30,000R and 40,000R before infection. The results show that a high degree of resistance is conferred against reinfection with a standard challenge dose of 32,000 sporulated oocysts following administration of a standard dose of 32,000 sporulated oocysts exposed to 5,000R, 10,000R or 20,000R before inoculation. However, this immunity is too low to prevent the appearance of detrimental effects when the challenge dose is increased to 64,000 sporulated oocysts per bird.

EXPERIMENT NINE.

THE PATHOGENIC AND IMMUNOGENIC EFFECTS OF A SINGLE INFECTION WITH DIFFERENT NUMBERS OF SPORULATED OOCYSTS OF *EIMERIA TENELLA* AFTER EXPOSURE TO LEVELS OF X-IRRADIATION RANGING FROM 10,000 TO 25,000 RONTGENS IN THREE WEEK OLD CHICKENS.

EXPERIMENTAL AIMS.

The experiment was designed to study the pathogenic effects of different numbers of irradiated oocysts of E. tenella in three week old chickens and also the degree of immunity conferred against reinfection with normal oocysts. Levels of irradiation ranging from 10,000R to 25,000R were selected for further investigation as earlier experimental results indicated conclusively that sporulated oocysts of E. tenella must be exposed to a minimum dose of 10,000R before administration to fully susceptible chickens in order to ensure that no significant pathogenic effects occurred after inoculation, while resistance was markedly reduced after exposure to 30,000R. The preliminary studies also indicated that an immunising dose of 32,000 sporulated oocysts was too low when the chickens were exposed to a high challenge dose of 64,000 sporulated oocysts 21 days after inoculation, although a high degree of immunity was conferred against a lower challenge dose of 32,000 sporulated oocysts. Therefore three additional levels of infection of 64,000, 128,000 and 256,000 sporulated oocysts per bird were compared with the standard dose of 32,000 sporulated oocysts at each level of X-irradiation when the chickens were reinfected with normal oocysts 21 days after inoculation. The challenge dose of 32,000 sporulated oocysts was selected to avoid an overwhelming infection which might mask differences between the immunity conferred by the different numbers of oocysts at each level of X-irradiation. It was also important to determine whether detrimental effects were associated with immunisation when the number of irradiated oocysts was increased in the inoculum.

The severity of the disease in each group of chickens was determined from observations made on the changes in the concentration of haemoglobin, on mortality, on clinical signs and on the growth rate after each infection.

Oocyst production was recorded during the patent period of the disease after the initial inoculation and on the seventh day following reinfection. Birds were killed for post-mortem examination on the fifth day after infection to establish if lesions were present during the acute phase of the disease when the number of irradiated oocysts was increased in the inoculum. The surviving birds were all killed on the seventh day after reinfection to determine whether there was any variation in the severity of the caecal lesions between each group which might indicate a significant difference in the immunity of the chickens.

MATERIALS AND METHODS.

Experimental Birds.

Broiler type hybrid cockerel chicks were used in the experiment. They were reared in complete isolation and were transferred to the experimental units the day before inoculation, where they were kept in metal cages with wire floors. The chicks given irradiated oocysts were housed in a separate room from those which received normal oocysts. The chicks of the fully susceptible challenge groups were retained in isolation until the day before infection. The chicks were fed on a proprietary chick food (British Oil and Cake Mills Ltd. - Baby Chick Crumbs) which were available ad lib., except on day 0 and day 21 when it was withdrawn seven and three hours respectively before administration of the infective dose.

Parasitology.

The culture of E. tenella used for the infection of the chickens on day 0 was 22 days old, with a total oocyst count of 1,375,000 per ml. and sporulation count of 70%.

Four 15 ml. aliquots of the culture were each exposed to the appropriate dose of X-irradiation one day before inoculation, i.e. day 0-1. Total oocyst counts were carried out on each portion of the irradiated culture on day 0 and also on a sample of the non-irradiated culture. Dilutions were made to give the appropriate number of oocysts per ml. for each respective group, by the addition of distilled water to the culture which was suspended in 2% potassium dichromate solution.

The culture administered to the chickens on day 21 was 45 days old, with a total oocyst count of 260,000 per ml. and sporulation count of 63%. Dilutions were made to give 15,000 sporulated oocysts per ml. by the addition of distilled water to an aliquot of the culture.

Administration of the Inoculum.

The infecting dose of irradiated oocysts was given in 2 ml. of water on day 0. The challenge dose of normal oocysts was given in 2 ml. of water on day 21. The inoculum was administered directly into the crop using an automatic dosing syringe. During inoculation the stock of oocysts from which the syringe was filled was kept in suspension by decanting the solution between two beakers.

The chickens were inoculated in the afternoon on day 0. The birds given irradiated oocysts were infected before those which received normal oocysts. The challenge dose of oocysts was administered to the chickens in the morning on day 21.

Experimental Design.

Ten birds were selected at random for each experimental group. The chickens were 24 days old on day 0.

The chickens in groups R.1 - R.4, R.5 - R.8, R.9 - R.12 and R.13 - R.16 received sporulated oocysts which had been exposed to 10,000R, 15,000R, 20,000R and 25,000R respectively on the day before inoculation.

The inoculum administered to each bird on day 0 contained 32,000 oocysts in groups R.1, R.5, R.9 and R.13, 64,000 oocysts in groups R.2, R.6, R.10 and R.14, 128,000 oocysts in groups R.3, R.7, R.11 and R.15, and 256,000 oocysts in groups R.4, R.8, R.12 and R.16. The chickens in groups S.1 and S.1/2 received 32,000 normal oocysts per bird on day 0.

All the surviving chickens, together with the fully susceptible chickens of group Ch.1 and Ch.2 received 32,000 normal oocysts on day 21.

Chickens were also infected on day 0 for post mortem examination on day 5. Two birds were inoculated to represent each experimental group, except R.3 and R.4.

Haemoglobin estimations were made on the chickens after inoculation on day 0, and on day 1, 4 and 6. Estimations were also made on the day before reinfection on day 20, i.e. C-1 and after challenge on day 4, 5, 6 and 7.

The birds were weighed before infection on day 0-1 and on day 12 and day 19. The birds were also weighed after reinfection on day 28.

The total oocyst production of each group was recorded daily during the patent period of the disease from day 7 to day 21. The oocyst production was also recorded after reinfection on day 28 before the birds were killed.

Post mortem examination was carried out on day 6 on the birds which died on day 5 after the initial infection, and on day 28 on the birds which died after the challenge infection. The surviving birds were killed for examination on day 28.

Clinical signs and mortality were also recorded.

RESULTS.

(1) Haematology.

The results (Table 4. 11 and 4. 12) of the haemoglobin estimations in the non-infected control group did not show any significant variation in the concentration of haemoglobin during the course of the experiment.

On day 1 and day 4, the results did not show any difference in the level of haemoglobin between any of the experimental groups.

On day 6 there was a marked decrease in the concentration of haemoglobin of approximately 4.5g. in the chickens of group E.1 and E.1/2. No change was recorded in the level of haemoglobin in the chickens of groups R.1 - R.16 which received irradiated oocysts.

On day 20 and day 25, i.e., C-1 and C+4, the results (Table 4. 12) did not demonstrate any variation in the concentration of haemoglobin between the non-infected control group, the susceptible challenge group, or the chickens in groups R.1 - R.16.

The first decrease in the level of haemoglobin after administration of the challenge dose of oocysts occurred on day 26, i.e. C+5, when there was a fall of approximately 1.5g. of haemoglobin in the chickens of group Ch.1 and Ch.2. The lowest value of haemoglobin was recorded in these chickens on day 27 when the decrease was 2.9g. of haemoglobin. No change occurred in the level of haemoglobin of the birds of groups R.1 to R.16 until day 27 when there was a slight decrease of haemoglobin in groups R.9 to R.16 which ranged between 1.6 to 1.8g. This was most marked in groups R.11, R.14 and R.16, although the results did not appear to demonstrate a significant variation between these groups. No decrease in the concentration of haemoglobin was recorded in the survivors of E.1 and E.1/2 or in the chickens of groups R.1 - R.8 after reinfection.

Table 4. 11.

The Mean Haemoglobin Concentration of the Chickens after Infection with Different Numbers of Sporulated Oocysts of *E. tenella* on day 0. The Inoculum Administered to Groups R.1 to R.16 was exposed to Different Levels of X-irradiation on the Day before Inoculation, Day 0-1

Group	Oocyst Dose	Rontgen Dose	Day After Inoculation (+1)	(4)	(6)
Control	nil	nil	9.4* ±0.7**	9.7 ±0.5	9.5 ±0.6
E.1.	32,000	"	9.0 ±0.6	9.5 ±0.7	4.7 ±1.2
E.1/2	"	"	8.9 ±0.5	9.3 ±0.6	4.8 ±1.5
R.1.	32,000	10,000	9.7 ±0.8	9.2 ±0.6	9.5 ±1.1
R.2.	64,000	"	9.1 ±0.6	9.2 ±0.4	9.1 ±0.3
R.3.	128,000	"	9.5 ±0.6	9.1 ±0.6	8.8 ±0.8
R.4.	256,000	"	9.9 ±0.8	9.1 ±0.5	9.1 ±0.8
R.5.	32,000	15,000	9.7 ±0.7	9.0 ±0.7	9.0 ±0.6
R.6.	64,000	"	9.2 ±0.4	9.0 ±0.9	8.5 ±0.5
R.7.	128,000	"	10.2 ±0.7	9.5 ±0.5	9.7 ±1.4
R.8.	256,000	"	9.9 ±1.0	9.9 ±1.0	9.2 ±0.5
R.9.	32,000	20,000	9.0 ±1.1	9.3 ±1.0	9.1 ±1.0
R.10.	64,000	"	9.1 ±0.6	9.0 ±0.4	9.0 ±0.6
R.11.	128,000	"	9.6 ±0.7	8.5 ±0.7	9.4 ±1.0
R.12.	256,000	"	9.4 ±0.8	9.6 ±1.2	8.9 ±0.8
R.13.	32,000	25,000	9.4 ±0.7	9.2 ±0.8	9.2 ±0.6
R.14.	64,000	"	9.3 ±0.7	9.2 ±0.8	9.3 ±0.9
R.15.	128,000	"	9.1 ±0.7	8.9 ±0.7	8.9 ±0.7
R.16.	256,000	"	10.0 ±0.9	9.7 ±0.8	8.9 ±0.8

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

** The standard deviation.

Table 4. 12.

The Mean Haemoglobin Concentration in the Blood of the Chickens after Reinfection with a Standard Challenge Dose of 32,000 Sporulated Oocysts of E. tenella on day 21.

Group.	Day After Inoculation.				
	(-1)	(4)	(5)	(6)	(7)
Con:	9.2* ±1.0**	9.4 ±0.6	9.2 ±0.9	9.3 ±0.8	9.2 ±0.8
Ch.1.	8.1 ±0.7	8.7 ±0.7	7.6 ±1.2	6.3 ±1.4	6.6 ±1.3
Ch.2.	8.4 ±0.6	9.1 ±0.6	7.2 ±1.5	6.7 ±2.1	6.0 ±1.7
E.1.	9.2 ±0.6	9.1 ±1.0	9.6 ±0.9	8.7 ±0.8	8.9 ±0.4
E.1/2	8.7 ±0.6	8.7 ±0.4	9.2 ±0.7	8.4 ±0.8	8.6 ±0.8
R.1.	10.0 ±0.8	8.6 ±0.6	9.3 ±0.6	9.0 ±1.0	8.9 ±0.6
R.2.	9.1 ±0.7	8.3 ±0.9	9.2 ±0.7	8.8 ±0.6	8.5 ±0.8
R.3.	9.3 ±0.7	8.4 ±0.6	9.0 ±0.5	8.8 ±0.8	8.1 ±1.7
R.4.	9.[illegible] ±0.4	8.9 ±0.8	9.5 ±0.4	8.9 ±0.7	8.7 ±0.6
R.5.	9.4 ±1.0	8.6 ±0.5	9.2 ±0.8	8.2 ±0.8	8.3 ±0.7
R.6.	9.3 ±0.7	8.4 ±0.8	9.0 ±0.8	8.4 ±0.8	8.5 ±1.0
R.7.	9.8 ±0.6	9.2 ±0.6	9.2 ±0.6	8.7 ±0.8	8.5 ±1.0
R.8.	9.2 ±0.5	8.7 ±0.8	9.0 ±0.7	8.4 ±0.9	8.6 ±0.7
R.9.	9.1 ±0.7	9.0 ±0.9	9.2 ±1.0	8.4 ±1.1	8.2 ±1.1
R.10.	8.9 ±0.7	8.9 ±0.5	9.1 ±0.6	8.1 ±0.6	8.5 ±0.9
R.11.	8.9 ±1.2	9.3 ±0.8	8.7 ±0.8	7.6 ±0.9	8.1 ±1.1
R.12.	8.8 ±0.4	8.6 ±0.5	8.5 ±1.0	7.9 ±1.5	8.1 ±1.6
R.13.	8.7 ±0.7	8.9 ±0.5	8.6 ±0.6	7.9 ±0.8	7.8 ±1.1
R.14.	8.8 ±0.6	9.0 ±0.7	8.4 ±0.9	7.1 ±0.9	7.5 ±1.1
R.15.	8.6 ±0.8	8.9 ±0.6	8.6 ±0.6	8.0 ±1.1	8.2 ±1.2
R.16.	8.5 ±0.6	8.9 ±0.7	8.2 ±0.9	7.2 ±1.3	7.1 ±1.2

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

** The standard deviation.

(2) Mortality.

Two deaths occurred in group R.1/2 on day 5. After administration of the challenge inoculum, one bird died in group Ch.2 and group R.14 on day 26, i.e. C+5, and on day 27, i.e. C+6 respectively.

(3) Clinical Findings.

Clinical signs were observed first on the morning of day 5 when slight haemorrhage was evident in groups R.1 and R.1/2. Symptoms of disease were marked in the afternoon of day 5, morbidity being severe and associated with evidence of severe haemorrhage. On day 6 the chickens still appeared very depressed, while haemorrhage was also quite marked. Evidence of blood in the faeces was not seen after day 6, although morbidity remained high on both day 7 and day 8.

No indication of haemorrhage was seen in the chickens which received irradiated oocysts on day 0, the chickens showing no sign of clinical disease after inoculation.

Following reinfection on day 21, the first clinical signs were recorded on day 26, i.e. C+5, when slight evidence of haemorrhage was seen in the chickens of groups Ch.1 and Ch.2. These birds were also slightly depressed in contrast to the chickens of groups R.1 - R.16 where no indication of morbidity occurred after challenge. On day 27 haemorrhage was quite marked in group Ch.1 and Ch.2, although no blood was present in the faeces on day 28. On day 26 a little blood was found in the cages of groups R.9 - R.16 while haemorrhage appeared quite severe in these chickens on day 27, although it was less pronounced than that seen in the fully susceptible chickens of group Ch.1 and Ch.2. Only very slight evidence of haemorrhage was found in group R.2, R.6 and R.7 following reinfection. No indication of haemorrhage was present in groups R.1, R.3, R.4, R.6, R.8, or in the survivors in group E.1 or E.1/2 after challenge.

<u>(4) Weight Gains.</u>

The results (Table 4. 13) showed no significant variation in the weight of the chickens in the experimental groups, with the exception of the birds in the non-infected control group which were slightly lower in weight on day C-1 compared with the other chickens.

On day 12 there was no significant difference between the mean weight gain of the non-infected control chickens and the chickens which received irradiated oocysts on day C, although the mean weight gain was slightly less in groups R.4, R.8, R.12 and R.16, compared with the value attained by the other groups inoculated on day C. However, less satisfactory weight gains were recorded in the birds of group E.1 and E.1/2, where the gain in weight equalled 85% and 80% of that recorded in the non-infected control group.

There was no significant difference between the gain in weight of the experimental groups on day 19.

On day 28 when the birds were weighed after reinfection the mean weight gains were slightly less in the fully susceptible chickens of group Ch.1 and Ch.2. There was no marked variation between the weight of the birds in R.1 - R.16 and the non-infected control chickens.

<u>(5) Pathology.</u>

<u>(i) Chickens Killed for Examination on Day 6.</u>

No lesions were found in the caeca of the chickens of groups R.1 or R.5 to R.16. In group R.2 the caecal mucosa appeared very slightly thickened, but there was no indication of haemorrhage present. Chickens were not available for post mortem examination from group R.3 and R.4.

Table 4. 13.

The Mean Weight Gains of the Chickens on Day 12, Day 19 and Day 28 to Illustrate the Effect on the Growth Rate Following Infection with Different Numbers of Sporulated Oocysts of *E. tenella* on Day 0 and Reinfection on Day 21 with a Standard Challenge Dose of 32,000 Oocysts. The Inoculum Administered to Groups R.1 to R.16 was Exposed to Different Levels of X-Irradiat[ion] on the Day before Inoculation, Day 0-1.

Group	Day 0-1	Gain Day 12	%Gain Control	Gain Day 19	%Gain Control	Gain Day 28	%Gain Control
Control	262x	283	100	503xx	100	251	100xxx
Ch.1	295			590	117	229	91
Ch.2	293			588	117	205	84
E.1.	309	241	85	496	99	309	123
E.1/2	296	227	80	500	99	325	130
R.1.	303	325	115	605	118	290	116
R.2.	308	349	123	641	128	302	120
R.3.	305	323	114	500	100	389	155
R.4.	301	301	106	564	112	259	103
R.5.	307	335	118	631	125	297	119
R.6.	307	330	117	645	128	276	110
R.7.	304	318	112	584	116	279	111
R.8.	299	251	89	516	103	318	127
R.9.	319	331	117	609	121	242	96
R.10.	308	334	118	588	117	272	100
R.11.	312	329	116	625	125	247	99
R.12.	302	266	94	545	108	295	118
R.13.	310	329	116	589	117	267	106
R.14	315	337	119	599	110	320	128
R.15.	314	337	119	606	121	260	104
R.16.	316	281	100	555	110	246	98

x Weight expressed in grammes.

xx The value on day 19 represents the total weight gain, day 19 - day 0-1.

xxx The value on day 28 represents the weight gain made between day 19 and day 28.

<u>(ii) Post Mortem Examination of the Chickens Killed on Day 28.</u>

Typical lesions of caecal coccidiosis were found in the birds from the fully susceptible challenge group Ch.1 and Ch.2 (Figures 17, 18 and 19). The walls of the caeca were markedly thickened with numerous petechial haemorrhages on the mucosa. The lumina contained extravasated blood and cellular debris which appeared very dry in consistency.

No significant lesions were present in the caeca of the birds from group S.1 and S.1/2 (Figure 19), the only indication of recent infection being shown by the presence of a very small amount of blood in the caecal contents. However, the caecal walls appeared slightly thickened, suggesting fibrosis, representing a residual lesion from the initial infection on day 0.

Very slight lesions were demonstrated in the caeca of only four chickens in group R.1, no indication of recent infection being seen in the other six birds of this group. The caecal walls were very slightly thickened and oedema was evident in the mucosa. Only a little blood was present in the lumen of some caeca while others contained blood-stained necrotic debris. Lesions were found in only three of the ten birds examined in group R.2. The changes appeared less pronounced compared with those in the chickens of group R.1. Only a very little blood was present in the contents of the caeca. The only evidence of recent infection seen in the chickens of group R.3 was indicated by slight thickening of the caecal walls in two of the ten birds examined and by the presence of a very small amount of blood in the caecal contents which appeared normal in consistency and appearance. No sign of haemorrhage was found in the caeca from the chickens of group R.4. The sole indication of infection was shown by the slight thickening of the caecal mucosa seen in one bird from the ten survivors.

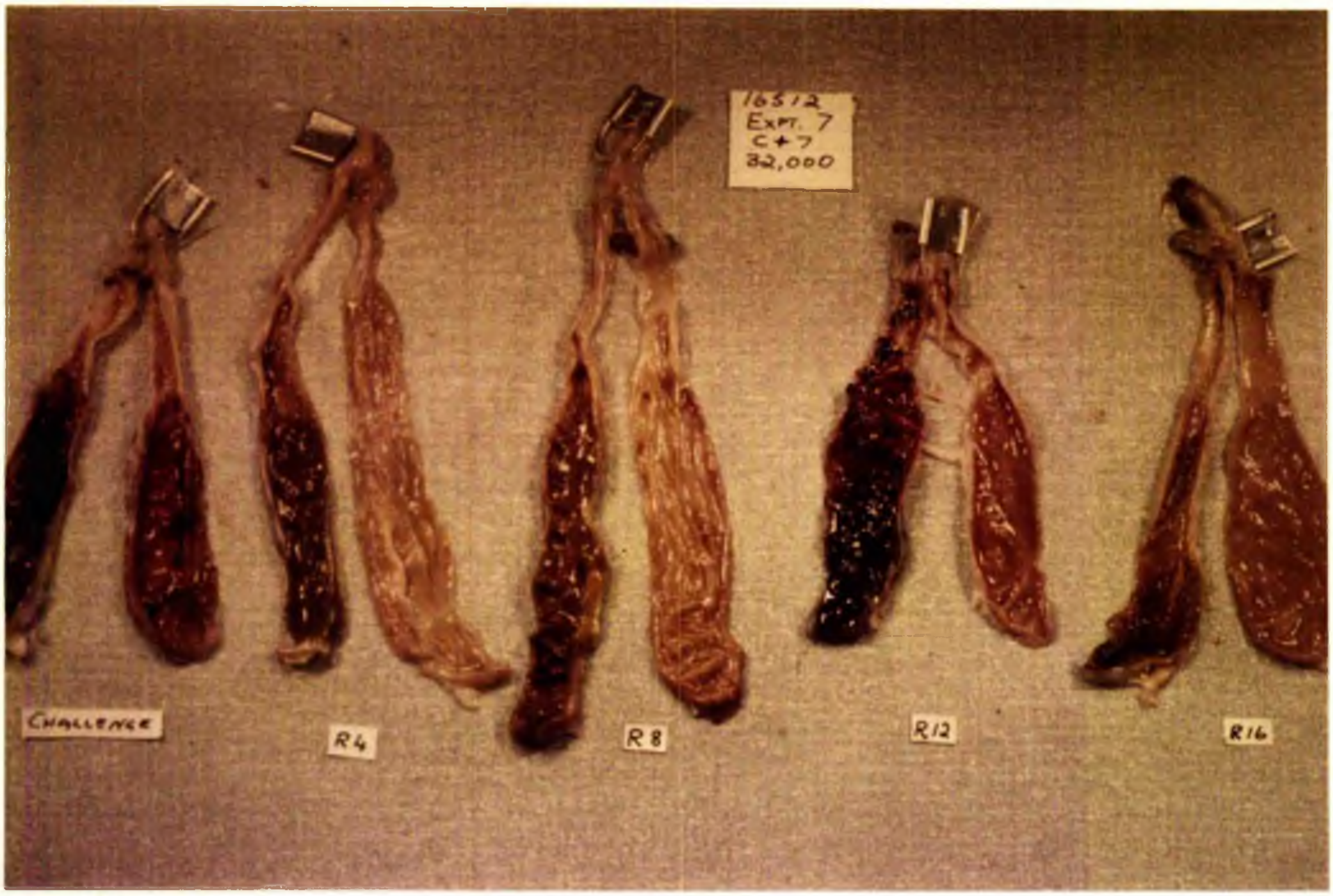
Lesions were demonstrated in two of the chickens of group R.5, being reflected by quite widespread thickening in the caecal walls and by the presence of a little blood in the caecal lumen which appeared slightly more pronounced than that seen in the four birds of R.1. Quite marked lesions were found in five of the ten chickens in group R.6, where they appeared more severe than those in R.5, R.7 or R.8. The caecal walls were quite obviously thickened and the lumen of the caeca contained an appreciable amount of blood in the contents. Very slight lesions were present in four chickens of group R.7 and in three chickens of group R.8. The caecal walls were slightly thickened and a very small amount of blood was seen in the caecal contents. The lesions seemed more pronounced in group R.8 where small haemorrhages were also found on the mucosa.

Quite marked changes were demonstrated in the caeca of the birds of groups R.9 to R.16 (Figure 17) in which approximately 50% of the chickens in each group had lesions present in the caeca. The caecal walls were obviously thickened and the caecal lumen contained extravasated blood and cellular debris. Lesions appeared slightly greater in group R.10 where numerous small haemorrhages were present on the mucosa, associated with widespread erosion of the caecal epithelium. However, the lesions in these chickens were all less severe than those in the chickens of group Ch.1 and Ch.2 where marked evidence of recent infection was demonstrated in all the surviving birds.

(iii) Post mortem Examination of the Chickens which Died After Inoculation on Day 0 and after Reinfection on Day 21.

Examination of the chickens which died on day 5 from group R.1/2 and on Day 26 and Day 27 from group Ch.2 and R.14 showed typical lesions of acute caecal coccidiosis.

FIGURE 17.



IMMUNITY CONFERRED BY OOCYSTS EXPOSED TO 10,000R, 15,000R, 20,000R OR 25,000R AGAINST REINFECTION WITH 32,000 NORMAL OOCYSTS.

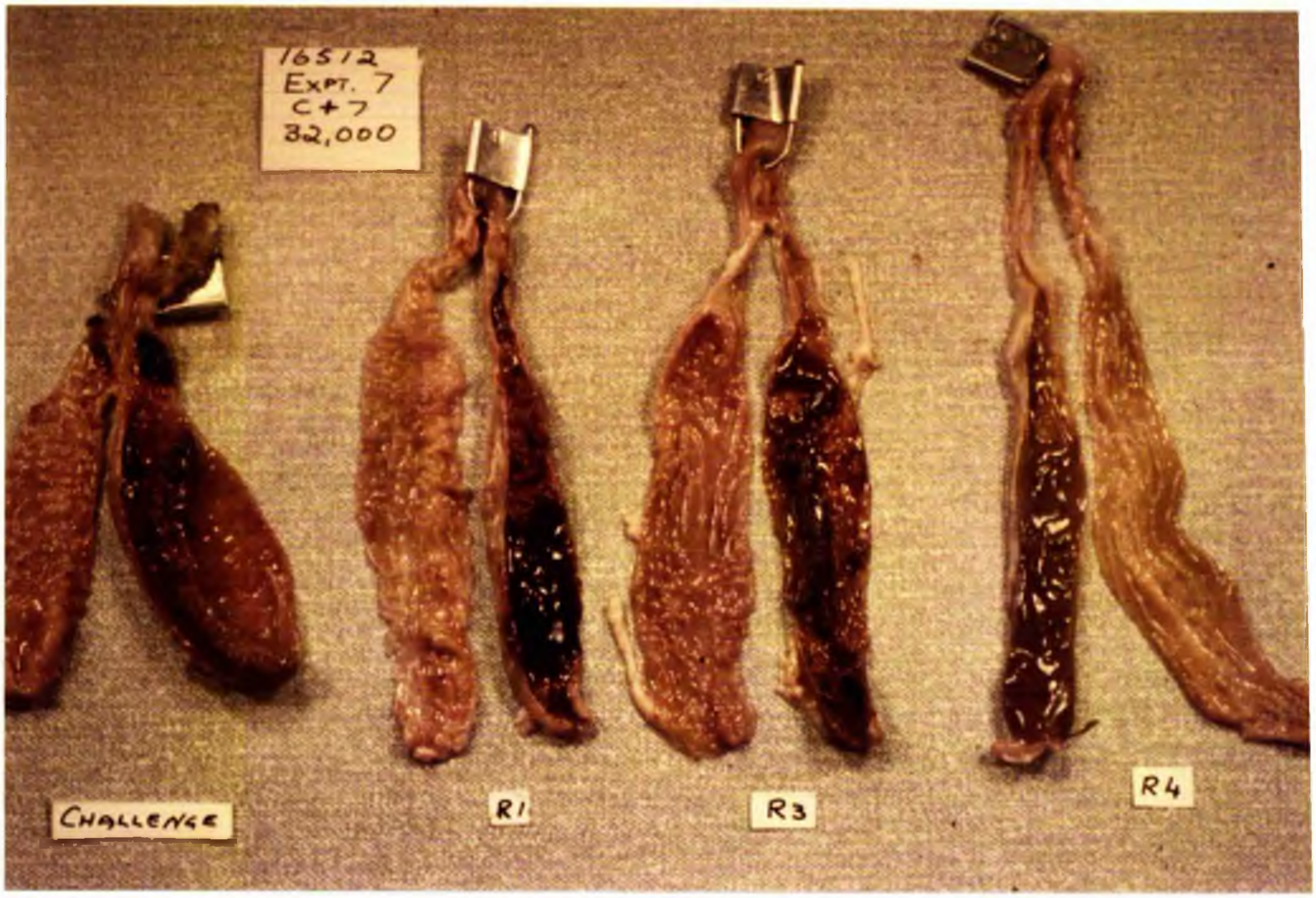
Note:-

(1) Typical signs of caecal coccidiosis in the caeca of fully susceptible bird on the left.

(2) Absence of lesions in birds immunised with oocysts exposed to 10,000R or 15,000R.

(3) Pronounced evidence of haemorrhage in birds immunised with oocysts exposed to 20,000R or 25,000R.

FIGURE 18.



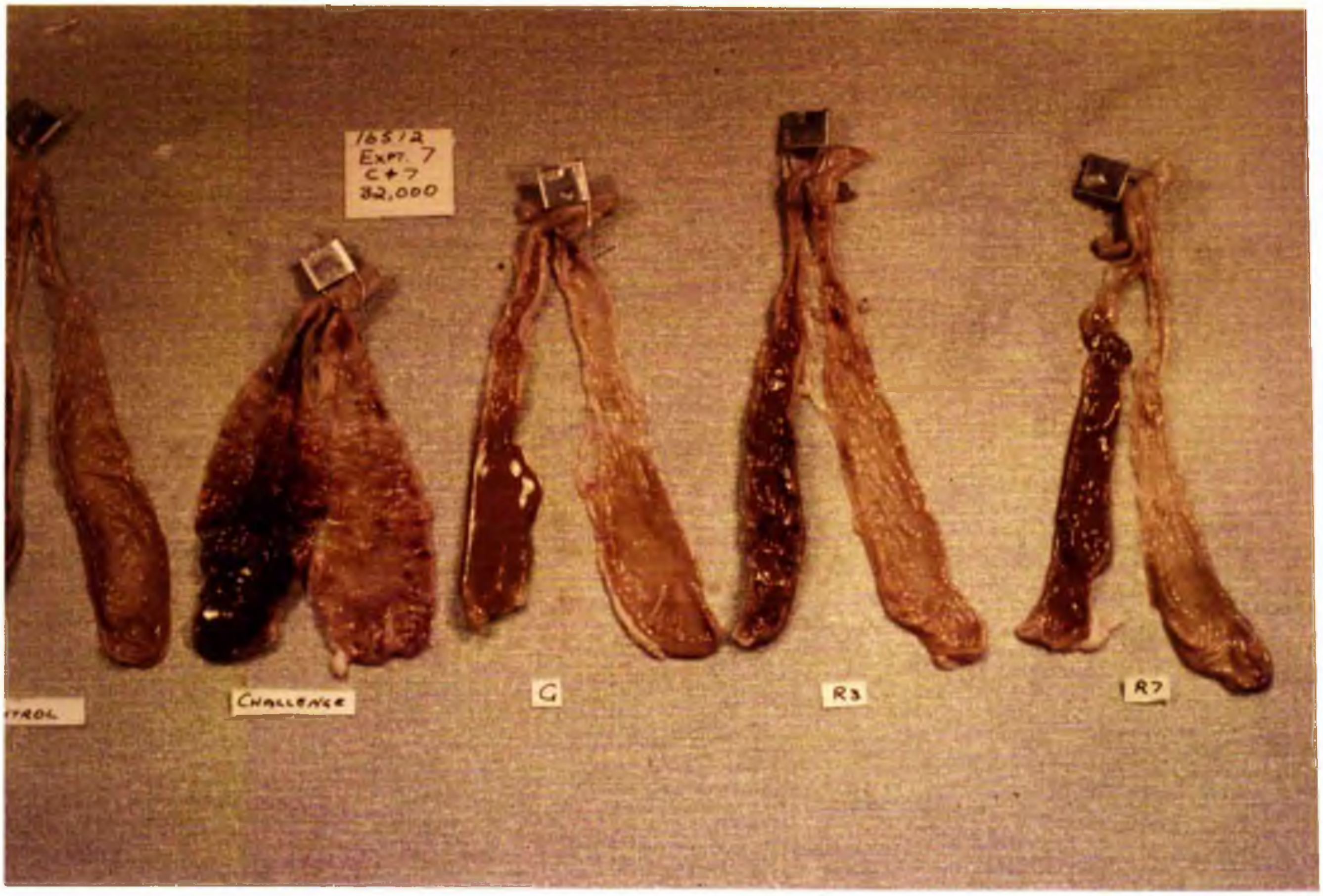
IMMUNITY CONFERRED BY DIFFERENT DOSES OF OOCYSTS, AFTER EXPOSURE TO 10,000R, AGAINST REINFECTION WITH 32,000 NORMAL OOCYSTS.

Note:-

(1) Typical signs of caecal coccidiosis in fully susceptible bird (left).

(2) Quite marked evidence of haemorrhage in bird receiving lowest immunising dose of 32,000 oocysts (R.1), compared with only traces of blood in the caeca of bird given 128,000 X-irradiated oocysts (R.3), and the complete absence of lesions in bird immunised with 256,000 oocysts (R.4).

FIGURE 19.



THE RESISTANCE CONFERRED BY 128,000 OOCYSTS, AFTER EXPOSURE TO 10,000R OR 15,000R, AGAINST REINFECTION WITH 32,000 NORMAL OOCYSTS.

Note:-

(1) The typical lesions of caecal coccidiosis in susceptible bird (Ch.) and the absence of lesions in birds immunised with normal oocysts (G) and those immunised with irradiated oocysts (R.3, R.7).

(2) Similarity of appearance between caeca from normal non-infected bird (extreme left) and those of immunised chickens (G, R.3, R.7).

(6) Oocyst Production.

The results (Table 4. 14) demonstrated a very significant difference between the total oocyst production of group E.1 and group R.1 to R.16. The total average oocyst production was 97 million per bird in group E.1, compared with only a total average output of approximately 1-3 million per bird in groups R.1 - R.4, and none from the majority of groups which received oocysts exposed to levels of X-irradiation above 10,000R. The oocyst production increased slightly from R.1 to R.4, being approximately 1, 1.5, 2 and 3 million per bird respectively. Less than 50,000 oocysts per bird were found in the samples from groups R.6, R.7, R.10 and R.15, while none were present in the faeces from groups R.5, R.8, R.9, R.11, R.12, R.13, R.14 and R.16 during the patent period of the disease after the initial infection.

The results (Table 4. 15) indicated quite a marked variation in the oocyst production on the first day of the patent period after reinfection, the samples being available only on day 7 before the chickens were killed for post mortem examination. The total average oocyst production was 91 million per bird in group Ch.1, compared with approximately 3 million per bird in groups R.1 - R.4, 20 million in R.5, 9 million in R.6 - R.8 and 40 million in groups R.9 - R.16, with the exception of R12 where the output was only 22 million per bird. In group E.1 the production was only 0.1 million oocysts per bird.

DISCUSSION.

The results (Table 4. 16) clearly demonstrated the attenuating effects of X-irradiation on the pathogenicity of sporulated oocysts of Eimeria tenella. There was no evidence of clinical disease after administration of sporulated oocysts exposed to Rontgen doses ranging from 10,000R to 25,000R before infection.

Table 4. 14.

The Average Total Daily Oocyst Production of the Chickens, Expressed in Millions of Oocysts per Bird, After Infection with Different Numbers of Sporulated Oocyst of *E. tenella* on Day 0. The Inoculum Administered to Groups R.1 to R.16 was Exposed to X-Irradiation on the Day before Infection.

Day	[illegible]	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16
7	32.1	0.1	0.2	0.2	0.2	-	-	-	-	-	-	-	-	-	-	-	-
8	28.7	0.4	0.5	0.6	1.6	-	*	-	-	-	*	-	-	-	-	*	-
9	9.1	0.3	0.4	0.6	0.6	-	-	-	-	-	-	-	-	-	-	*	-
10	3.6	0.1	0.2	0.3.	0.3	-	-	*	-	-	-	-	-	-	-	-	-
11	1.9	0.1	0.1	0.1	0.1	-	-	-	-	-	*	-	-	-	-	-	-
12	7.4	*	*	0.1	*	-	-	-	-	-	*	-	-	-	-	-	-
13	3.4	-	*	*	*	-	-	-	-	-	-	-	-	-	-	-	-
14	3.3	*	*	*	*	-	-	-	-	-	-	-	-	-	-	-	-
15	3.0	*	*	*	*	-	-	-	-	-	-	-	-	-	-	-	-
16	1.3	*	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
17	1.4	*	-	*	*	-	-	-	-	-	-	-	-	-	-	-	-
18	0.7	-	-	*	*	-	-	-	-	-	-	-	-	-	-	-	-
19	0.6	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
20	0.4	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
21	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total** per Bird	97.0	0.9	1.4	1.9	2.9	0	*	*	0	0	*	0	0	0	0	*	0

* Indicates that oocyst production was less than 50,000 per bird.

** The total production of oocysts per bird, calculated to nearest million oocysts.

Table 4. 16.

The pathogenic Effects of *E. tenella* in Chickens Following Inoculation with Different Numbers of Sporulated Oocysts on Day 0, the Inoculum Given to Groups R.1 - R.16 being Exposed to Different Levels of X-Irradiation on Day 0-1, and Subsequently after Reinfection with a Standard Challenge Dose of 32,000 Sporulated Oocysts per Bird on Day 21.

Group	Oocyst Dose	Rontgen Dose	Haemoglobin* Decrease (1)	Haemoglobin* Decrease (2)	Mortality (1)	Mortality (2)	% Weight Gain** Day 12	% Weight Gain** Day 28	Oocyst*** Output (1)	Oocyst*** Output (2)
Control	nil	nil	-	-	-	-	100	100	-	-
Ch.1	"	"	-	2.4	-	-	-	91	-	91
Ch.2	"	"	-	3.4	-	1	-	84	-	91
E.1.	32,000	"	4.8	-	-	-	85	128	97	.1
E.1/2	32,000	"	4.5	-	2	-	80	130	-	-
R.1.	32,000	10,000	-	-	-	-	115	116	1	4
R.2.	64,000	"	-	-	-	-	123	120	1	3
R.3.	128,000	"	-	-	-	-	114	166	2	3
R.4.	256,000	"	-	-	-	-	106	103	3	2
R.5.	32,000	15,000	-	-	-	-	118	119	-	20
R.6.	64,000	"	-	-	-	-	117	110	-	10
R.7.	128,000	"	-	-	-	-	112	111	-	9
R.8.	256,000	"	-	-	-	-	89	127	-	8
R.9.	32,000	20,000	-	0.8	-	-	117	96	-	43
R.10.	64,000	"	-	0.8	-	-	118	100	-	33
R.11.	128,000	"	-	1.3	-	-	116	99	-	34
R.12.	256,000	"	-	0.7	-	-	94	118	-	22
R.13.	32,000	25,000	-	1.0	-	-	116	106	-	44
R.14.	64,000	"	-	1.8	-	1	119	128	-	41
R.15.	128,000	"	-	0.6	-	-	119	104	-	37
R.16.	256,000	"	-	1.4	-	-	100	98	-	41

* Haemoglobin expressed as grammes per 100 ml. of blood. (1) and (2) represent the decrease recorded following the inital and challenge infections.

** The gain in weight expressed as a percentage of the gain recorded in the non-infected control chickens. The value on day 29 represents the gain made between day 19 and day 28.

*** The average total oocyst production per bird to nearest million, recorded between day 7 and day 21 inclusive, and the output on day 28 alone being tabulated under (1) and (2) respectively.

Satisfactory weight gains were recorded in the birds after inoculation which equalled those attained by the non-infected control chickens (Table 4. 13). Post mortem examination on day 5 confirmed the absence of any detrimental effects associated with immunisation with irradiated oocysts. No lesions were found in the caeca of th chickens, in contrast to the very severe changes demonstrated in the chickens of group E.1 and E.1/2 which received normal oocysts on day 0. The pathogenicity of the culture before X-Irradiation was also emphasised by the marked fall of 4.5g. of haemoglobin (Table 4. 11), by the mortality and high morbidity and by the depression of the growth rate (Table 4. 13) which were recorded in group E.1 and E.1/2 after infection.

The post mortem findings on day 28 indicated a high level of resistance to reinfection in the chickens which received oocysts exposed to 10,000R on day 0. Very slight lesions were found in the caeca of some of the birds which received 32,000 or 64,000 oocysts per bird on day 0, while no significant lesions were seen in the chickens given the higher levels of infection of 128,000 or 256,000 oocysts per bird (Figure 18). This suggested the development of a slightly greater degree of immunity when the inoculum was increased from 64,000 to 128,000 oocysts per bird. Slight lesions were found in the caeca of some birds in each group which received oocysts exposed to 15,000R on day 0. The lesions appeared most severe in the chickens inoculated with 64,000 oocysts, although the oocyst production on the first day of the patent period was greater in the birds given 32,000 oocysts. Comparison of the severity of the caecal lesions in the chickens of R.1 - R.4 and R.5 - R.8 suggested that immunity was greater in the former birds which received oocysts exposed to 10,000R than in the chickens inoculated with oocysts exposed to 15,000R. This difference was also reflected in the oocyst production on day 28 when the average production was approximately 4 -2 and 20 - 8 million per bird respectively.

Post mortem examination and the oocyst production on day 28 both confirmed that resistance to reinfection was significantly lower after immunisation with oocysts exposed to either 20,000R or 25,000R (Figure 17). This was emphasised by the death of one bird in group R.14 on day 27. No definite variation in the resistance of these birds was indicated between those receiving either oocysts exposed to 20,000R or 25,000R on day 0, nor was any difference suggested between the immunity conferred by the different number of oocysts in the inoculum, although the oocyst production was slightly lower on day 28 in the chickens of group R.12 which received 256,000 oocysts exposed to 20,000R.

These results demonstrate conclusively that good immunity is conferred by the administration of oocysts exposed to 10,000R without detrimental effects during immunisation to reinfection with 32,000 sporulated oocysts 21 days after inoculation. Immunity appears slightly higher when the inoculum is increased from 32,000 to 256,000 oocysts per bird. Resistance is also developed after administration of oocysts exposed to 15,000R but the level of immunity is slightly lower compared with that conferred by oocysts exposed to 10,000R. Resistance is significantly lower when the oocysts are exposed to either 20,000R or 25,000R before inoculation.

The demonstration of resistance in birds given oocysts exposed to 15,000R, 20,000R or 25,000R, which passed no oocysts during the patent period after vaccination, suggests that the development of the parasite is only partially inhibited by these levels of X-irradiation, as it is generally accepted that schisogony is essentially for the stimulation of acquired immunity. Therefore the attenuating effect of X-irradiation on the pathogenicity of the sporulated oocysts of E. tenella is not due to a lethal effect alone on the viability of the oocysts leading to a simple reduction in the number of oocysts in the infective dose.

The results of the haemoglobin estimation (Table 4. 12) after reinfection suggested a variation in the resistance between the chickens of groups R.1 - R.8 on the one hand and R.9 - R16 on the other, although the decrease in the level of haemoglobin which occurred only in the latter groups was very slight, ranging from 0.6g. to 1.8g. The change in the level of haemoglobin in these birds was less than that of 2.4g. to 3.4g. recorded in the susceptible birds of group Ch.1 and Ch.2.

Good immunity to reinfection was demonstrated in the survivors of group E.1 and E.1/2 (Figure 19) which received normal oocysts on day 0, but the disadvantages of this method of immunisation were clearly shown by the increase in the level of haemoglobin, by the mortality and severe morbidity and the high oocyst production which were recorded in these chickens after inoculation.

The only indication of infection after immunisation was shown by the presence of a relatively small number of oocysts in the faeces of groups R.1 - R.4 during the patent period of the disease. The average total oocyst production was 0.9, 1.4, 2 and 3 million per bird in each group respectively, in contrast to 97 million per bird in group E.1(Table 4. 14). The results clearly emphasise that no detrimental effects occur when the infective dose is increased from 32,000 to 256,000 irradiated oocysts per bird. When the inoculum was exposed to 15,000R, 20,000R or 25,000R, oocysts were only present in the samples of one or two groups, the output being less than 50,000 per bird.

The pathogenicity of the challenge dose of oocysts was confirmed by the decrease in the concentration of haemoglobin, by the morbidity and mortality and the high oocyst production recorded in the fully susceptible chickens of Groups Ch.1 and Ch.2.

group Ch.1 and Ch.2.

The difference in the degree of immunity conferred by oocysts exposed to 10,000R or 15,000R and 20,000R or 25,000R was clearly reflected in the clinical observations when only very slight evidence of haemorrhage was seen after challenge in the groups R.1 - R.8, in contrast to R.9 - R.16 where haemorrhage appeared quite severe.

EXPERIMENT TEN.

IMMUNISATION AGAINST *EIMERIA TENELLA* INFECTION BY SINGLE AND DOUBLE VACCINATION WITH IRRADIATED OOCYSTS IN 3 WEEK OLD CHICKENS.

EXPERIMENTAL AIMS.

The experiment was designed to study the immunity conferred by single and double vaccination with irradiated oocysts of E. tenella against reinfection with normal oocysts. Earlier experimental observations had demonstrated that resistance to reinfection was developed after infection with a single dose of sporulated oocysts exposed to X irradiation before inoculation without evidence of any detrimental effects during immunisation. No indication of clinical disease was seen when the birds were challenged with 32,000 oocysts but the degree of resistance was not high enough to prevent the appearance of significant pathogenic effects when the challenge dose was raised to 64,000 oocysts per bird. Therefore it was important to see whether the level of immunity could be increased by the administration of two doses of vaccine.

Rontgen levels of 10,000 R, 12,500 R and 15,000 R were selected for observation as the previous results had shown that oocysts must be exposed to a minimum dose of 10,000 R to prevent the appearance of deleterious effects after inoculation, while resistance was significantly reduced if the Rontgen level exceeded 15,000 R.

Several doses of oocysts ranging from 32,000 to 500,000 per bird were selected for comparison. The immunising effect of the same total number of oocysts administered either as one single infection or as two smaller doses of oocysts was also studied by comparison of the appropriate groups following single and double vaccination.

The challenge dose of 32,000 sporulated oocysts was chosen to avoid an over-whelming infection which might mask a variation in the immunity conferred by the different methods of immunisation.

The severity of the disease in each group of chickens was determined from observations made on the changes in the concentration of haemoglobin, on mortality on clinical signs, on growth rate and on oocyst production after both vaccination and reinfection. Birds were killed for postmortem examination after reinfection to determine whether there was any variation in the severity of the caecal lesions between each group which might also indicate a difference in the immunity of the chickens.

MATERIALS AND METHODS.

Experimental Birds:

Broiler type cockerel chicks were used in the experiment. They were reared in complete isolation and were transferred to the experimental units the day before inoculation, where they were kept in metal cages with wire floors. The chicks given irradiated oocysts were housed in a separate room from those which received normal oocysts. The chicks in Group N.2 and the fully susceptible challenge groups were retained in isolation until the day before infection, i.e. day 13 and day 27 respectively. The chicks were fed on a proprietary chick food (British Oil and Cake Mills Ltd. - Baby Chick Crumbs), which were available ad lib except on day 0 and day 14, when it was withdrawn five hours before administration of the vaccine and on day 28 when food was removed from the chickens three hours before infection with the challenge dose of oocysts.

Parasitology:

The culture of E. tenella used for infection of the chickens on day 0 was thirty days old with a total oocyst count of 1,487,500 per ml. and a sporulation count of 80%.

Two 15 ml. aliquots of the culture were exposed to each appropriate dose of X irradiation one day before inoculation, i.e. day 0-1. Total oocyst counts were carried out on each portion of the irradiated culture on day 0 and also on the nonirradiated culture. Dilutions were made to give the correct number of oocysts per ml. for each respective group, by the addition of distilled water to the culture which was suspended in 2% potassium dichromate solution.

The culture administered to the chickens on day 14 was 44 days old with a total oocyst count of 1,487,500 oocysts per ml. and a sporulation count of 80%.

Three 15 ml. aliquots of the culture were each exposed to the appropriate dose of X irradiation one day before inoculation, i.e. day 13. Total oocyst counts were made on each portion of the irradiated culture on day 14 and also on the portion of non-irradiated culture. Dilutions were made in the usual manner to give the appropriate number of oocysts per ml. for each respective group.

The culture administered to the chickens on day 28 was 58 days old with a total oocyst count of 1,308,500 oocysts per ml. and a sporulation count of 80%. Dilutions were made to give 16,000 sporulated oocysts per ml. by the addition of distilled water to an aliquot of the culture.

Administration of the Inoculum:

The infecting dose of irradiated oocysts was given in 2 ml. of water on day 0 and on day 14. The challenge dose of normal oocysts was given in 2 ml. of water on day 28. The inoculum was administered directly into the crop using an automatic dosing syringe. During inoculation the stock of oocysts from which the syringe was filled was kept in suspension by decanting the solution between two beakers.

The chickens were inoculated in the afternoon on day 0 and day 14. The birds given irradiated oocysts were infected before those which received normal oocysts. The challenge dose of oocysts was administered to the chickens in the morning of day 28.

<u>Experimental Design:</u>

Ten chickens were selected at random for each experimental group, with the exception of group E.2 which contained only five chickens. The chickens were 22 days old on day 0.

The chickens in groups R.1 - R.8, R.9 - R.16 and R.17 - R.24 received sporulated oocysts which had been exposed to 10,000R, 12,500R and 15,000R respectively on the day before inoculation. The chickens in groups E.1, E.1/2 and E.2 received normal oocysts.

The inoculum administered to each bird on day 0 contained 32,000 oocysts in groups E.1, E.1/2, R.1, R.2, R.9, R.10, R.17 and R.18; 64,000 oocysts in groups R.3, R.11 and R.19; 128,000 oocysts in R.4, R.5, R.12, R.13, R.20 and R.21; 256,000 oocysts in groups R.6, R.7, R.14, R.15, R.22 and R.23; 500,000 oocysts in groups R.8, R.16 and R.24.

The chickens in groups R.2, R.3, R.5, R.7, R.10, R.11, R.13, R.15, R.18, R.19, R.20 and R.23 were given a second dose of irradiated oocysts on day 14, the infective dose containing the same number of oocysts as the inoculum which was administered to the chickens on day 0. The chickens in group E.2 received 32,000 normal oocysts on day 14.

The chickens in groups R.1 - R.24, E.1/2 and the fully susceptible chickens in groups Ch.1 and Ch.2 received 32,000 normal oocysts on day 28.

Chickens were also infected on day 0 for postmortem examination on day 35. Birds were inoculated to represent groups R.11, R.14, R.15, R.16, R.19, R.22, R.23 and R.24.

Haemoglobin estimations were made on the chickens after inoculation on day 0, on day 2 and day 6. Following reinfection on day 28 estimations were made on day 29 and day 34, i.e. C+1 and C+6. Estimations were carried out on the chickens of group E.2 on day 16 and day 20, i.e. day 2 and day 6 after infection.

The chickens were weighed before inoculation on day 0-1 and on day 12 and day 28. The birds were also weighed on the twelfth day after challenge on day 40 and before slaughter on day 49.

The total oocyst production of each group was recorded daily during the patent period of the disease after both the immunising inoculations and the challenge infection between day 7 to day 28 and day 36 to day 49 respectively. Oocyst production was not recorded in group E.1, E.2 or Ch.2, the oocyst production of E.1/2 and Ch.1 representing E.1 and Ch.2 respectively.

Postmortem examination was carried out on day 6 on the birds which died on day 5 and 6 after the initial infection. The chickens of group E.2 were killed for postmortem examination on day 21, i.e. the seventh day after infectio[n] The birds of Ch.2 and E.1 were killed for examination on day 35, i.e. the seventh day after reinfection.

Clinical signs and mortality were also recorded.

RESULTS:

(1) Haematology:

The results (Table 4. 16) of the haemoglobin estimations did not show any significant variation in the concentration of haemoglobin between the non-infecte[d] control chickens and the birds in groups R.1 - R.24 during the course of the experiment. The level of haemoglobin was very slightly higher in the chickens on day 2 and day 6 compared with the values recorded on day 29 and day 34.

On day 6 there was a marked decrease of approximately 4 g. and 5.6 g. of

TABLE 4. 16.

The Mean Haemoglobin Concentration in the Blood of the Chickens after Infection with Different Numbers of Sporulated Oocysts of E. tenella on Day 0, and after Reinfection with a Standard Challenge Dose of 32,000 Sporulated Oocysts per Bird on Day 28. The Oocysts administered to Groups R.1 to R.8; R.9 to R.16; and R.17 to R.24 on Day 0 and Appropriate groups + on Day 14 were exposed to 10,000; 12,500; and 15,000 Rontgen Doses of X Irradiation before Inoculation.

GROUP:	DAY AFTER INOCULATION:			
	0+2	0+6	C+1	C+6
	* **			
Control	8.4 ± 0.5	8.3 ± 0.7	7.6 ± 0.8	7.6 ± 0.7
Ch. 1.	9.1 ± 0.8		7.9 ± 0.5	6.5 ± 1.4
Ch. 1/2.	8.4 ± 0.7		7.3 ± 0.5	6.5 ± 1.7
E. 1.	8.2 ± 0.8	4.0 ± 1.3	7.8 ± 1.0	7.6 ± 0.9
E. 1/2.	8.8 ± 0.6	3.2 ± 1.2	7.8 ± 0.4	7.8 ± 1.2
E. 2.	9.0 ± 0.6	5.0 ± 1.5		
R. 1.	9.6 ± 0.7	9.2 ± 0.9	8.0 ± 0.9	8.2 ± 0.8
R. 2.+	9.1 ± 0.6	8.7 ± 0.6	8.0 ± 0.8	8.3 ± 0.7
R. 3.+	8.7 ± 0.4	8.7 ± 0.5	7.7 ± 0.3	8.1 ± 0.4
R. 4.	8.8 ± 0.5	8.4 ± 0.7	8.0 ± 0.4	8.2 ± 0.6
R. 5.+	9.3 ± 0.4	8.7 ± 0.4	8.1 ± 0.4	8.4 ± 0.4
R. 6.	9.1 ± 0.9	8.6 ± 1.0	8.6 ± 1.0	8.6 ± 1.2
R. 7.+	9.3 ± 0.4	8.6 ± 0.6	8.1 ± 0.6	8.2 ± 0.6
R. 8.	9.2 ± 0.8	8.4 ± 0.6	7.8 ± 0.9	8.3 ± 0.7
R. 9.	9.3 ± 0.7	8.5 ± 0.7	8.0 ± 0.5	7.9 ± 0.9
R. 10.+	9.3 ± 0.7	8.6 ± 0.9	7.8 ± 0.8	7.6 ± 0.9
R. 11.+	8.9 ± 0.4	8.5 ± 0.4	7.5 ± 0.6	7.5 ± 0.8
R. 12.	9.0 ± 0.6	8.6 ± 0.5	7.7 ± 0.5	7.8 ± 0.6
R. 13.+	9.0 ± 0.6	8.6 ± 0.5	7.8 ± 0.7	7.8 ± 1.0
R. 14.	8.8 ± 0.8	8.0 ± 0.6	7.4 ± 0.6	7.2 ± 0.6
R. 15.+	8.9 ± 0.5	8.1 ± 0.6	7.7 ± 0.6	7.6 ± 0.5
R. 16.	8.6 ± 0.4	7.9 ± 0.5	7.3 ± 0.5	7.4 ± 0.9
R. 17.	8.8 ± 0.8	8.7 ± 0.6	7.7 ± 0.6	7.4 ± 0.6
R. 18.+	8.5 ± 0.6	8.3 ± 0.6	8.0 ± 0.5	7.4 ± 0.6
R. 19.+	8.6 ± 0.8	8.4 ± 0.7	8.1 ± 0.8	7.6 ± 0.7
R. 20.	8.5 ± 0.8	8.4 ± 0.8	7.8 ± 0.7	7.5 ± 0.7
R. 21.+	9.2 ± 1.0	8.1 ± 0.7	7.8 ± 0.6	8.3 ± 0.6
R. 22.	8.6 ± 0.6	8.0 ± 0.5	7.5 ± 0.4	7.4 ± 0.5
R. 23.+	8.9 ± 0.6	8.1 ± 0.9	7.7 ± 0.9	7.5 ± 0.8
R. 24.	8.8 ± 0.6	7.4 ± 0.5	7.9 ± 0.6	7.6 ± 0.7

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

** The standard deviation.

haemoglobin in groups E.1 and E.1/2 respectively. There was also a decrease of 4 g. in group E.2 on day 20, i.e. day 6 after infection.

Following reinfection on day 28 a small decrease of approximately 1 g. of haemoglobin was recorded in the fully susceptible chickens of groups Ch.1 and Ch

No change occurred in the concentration of haemoglobin of the chickens in groups R.1 to R.24 following either inoculation with irradiated oocysts or after reinfection with normal oocysts.

(2) Mortality:

Three deaths occurred in group E.1 and one death occurred in group E.1/2 on day 5. A second bird died in group E.1/2 on day 6, giving a total mortality of 30% and 20% in groups E.1 and E.1/2 respectively. (Table 4. 21.)

(3) Clinical Findings:

No evidence of clinical disease was seen in the chickens of groups R.1 - R.2 after administration of the irradiated vaccine.

Marked clinical symptoms were observed on day 5 and 6 in the chickens of groups E.1 and E.1/2. Morbidity was pronounced, the chickens appearing very depressed, while haemorrhage was also severe, being reflected in the palor of the mucous membranes of the birds. Haemorrhage was only slight on day 7 althoug the birds still seemed quite depressed.

Clinical symptoms were also observed in the chickens of group E.2 after infection on day 14, slight evidence of haemorrhage being seen on day 19 and day 20 when the birds also appeared rather depressed. However, morbidity was less pronounced than that seen in groups E.1 and E.1/2.

No indication of morbidity was observed in the chickens of groups E.1, E.1/2 or R.1 - R.24 after reinfection on day 28. A very slight indication of haemorrhage was seen in the pens of groups R.1, R.19 and R.21 on day 34. Evidence of haemorrhage was slightly more pronounced in groups R.9, R.17, R.18, R.22 and R.24, blood being found in the faeces on both day 33 and day 34. In contrast to these groups haemorrhage was quite marked in the fully susceptible chickens of group Ch.1 and Ch.2 on day 33 and 34, when these birds also appeared slightly depressed.

(4) Weight Gains:

The results (Table 4.17) showed no significant variation in the weight of the chickens in each group on the day before infection, day 0 - 1.

There was no significant difference in the mean weight gains of chickens between the noninfected control group and groups R.1 - R.24 following either inoculation with irradiated oocysts or reinfection with normal oocysts. The mean weight gains recorded in groups R.1 - R.24 equalled those attained by the non-infected control group on day 12, day 28, day 40 and day 49, with only one exception on day 12 when the mean weight gain was slightly less satisfactory in group R.24.

On day 12 the gain in weight was markedly lower in the birds which received normal oocysts on day 0 compared with the non-infected control group and the chickens of groups R.1 - R.23. The growth rate appeared most depressed in group E.1/2 where the mean weight gain was only 290 g. compared with 382 g. in the control group and 312 g. in group E.1. The retardation of growth was still evident in mean weight of the chickens of group E.1/2 which was recorded on day 28 and day 49.

There was no significant difference between the mean weight gain of the chickens in group Ch.1 and the noninfected control group (after reinfection on day 40 or day 49).

TABLE 4. 17.

The Mean Weight Gains of the Chickens on Day 12, Day 40 and Day 49 to Illustrate the Effect on the Growth Rate after Infection with Different Numbers of Sporulated Oocysts of *E. tenella* on Day 0 and Day 14 and after Reinfection with a Standard Challenge Dose of 32,000 Sporulated Oocysts on Day 28. The Oocysts Administered to Groups R.1 to R.8; R.9 to R.16; and R.17 to R.24 on Day 0 and Appropriate Groups * on Day 14 were Exposed to 10,000, 12,500 and 15,000 Rontgen Doses of X Irradiation before Inoculation

Group:	Day 0-1	Wt.Gain Day 12.	Day 28	Wt.Gain Day 40.	Day 49	Wt.Gain Day 49.
Control	336*	382	1175	551	1916	1581**
Ch. 1.	342		1234	569	1946	1603
Ch. 1/2.	359		1344	-	-	-
E. 1.	377	312	1254			
E. 1/2.	374	290	1116	609	1869	1495
R. 1.	378	356	1253	538	1943	1566
R. 2.*	381	361	1318	584	2047	1666
R. 3.*	393	367	1274	603	2004	1612
R. 4.	384	382	1266	545	1973	1589
R. 5.*	367	371	1261	571	1982	1625
R. 6.	374	359	1252	581	1967	1593
R. 7.*	340	393	1257	594	1978	1638
R. 8.	362	349	1251	582	2000	1638
R. 9.	361	368	1269	572	2007	1646
R. 10.*	337	366	1179	469	1783	1446
R. 11.*	346	347	1193	617	2004	1657
R. 12.	368	362	1253	542	1959	1591
R. 13.*	388	379	1288	512	1956	1568
R. 14.	385	386	1264	563	1974	1584
R. 15.*	360	362	1226	584	1963	1603
R. 16.	365	361	1273	557	1990	1625
R. 17.	349	338	1176	513	1853	1504
R. 18.*	360	389	1270	522	2031	1672
R. 19.*	367	374	1266	663	2127	1760
R. 20.	343	361	1226	570	1937	1596
R. 21.*	350	366	1262	588	1976	1626
R. 22.	349	340	1227	533	1930	1581
R. 23.*	358	346	1237	566	1963	1596
R. 24.	362	319	1243	569	1970	1608

* Weight in grammes.

** Represents total weight gain, i.e. Day 49 - Day 0-1.

(5) Pathology:

(i) Chickens Killed for Postmortem Examination on day 35.

Typical lesions of caecalcoccidiosis were found in the caeca of the fully susceptible chickens of group Ch.2, which were killed for examination on the seventh day after infection (Figures 20 and 21). The lesions were very severe in 3 of the 10 birds, the caecal walls being either markedly thickened or showing evidence of widespread erosion of the mucosa and containing large cores of necrotic debris in the lumens. Quite severe changes were found in the other 7 birds of this group reflected by the markedly thickened caecal walls and the presence of numerous small haemorrhages on the caecal mucosa, which were associated with slight erosion of the caecal epithelium. The lumina contained small amounts of extravasated blood and cellular debris.

No evidence of recent infection was found in the caeca of the six survivors from E.1 although the walls were slightly thickened indicating fibrosis associated with lesions from the initial infection on day 0.

Evidence of the challenge infection was shown by very slight thickening of the caecal mucosa in the chickens of group R.14, R.22 and R.23, while a little blood was also found in the caeca of one bird in group R.16 (Figure 20 and Figure 21). The changes were more pronounced in group R.24, the caecal walls being quite markedly thickened with evidence of slight erosion of the mucosa. The caecal lumina also contained a small amount of extravasated blood and cellular debris.

No lesions were present in the birds examined from groups R.11, R.15 or R.19 (Figure 20 and Figure 21).

(ii) Postmortem Examination of the Chickens which Died on Day 5 and Day 6:

Examination of the chickens which died on day 5 and day 6 from group E.1 and E.1/2 after inoculation on day 0 with normal oocysts demonstrated typical

FIGURE 20.

IMMUNITY CONFERRED BY SINGLE OR DOUBLE VACCINATION WITH OOCYSTS EXPOSED TO 12,500 ROENTGENS AGAINST REINFECTION WITH 32,000 NORMAL OOCYSTS.

Note:-

(1) Typical lesions of acute caecal coccidiosis in fully susceptible chicken (Ch.).

(2) The absence of lesions in the birds immunised with two doses of 64,000 (R.11), one dose of 256,000 (R.14) or two doses of 256,000 (R.15) X-irradiated oocysts.

(3) The evidence of haemorrhage in bird immunised with single dose of 500,000 X-irradiated oocysts (R.16). The low resistance in this bird may be associated with possible potassium dichromate intoxication during immunisation.

FIGURE 21.

IMMUNITY CONFERRED BY SINGLE OR DOUBLE VACCINATION WITH OOCYSTS EXPOSED TO 16,000R AGAINST REINFECTION WITH 32,000 NORMAL OOCYSTS.

Note:-

(1) Typical lesions of caecal coccidiosis in fully susceptible bird, in contrast to no evidence of infection in caeca of birds immunised with either two doses of 64,000 (R.19), one dose of 256,000 (R.22) or two doses of 256,000 X-irradiated oocysts (R.23).

(2) Very slight evidence of haemorrhage in caeca of bird immunised with one dose of 500,000 irradiated oocysts (R.24). It is possible that the lower immunity of this bird may be associated with toxicity from potassium dichromate during immunisation.

lesions of acute caecal coccidiosis.

(iii) <u>Postmortem Examination of the Chickens of Group E.2 killed on day 21.</u>

Examination of the five chickens of group E.2 on the seventh day after infection demonstrated severe lesions of caecal coccidiosis.

6. <u>Oocyst Production:</u>

The results (Table 4. 18) demonstrated a very significant difference between the total oocyst production of group E.1/2 and groups R.1 - R.24 (after infection on day 0). The average total oocyst production was 77 million per bird in group E.1/2 in contrast to only 1 - 9 million per bird in groups R.1 - R.8, and 0.1 - 2 million per bird in groups R.9 - R.24. The results showed a slight variation between the oocyst production of the chickens in groups R.1, R.2 and R.3 on the one hand and those in groups R.4, R.5, R.6, R.7 and R.8 on the other hand, the average total production per bird being approximately 2 and 8 million oocysts respectively. There was no significant variation between the oocyst production of groups R.9 - R.24 which ranged from 0.1 - 2 million oocysts per bird being slightly lower than that of groups R.4 - R.8. No significant increase in oocyst production occurred after the administration of the second dose of irradiated oocysts on day 14.

The results (Table 4. 19) showed a significant difference in the oocyst production of the fully susceptible chickens of group Ch.1 and the chickens of groups R.1 - R.24 after administration of the challenge dose of oocysts on day 28. The average total oocyst production per bird was 119 million in group Ch.1, compared with an output ranging from 1 - 27 million oocysts in groups R.1 - R.8, 5 - 31 million oocysts in groups R.9 - R.16, and 7 - 85 million oocysts in groups R.17 - R.24.

Table 4. 18.

The Average Total Daily Oocyst Production of the Chickens, Expressed in Millions of Oocysts per Bird, after Infection with Different Numbers of Sporulated Oocysts on Day 0 and Day 14 (Groups +). The Oocysts Administered to Groups R.1 to R.8, R.9 to R.16 and R.17 to R.24 on Day 0 and Appropriate Groups + on Day 14 were Exposed to 10,000, 12,5000 and 15,000 Rontgen Doses of X-Irradiation before Inoculation.

Day	E1/2	R1/R2+	R.3+	R4/R5+	R6/R7+	R8	R9/R10
7	38.3	0.2	0.4	1.4	1.0		*
8	10.0	0.4	0.6	4.0	3.3	3.6	*
9	10.1	0.4	0.6	1.5	2.1	3.5	*
10	2.3	0.1	0.2	0.3	0.5	1.0	*
11	1.7	*	*	0.3	0.2	0.2	*
12	2.0	*	*	*	0.1	0.1	-
13	0.3	*	*	*	*	*	-
14	1.7	-	*	*	*	*	-
15	1.7	-	*	*	0.1	*	*
16	2.4	-	*	0.1	*	*	-
17	0.5	*	*	0.1	0.1	*	*
18	0.3	*	0.1	0.1	*	0.3	-
19	0.2	*	0.1	0.1	0.1	0.5	-
20	0.2	*	0.1	0.1	*	0.3	-
21	0.5	*	*	*	*	0.2	*
22	0.7	*	0.1	*	*	*	*
23	0.8	*	*	*	*	*	*
24	0.7	*	*	*	-	-	*
25	1.0	-	-	-	-	-	*
26	0.8	-	-	*	*	-	*
27	0.3	-	-	*	-	-	-
28	0.7	-	-	-	-	*	-
Total/Bird**	77	1	2	8	8	9	.1

* Indicates that oocyst production was less than 50,000 per bird.

** The total production of oocysts per bird calculated to nearest million oocysts.

R11	R12/13	R14/R15	R16	R17/R18	R19	R20/21	R22/R23	R24
·	0.1	0.3			·	·	·	
0.1	0.3	1.1	1.3	0.2	·	-	0.1	·
0.1	0.1	0.6	0.7	0.1	·	·	0.1	·
·	·	0.2	0.3	0.1	·	·	·	-
-	-	0.1	0.1	·	·	-	·	·
·	-	·	·	·	·	-	·	·
·	·	·	·	-	·	-	-	-
-	-	·	·	-	·	-	·	-
-	·	·	·	-	·	-	·	-
-	-	·	-	-	-	-	-	-
·	-	-	-	·	·	-	-	-
-	-	-	·	-	-	-	-	-
-	-	·	·	-	·	-	·	·
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-	-	-	-	·	-	-	-	·
-	-	-	-	-	-	-	-	·
-	-	-	-	-	·	·	-	-
-	-	-	-	-	-	·	-	-
·2	·5	2	2	·4	·2	·1	·3	·2

Table 4. 19.

The Average Total Daily Oocyst Production of the Chickens, Expressed in Millions of Oocysts per Bird, after Reinfection with a Standard Challenge Dose of 32,000 Sporulated Oocysts of E. tenella on Day 28.

Day	Ch.	E.1	R.1	R.2+	R.3+	R.4	R.5+	R.6	R.7+	R.8	R.9	R.10+
6	6.0	-	0.3	0.1	*	-	-	-	-	*	0.7	-
7	60.9	*	2.3	3.5	1.9	1.0	0.1	0.2	0.3	1.5	8.4	2.6
8	15.6	0.2	3.2	5.1	1.8	1.4	1.3	1.7	0.1	4.1	1.9	4.2
9	2.1	0.3	4.5	7.7	1.8	0.8	0.8	1.8	0.1	2.6	5.8	4.3
10	3.2	0.2	4.3	2.0	0.4	0.3	0.2	0.5	0.1	0.6	6.3	1.9
11	6.1	0.1	4.2	0.7	0.2	0.1	0.1	0.2	*	0.4	3.6	1.3
12	4.4	*	3.5	0.6	0.2	*7	*	*	*	0.3	1.5	0.3
13	4.6	-	1.2	0.3	*	*	*	*	-	0.1	1.2	0.1
14	7.7	*	0.8	0.3	*	*	*	*	-	0.1	1.1	*
15	2.8	*	0.8	*	*	*	*	-	-	*	0.2	-
16	1.7	-	0.5	*	*	-	-	-	-	*	*	*
17	1.8	-	0.6	-	-	-	-	-	-	-	*	-
18	0.9	-	0.4	-	-	-	-	-	-	-	*	-
19	0.2	-	0.1	-	-	-	-	-	-	-	-	-
20	0.5	-	*	-	-	-	-	-	-	-	-	-
** Total per Bird	119	1	27	20	6	4	3	5	1	10	31	15

* Indicates that oocyst production was less than 50,000 per bird.

** The total production of oocysts per bird, calculated to the nearest million oocysts.

\+ Groups which received double vaccination.

R.11+	R.12	R.13+	R.14	R.15+	R.16	R.17	R.18+	R.19+	R.20	R.21	R.22	R.23+	R.24
-	0.1	-	-	-	0.2	0.7	1.4	0.1	0.3	-	0.1	-	0.3
1.5	6.3	8.5	0.3	3.6	0.3	40.1	13.9	6.9	21.3	2.4	15.3	0.7	33.6
2.2	3.3	0.5	4.6	0.3	1.8	8.0	14.1	7.3	8.6	4.2	11.1	2.3	7.3
4.7	6.5	1.5	4.0	0.5	2.0	12.9	17.8	10.4	11.3	6.4	10.7	1.9	8.5
1.7	5.4	0.9	1.7	0.2	2.5	8.2	13.3	7.0	8.3	2.6	6.3	1.2	4.3
1.6	3.3	0.3	0.5	*	0.5	6.6	3.1	2.9	4.7	0.8	4.6	0.5	1.9
0.8	1.5	0.3	0.1	-	0.2	2.1	1.4	1.0	3.1	0.4	1.8	0.2	0.9
0.3	1.4	0.1	*	*	0.1	3.0	1.0	0.4	2.4	0.1	0.2	0.1	1.2
0.1	0.3	0.1	0.1	-	0.1	2.4	0.6	0.2	1.6	*	0.1	0.1	1.6
0.1	0.1	-	*	-	*	0.8	0.1	0.1	1.7	*	*	-	0.7
*	0.2	*	-	-	*	0.2	*	*	0.5	*	-	-	0.2
-	0.1	-	-	-	-	0.1	*	*	0.2	-	*	*	0.3
-	*	*	-	-	-	*	*	*	0.2	-	-	*	*
*	0.1	-	-	-	-	-	*	-	0.1	-	-	-	*
-	0.1	-	-	-	-	-	-	-	*	-	-	-	-
13	30	12	11	5	9	85	62	36	64	15	50	7	61

These results demonstrated a very marked difference in the level of immunity conferred by the various doses of irradiated oocysts at each rontgen level and also between the efficacy of single and double vaccination. Following reinfecti[on] the oocyst production was lower in the groups which received double vaccination compared with the corresponding groups which were given only a single dose of irradiated oocysts. The oocyst production after reinfection also appeared to show a relationship with the magnitude of the immunising dose of oocysts administered to the birds on day 0 and day 14. The total output per bird tended to be significantly reduced as the number of oocysts in the vaccine was increased. The oocyst production also tended to be less when the same total number of oocysts in the vaccine were administered in two smaller doses compared with administration in one large dose of vaccine.

These results are illustrated clearly by the comparison of the oocyst production after reinfection between the appropriate groups (Table 4.20).

TABLE 4. 20.

The Average Total Oocyst Production of the Chickens, expressed in Millions of Oocysts per Bird, after reinfection on Day 28 with a Standard Dose of 32,000 Sporulated Oocysts of E. tenella.

Rontgen Dose	10,000R.	12,500 R.	15,000 R.
Oocyst Dose		Single Vaccination*	
32,000	27	31	85
128,000	4	30	64
256,000	6	11	80
500,000	10	9	81
Oocyst Dose		Double Vaccination**	
32,000	20	15	62
64,000	6	13	36
128,000	3	12	15
256,000	1	5	7

* Administration of the vaccine on day 0.

** Administration of the vaccine on day 0 and day 14.

Table 4. 21

The Pathogenic Effect of E. tenella in Chickens Following Infection with Different Numbers of Sporulated Oocysts on Day 0 and also on Day 14 R* Groups; and Subsequently after Reinfection on Day 28 with a Standard Challenge Dose of 32,000 Sporulated Oocysts per Bird. The Oocysts Administered to Groups R.1 to R.24 being Exposed to X Irradiation before Inoculation.

Group	Oocyst Dose	Rontgen Dose	Haemoglobin* Decrease		Mortality		Weight Gain		Oocyst** Output	
			1	2	1	2	Day 12	Day 40	1	2
Control	-	-	-	-	-	-	382	551	-	-
Ch. 1	-	-	-	1.5	-	-		569	-	119
Ch. 1/2	-	-	-	1.0	-	-		-	-	-
E. 1	32,000	-	4.2	-	3	-	312	-	-	-
E. 1/2	32,000	-	5.6	-	2	-	290	609	77	1
E. 2	32,000	-	4.0	-	-	-	-	-	-	-
R. 1	32,000	10,000	-	-	-	-	356	538	1	27
R. 2	32,000*	"	-	-	-	-	361	584	1	20
R. 3	64,000*	"	-	-	-	-	367	603	2	6
R. 4	128,000	"	-	-	-	-	382	546	8	4
R. 5	128,000*	"	-	-	-	-	371	571	8	3
R. 6	256,000	"	-	-	-	-	339	581	8	5
R. 7	256,000*	"	-	-	-	-	393	594	8	1
R. 8	500,000	"	-	-	-	-	349	582	9	10
R. 9	32,000	12,500	-	-	-	-	368	572	.1	31
R. 10	32,000*	"	-	-	-	-	366	469	.1	15
R. 11	64,000*	"	-	-	-	-	347	617	.2	13
R. 12	128,000	"	-	-	-	-	362	542	.5	30
R. 13	128,000*	"	-	-	-	-	379	512	.5	12
R. 14	256,000	"	-	-	-	-	386	563	2	11
R. 15	256,000*	"	-	-	-	-	362	584	2	5
R. 16	500,000	"	-	-	-	-	361	557	2	9
R. 17	32,000	15,000	-	-	-	-	338	513	.4	85
R. 18	32,000*	"	-	-	-	-	389	522	.4	62
R. 19	64,000*	"	-	-	-	-	374	663	.2	36
R. 20	128,000	"	-	-	-	-	361	570	.1	64
R. 21	128,000*	"	-	-	-	-	366	588	.1	15
R. 22	256,000	"	-	-	-	-	340	533	.3	50
R. 23	256,000*	"	-	-	-	-	345	556	.3	7
R. 24	500,000	"	-	-	-	-	319	569	.2	61

* Haemoglobin expressed as grammes per 100 ml. of blood (1) and (2) represent the decrease recorded following the initial and challenge infection.

** The average total oocyst production per bird to nearest million, recorded after vaccination (1) and reinfection (2) respectively.

DISCUSSION.

The results (Table 4. 21) demonstrated that immunity to reinfection with normal oocysts was conferred by vaccination with irradiated oocysts without any evidence of deleterious effects during immunisation. Satisfactory weight gains were recorded (Table 4. 17) in the chickens both after administration of the vaccine and after reinfection. The only indication of infection after inoculation was shown by the presence of a relatively small number of oocysts in the faeces (Table 4. 18) during the patent phase of the disease. The oocyst production increased from approximately 2 to 8 million per bird after administration of oocysts exposed to 10,000 R. when the infective dose was raised from 64,000 to 128,000 oocysts per bird, although no further increase occurred in production of oocysts when the infective dose was raised to 256,000 and 500,000 oocysts per bird. No significant variation was shown between the oocyst producti of the birds receiving oocysts exposed to either 12,500 R. or 15,000 R., which ranged from 0.1 to 2, million per bird after vaccination. There was no differenc in the oocyst production after inoculation between the chickens receiving one dose of vaccine and those receiving two doses of vaccine, no increase occurring in the number of oocysts demonstrated in the faeces after administration of the second dose of vaccine on day 14. No change was recorded in the concentration of haemoglobin of the vaccinated chickens (Table 4. 16) in contrast to a decrease of 4 g. - 5 g. of haemoglobin in the chickens which received normal oocysts on day 0 and on day 14. The absence of any pathogenic effects associated with immunisation was emphasised when no evidence of morbidity was observed in the chickens after vaccination in contrast to the severe clinical symptoms recorded in the birds given normal oocysts.

The pathogenicity of the challenge dose of oocysts was confirmed by the fall in the concentration of haemoglobin (Table 4. 16) in the fully susceptible chickens on day 34, and by the severe lesions demonstrated in the caeca of these birds at postmortem on day 35. It was also confirmed by the high total oocyst production of 119 million per bird during the patent period of the infection.

Following reinfection no change occurred in the level of haemoglobin of the vaccinated chickens (Table 4. 16) although slight evidence of haemorrhage was observed in the chickens which received oocysts exposed to 15,000 R. with the exception of the birds which were inoculated with two doses of 256,000 oocysts per bird. Very slight haemorrhage was also seen in the chickens vaccinated with a single dose of 32,000 oocysts, being least in the group which received oocysts exposed to 10,000 R. Postmortem examination on the seventh day after reinfection indicated that immunity was less satisfactory in the chickens given only one dose of irradiated oocysts compared with that conferred by two doses of irradiated oocysts. This was emphasised when quite severe lesions were found in the caeca of birds vaccinated with a single dose of 500,000 oocysts in contrast to the very slight lesions demonstrated in the caeca of birds inoculated with two doses of 256,000 oocysts at the same rontgen level of 15,000 R., each group of chickens receiving the same total number of oocysts during vaccination. No lesions were found in the caeca of the chickens which received two doses of 256,000 oocysts exposed to 12,500 R. which suggested that a higher level of resistance to reinfection was conferred by oocysts exposed to 12,500 than by oocysts exposed to 15,000 R. This difference was also reflected in the postmortem findings on the chickens examined from the groups which received two doses of 128,000 oocysts per bird after exposure to 12,500 R. and 15,000 R.

The results (Table 4.20) showed a very marked variation in the total oocyst production of the chickens after administration of the challenge dose of oocysts on day 28. They demonstrated that the highest level of resistance was developed after administration of two doses of oocysts exposed to 10,000 R. Immunity also appeared satisfactory after vaccination with two doses of oocysts exposed to 12,500 R. The results indicated that resistance to reinfection was lower when chickens received oocysts exposed to 15,000 R. except after administration of two doses of either 128,000 or 256,000 oocysts per bird, when the oocyst production of the chickens showed no variation from that of birds given a similar number of oocysts exposed to 12,500 R. The results confirmed the difference indicated by the clinical observations and the postmortem findings between the immunity conferred by single and double vaccination showing conclusively that the highest degree of resistance was produced in the chickens receiving two doses of vaccine. This was most clearly demonstrated in the groups which received oocysts exposed to 12,500 R. and 15,000 R. being less obvious in the groups vaccinated with oocysts exposed to 10,000 R. The beneficial effects of double vaccination were emphasised when the level of immunity conferred by the same number of oocysts was compared between chickens given one large single dose of 500,000 oocysts and those receiving two doses of 256,000 oocysts, the greater resistance in the latter birds indicating the significance of the two doses of vaccine rather than the total number of oocysts administered in the inoculum to the chickens. This difference was also reflected in the oocyst production of the chickens which received lower doses of oocysts during immunisation. However, the results suggested a definite relationship between the number of oocysts in the vaccine administered to the chickens and the number of oocysts produced by the chickens during the patent period of the disease after reinfection. The total oocyst production after challenge tended to decrease as the number of oocysts was increased in the inoculum. This observation appeared to indicate a difference in the level of immunity conferred by different doses of irradiated oocysts, which was further emphasised when the oocyst production of the corresponding groups was compared at each level of X irradiation.

Good immunity was also demonstrated in the survivors of the group which received normal oocysts on day 0, although this method of immunisation was contraindicated by the severe detrimental effects which occurred after inoculation However, the results suggested that the resistance to reinfection of the chickens vaccinated with two doses of 256,000 oocysts exposed to 10,000 R. equalled that of the birds given normal oocysts, no difference being recorded in the oocyst production of these groups after challenge. This observation emphasised the practical implications of immunisation with X irradiated oocysts as the resistance in these groups appeared high, less than one million oocysts being produced per bird after reinfection. This fact also suggested an important effect on the epidemiology of the disease in the field. The results indicated a significant difference in the number of oocysts which would be present in the litter under intensive management after infection of fully susceptible chickens and immune birds, illustrated by the total oocyst production per bird of 119 million and less than one million recorded in the susceptible and vaccinated chickens respectively after administration of the challenge infection.

These observations demonstrate that the greatest level of immunity is conferred by double vaccination with oocysts exposed to 10,000 R. before inoculation.

EXPERIMENT ELEVEN.

IMMUNISATION AGAINST *EIMERIA TENELLA* INFECTION BY SINGLE AND DOUBLE VACCINATION WITH IRRADIATED OOCYSTS IN EIGHT DAY OLD CHICKENS.

EXPERIMENTAL AIMS.

The experiment was designed to study the immunity conferred by single and double vaccination with irradiated oocysts of *E. tenella* against reinfection with normal oocysts in 8 day old chicks.

Previous experimental observations showed that a high level of resistance to reinfection was developed after double vaccination with sporulated oocysts exposed to 10,000 R in 3 week old chickens. It was important to confirm that similar results could be reproduced in young chicks, since under field condition it was necessary to ensure that resistance to the disease could be developed at an early age before a significant challenge infection was established in the litter under intensive methods of management.

Rontgen levels of 10,000 R and 12,500 R were selected for observation as the previous results had shown that oocysts must be exposed to a minimum dose of 10,000 R to prevent the appearance of deleterious effects after inoculation, while resistance was significantly reduced if the rontgen level exceeded 12,500 R.

Several doses of oocysts ranging from 32,000 to 256,000 per bird were selected for comparison. The immunising effect of the same total number of oocysts administered either as one single infection or as two smaller doses of oocysts was also studied by the comparison of appropriate groups following single and double vaccination to confirm the previous results, which appeared to indicate conclusively that the latter method of vaccination conferred the highest degree of resistance to reinfection.

The challenge doses of 32,000 and 64,000 normal oocysts were selected to represent low and high levels of infection, as it was necessary to determine both if there was a significant variation in the resistance associated with the different methods of vaccination and also establish the degree of immunity to a heavy challenge infection.

The severity of the disease in each group of chickens was determined from observations made on mortality, on clinical signs, on growth rate and on oocyst production after both vaccination and reinfection. Haemoglobin estimations were carried out on all the experimental chickens after reinfection, and also on the chickens which received normal oocysts on day 0 and day 8, to confirm the pathogenicity of the culture exposed to X-irradiation. Post mortem examinations were also made on the seventh day after reinfection to determine whether there was any variation in the severity of the caecal lesions between each group of chickens which might indicate a difference in the immunity of the chickens.

MATERIALS AND METHODS.

Experimental Birds.

Broiler type cockerel chicks were used in the experiment. They were reared in complete isolation and were transferred to the experimental units the day before inoculation, where they were kept in electrically heated brooders with wire floors. They were transferred to metal cages with wire floors on day 14. The chicks given irradiated oocysts were housed in a separate room from thos which received normal oocysts. The chicks in group E.2A and E.2B and the chickens for the fully susceptible challenge groups were retained in isolation until the appropriate day before inoculation. The chicks were fed on a special high energy broiler crumb containing high levels of the vitamin B complex and minerals (B.O.C.M. Special Ration E.206). The food was available ad lib. except on day 0 and day 8 when it was withdrawn six and five hours respectively before administration of the vaccine, and on day 22 when food was removed from the chickens three hours before infection with the challenge dose of normal oocysts.

The chicks were eight days old on day 0.

Parasitology.

The culture used for infection of the chickens on day 0 was 56 days old, with a total oocyst count of 250,000 per ml. and a sporulation count of 80%.

Four 15 ml. aliquots of the culture were exposed to each appropriate dose of X-irradiation of 10,000R and 12,500R two days before inoculation, i.e. day 0-2. Total oocyst counts were carried out on the culture after X-irradiation on the day before infection, i.e. day 0-1. Dilutions were made to give the correct number of oocysts per ml. for each respective group by the addition of distilled water to the culture, which was suspended in 2% potassium dichromate solution. The dilutions were carried out on day 0.

The culture administered to the chickens on day 8 was 92 days old, with a total oocyst count of 1,500,000 oocysts per ml. and a sporulation count of 80%.

Two 15 ml. aliquots of the culture were exposed to each appropriate Roentgen level of 10,000R and 12,500R the day before inoculation, i.e. day 7. Total oocyst counts were made on the irradiated culture on day 8 and also on the non-irradiated culture. Dilutions were made in the usual manner to give the correct number of oocysts per ml. for each respective group.

The culture administered to the chickens on day 22 was 106 days old, with a total oocyst count of 1,325,000 per ml. and a sporulation count of 80%. Dilutions were made to give 16,000 and 32,000 sporulated oocysts per ml. respectively by the addition of distilled water.

Administration of the Inoculum.

The infecting dose of irradiated oocysts was given in 1 ml. of water on day 0 and on day 8. The challenge dose of normal oocysts was administered in 2 ml. of water on day 22. The birds receiving two doses of irradiated oocysts were inoculated on day 0 and on day 8. The chickens receiving one dose of irradiated oocysts were inoculated on day 8. The inoculum was administered directly into the crop using an automatic dosing syringe. During inoculation the stock of oocysts from which the syringe was filled was kept in suspension by decanting the solution between two beakers.

The chickens were inoculated in the afternoon of day 0 and day 8. The birds given irradiated oocysts were infected before those which received normal oocysts. The challenge dose of oocysts was administered to the chickens in the morning of day 22.

Experimental Design.

Ten chickens were selected at random from each experimental group, with the exception of the groups E.1A, E.1B, E.2A and E.2B. The latter groups each contained five birds. The birds in group E.1A and E.1B were selected at random from the survivors of groups E.1 and E.1/2 which received normal oocysts on day 0 to confirm the pathogenicity of the culture exposed to X-irradiation on day 0-2. The birds in groups E.2A and E.2B were selected at random on day 21 before administration of the challenge dose of oocysts from the chickens of group E.2 which received normal oocysts on day 8 to confirm the pathogenicity of the culture exposed to X-irradiation on day 7.

The chickens in groups R.1A - R.6B and R.7A - R.12B received oocysts which had been exposed to 10,000R and 12,500R respectively before inoculation. The chickens of groups E.1, E.1/2 and E.2 received normal oocysts.

The chickens receiving two doses of irradiated oocysts were inoculated on day 0 and on day 8; the birds given one dose of irradiated oocysts were inoculated on day 8.

The inoculum administered to each bird on day 0 contained 32,000 oocysts in groups R.1, R.1/2, R.3A, R.3B, R.9A and R.9B, 64,000 oocysts in groups R.4A, R.4B, R.10A and R.10B, 128,000 oocysts in groups R.5A, R.5B, R.11A and R.11B, and 256,000 oocysts in groups R.6A, R.6B, R.12A and R.12B.

The inoculum administered to each bird on day 8 contained 32,000 oocysts in groups R.2, R.3A, R.3B, R.9A and R.9B, 64,000 oocysts in groups R.4A, R.4B, R.10A and R.10B, 128,000 oocysts in groups R.1A, R.1B, R.5A, R.5B, R.7A, R.7B, R.11A and R.11B, and 256,000 oocysts in groups R.2A, R.2B, R.6A, R.6B, R.8A, R.8B, R.12A and R.12B.

The chickens in the groups/A and chickens in groups/B, together with the fully susceptible chickens of groups Ch.A and Ch.B received a challenge dose of 32,000 and 64,000 normal oocysts respectively on day 22.

Chickens were also infected on day 0 and day 8 for post mortem examination on day 29 after reinfection on day 22. Birds were inoculated to represent each group, except R.3A, R.3B, R.9A, R.9B, R.10A and R.12B.

Haemoglobin estimations were made on the chickens of groups R.1, R.1/2 and the non-infected control chickens after inoculation on day 3 and on day 6. They were also made on group R.2 and the non-infected control birds on day 10 and day 14, i.e. day 2 and day 6 after inoculation. Haemoglobin estimations were carried out on all the groups after reinfection on day 23 and day 28, i.e. day 0 and day 6 after inoculation.

The chickens receiving single and double vaccination were weighed on the day before inoculation on day 7 and day 0-1 respectively. The chickens inoculated on day 0 were re-weighed on day 10. All groups were weighed on day 21 before the administration of the challenge dose of oocysts and after reinfection on day 31 and day 42, with the exception of the replicate groups Ch.A and Ch.B which were not re-weighed on day 42.

The total oocyst production of each group was recorded daily during the patent phase of the disease after both the immunising inoculations and the challenge infection between day 7 and day 22 and between day 29 and day 42 respectively. Oocyst production was recorded in only one of each replicate challenge group.

Post mortem examination was carried out on day 5 on the birds which died due to inoculation on day 0,and on day 7 on the birds which died on day 6 and day 7 after the initial infection. Birds were also examined on day 28, having died on day 27, after the challenge infection. Birds were killed for examination on day 29 from each group, except group R.3A, R.3B, R.9A, R.9B, R.10A and R.12B.

Clinical signs and mortality were also recorded.

RESULTS.

(1) Haematology.

The results (Table 4. 22) of the haemoglobin estimation did not show any variation in the concentration of haemoglobin in the non-infected control chickens on day 5 and day 6, in contrast to a marked fall of approximately 3.6g to 4g of haemoglobin in the chickens of group E.1 and E.1/2 on day 6. The results also demonstrated a decrease of 3.5g. of haemoglobin in group E.2 on day 14.

The results (Table 4.23) of the haemoglobin estimation did not show any significant variation between the non-infected control group, the surviving birds in E.1A - E.2B, the vaccinated chickens of groups R.1A - R.12B and the fully susceptible chickens of groups Ch.A, Ch.A/2, Ch.B and Ch.B/2 on day 23.

There was no significant difference in the concentration of haemoglobin in groups E.1A - E.2B, R.1A - R.2B and the non-infected control group on day 28, in contrast to a decrease of approximately 1g. and 2.5g. in groups Ch.A and Ch.B respectively. A similar decrease in the level of haemoglobin occurred in the replicate groups Ch.A/2 and Ch.B/2.

(2) Mortality.

One death occurred in group R.12A, R.12B and R.6A on day 2. One chicken died in group R.6B on day 3.

One death was recorded in group E.1 and E.1/2 on day 6. A second chicken died in group E.1/2 on day 7.

One bird died in group Ch.B on day 27 after administration of the challenge dose of oocysts on day 22.

(3) Clinical Findings.

Following the administration of the vaccine on day 0, 1 or 2, chicks appeared slightly depressed in groups R.6A, R.6B, R.12A and R.12B, until day 4 when no further evidence of morbidity was seen in these groups.

No indication of clinical disease was observed in the vaccinated chicks on day 5 or day 6, in contrast to the high morbidity and severe haemorrhage recorded in the birds of group E.1 and E.1/2.

Quite severe haemorrhage was also seen in the chickens of group E.2 on day 13 and day 14, after inoculation on day 8. Morbidity was also quite marked although no deaths occurred during the acute phase of the disease. No evidence of infection was observed in the chickens which received irradiated oocysts on day 8.

Table 4. 22

The Mean Haemoglobin Concentration of the Chickens Infected with a Standard Dose of 32,000 Sporulated Oocysts of E. tenella on Day 0 and Day 8. Group E.1, E.1/2 and E.2 respectively to Check the Pathogenicity of the Culture which was Exposed to X Irradiation before Inoculation of Groups E.1 to E.12.

Group	Day 5	Day 6	Haemoglobin Decrease	"p"
Control	8.3 ** ± 0.7 *	8.2 ± 0.6	Nil	
E.1	8.3 ± 0.6	4.2 ± 0.5	4.1	0.001 ***
E.1/2	7.6 ± 0.8	4.0 ± 1.0	3.6	0.001
	Day 10	Day 14		
Control	8.5 ± 0.7	8.5 ± 0.7	nil	
E.2	8.3 ± 0.8	4.8 ± 1.7	3.5	0.001

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

- The Standard Deviation.

* * * The probability calculated by the "t-test".

After the administration of the challenge dose of oocysts on day 22, slight haemorrhage was recorded in group Ch.A and Ch.A/2 on day 27 and 28, in contrast to the severe symptoms of caecal coccidiosis seen in groups Ch.B and Ch.B/2, where the chickens appeared very depressed due to severe haemorrhage which was most pronounced on day 28.

No indication of morbidity was observed in the chickens of groups R.1A - R.2B and R.1A - R.12B after reinfection. Very slight evidence of haemorrhage was found in groups R.2B and R.12B on day 27 and in groups R.1B and R.5B on day 28. Evidence of haemorrhage was slightly more pronounced in groups R.2B, R.7B and R.12B on day 27 and in groups R.2B, R.7B, R.8B and R.12B on day 28.

(4) Weight Gains.

The results (Table 4. 24) showed no significant variation in the weight of the chickens before inoculation on day 0 and day 8.

The mean weight gains of the chickens vaccinated with irradiated oocysts on day 0 and day 8 equalled those attained by the non-infected control chickens, with the exception of the chickens in groups R.5A, R.5B, R.6A, R.6B, R.11A, R.11B, R.12A and R.12B which received 128,000 or 256,000 oocysts per bird on day 0. The mean weight gain was significantly lower on day 10 in these chickens, the retardation of growth being reflected in the total weight gain recorded on day 42, although there was no difference in the weight gains of these groups compared with the non-infected control birds on day 31 after reinfection.

The mean weight gain of the birds in groups R.1 And R.1/2 was slightly lower on day 10 than that of the non-infected control group. It was still

Table 4. 23

The Mean Haemoglobin Concentration in the Blood of the Chickens after Reinfection with a Standard Challenge Dose of 32,000 or 64,000 Sporulated Oocysts per Bird on Day 22 in Groups /A and /B respectively.

Group	Oocyst Dose	Rontgen Dose	Day 23	Day 28	Haemoglobin Decrease *
Control	-	-	7.7 ±0.3	7.7 ±0.6	-
Ch.A	-	-	7.6 ±0.3	6.4 ±0.5	1.2
Ch.A/2	-	-	7.5 ±0.6	6.0 ±0.9	1.5
Ch.B	-	-	8.1 ±0.8	5.5 ±1.6	2.6
Ch.B/2	-	-	7.2 ±0.8	4.7 ±1.3	2.5
E.1A	32,000	-	8.3 ±0.8	7.5 ±0.5	-
E.1B	32,000	-	8.2 ±0.4	7.6 ±0.6	-
E.2A	32,000	-	8.0 ±0.7	7.6 ±0.5	-
E.2B	32,000	-	7.9 ±0.3	7.1 ±0.4	-
R.1A	128,000	10,000	7.8 ±0.8	7.6 ±0.6	-
R.1B	128,000	"	7.7 ±0.7	7.3 ±0.7	-
R.2A	256,000	"	7.3 ±0.7	7.3 ±0.5	-
R.2B	256,000	"	7.8 ±0.3	7.2 ±0.4	-
R.3A	32,000 +	"	8.2 ±0.6	7.6 ±0.6	-
R.3B	32,000 +	"	7.4 ±0.5	7.5 ±0.3	-
R.4A	64,000 +	"	8.1 ±0.6	8.0 ±0.5	-
R.4B	64,000 +	"	7.9 ±0.4	7.9 ±0.7	-
R.5A	128,000 +	"	7.9 ±0.5	8.0 ±0.6	-
R.5B	128,000 +	"	7.7 ±0.5	7.8 ±0.6	-
R.6A	256,000 +	"	7.8 ±0.4	7.5 ±0.5	-
R.6B	256,000 +	"	7.7 ±0.6	7.0 ±0.4	-
R.7A	128,000	12,500	7.4 ±0.7	7.1 ±0.7	-
R.7B	128,000	"	7.4 ±0.8	6.7 ±0.6	-
R.8A	256,000	"	7.1 ±0.4	6.7 ±0.5	-
R.8B	256,000	"	7.0 ±0.7	6.6 ±0.6	-
R.9A	32,000 +	"	8.0 ±0.6	7.4 ±0.6	-
R.9B	32,000 +	"	7.7 ±0.6	7.4 ±0.7	-
R.10A	64,000 +	"	7.6 ±0.9	6.9 ±0.5	-
R.10B	64,000 +	"	7.6 ±0.5	7.2 ±0.8	-
R.11A	128,000 +	"	7.6 ±0.5	7.2 ±0.6	-
R.11B	128,000 +	"	7.5 ±0.4	7.2 ±0.5	-
R.12A	256,000 +	"	7.1 ±0.7	7.4 ±0.6	-
R.12B	256,000 +	"	7.5 ±0.6	7.2 ±0.7	-

* Mean haemoglobin concentration expressed as grammes per 100 ml. of blood.

evident on day 21 and was also reflected in the total weight gain on day 42. No marked difference was seen in the weight gains of the chickens in group E.2, although the weight of these birds appeared slightly lower on day 21 than than of the non-infected control chickens.

After administration of the challenge dose of oocysts on day 22 there was no indication of any effect on the growth rate of the birds in group Ch.A, in contrast to a definite depression in the weight gain of the birds in group Ch.B on day 31. This was not so evident in the replicate group Ch.B/2, where the mean weight gain appeared only slightly less than that of the non-infected control chickens. There was also a marked depression in the mean weight gain of group E.1B on day 31.

No significant variation was seen between the mean weight gains of the vaccinated chickens and the non-infected control group after administration of the challenge dose of oocysts on day 22.

Table 4. 24

The Mean Weight Gains of the Chickens on Day 10, Day 31 and Day 42, to Illustrate the Effect on the Growth Rate After Vaccination with Different Numbers of Sporulated Oocysts of E. tenella, and after Reinfection with a Standard Challenge Dose of 32,000 and 64,000 Sporulated Oocysts per Bird in Groups /A and /B respectively, on Day 22. The Oocysts administered to Groups R.1 to R.6 and R.7 to R.12 on Day 0 and Day 8 were Exposed to 10,000 and 12,500 Rontgen Doses of X Irradiation before Inoculation Respectively. Groups R.1, R.2, R.7 and R.8 were given Oocysts on Day 8 Only.

Group	Day 0-1	Wt. Gain Day 10	Day 21	Wt. Gain Day 31	Day 42	Wt. Gain Day 42 ****
Control	100 *	166	542	328	1301	1202
Ch.A	243 **	-	617	-	-	-
Ch.A/2	226 **	-	572	298	1287	-
Ch.B	229 **	-	566	219	-	-
Ch.B/2	239 **	-	666	286	1371	-
E.1A	101	110	448	294	1166	1065
E.1B	101	110	489	177	1133	1032
E.1/2	100	145	-	-	-	-
E.2A	240 **	-	513	337	1236	-
E.2B	240 **	-	511	326	1281	-
R.1A	230 **		615	373	1426	1196 ***
R.1B	241 **	-	526	369	1428	1186 ***
R.2A	251 **	-	546	364	1327	1096 ***
R.2B	232 **	-	547	361	1322	1091 ***
R.3A	102	196	622	384	1187	1086
R.3B	101	182	584	376	1413	1312
R.4A	104	181	580	376	1426	1322
R.4B	103	173	570	363	1378	1275
R.5A	104	151	526	363	1369	1256
R.5B	96	106	481	287	1269	1173
R.6A	100	66	409	333	1181	1082
R.6B	102	66	447	367	1287	1186
R.7A	215 **	-	569	368	1383	1168 ***
R.7B	222 **	-	547	315	1312	1090 ***
R.8A	230 **	-	496	376	1336	1106 ***
R.8B	236 **	-	534	345	1366	1131 ***
R.9A	97	166	540	366	1349	1252
R.9B	99	166	536	328	1291	1191
R.10A	101	172	565	383	1414	1313
R.10B	103	174	574	367	1394	1291
R.11A	100	106	481	346	1266	1166
R.11B	97	97	446	326	1204	1107
R.12A	103	95	453	359	1261	1158
R.12B	97	86	421	296	1130	1033

* Weight expressed in grammes.

** Weight on Day 7

*** Total weight gain between Day 7 and Day 42

**** Total weight gain between Day 0-1 and Day 42.

5. Pathology

(i). Postmortem Examination of the Birds Killed on Day 29 after Reinfection on Day 22.

Quite severe lesions were found in the caeca of the chickens from group ChA. (Figure 22). The caecal walls were markedly thickened, the mucosa being raised into longitudinal and horosontal ridges giving a corrugated appearance. The caeca were not enlarged although extravasated blood and tissue debris were present in the lumen. The changes were far more severe in group Ch.B. (Figure 23) The caeca had very thin walls and showed evidence of widespread erosion of the mucosa. This was emphasised by the tremendous distension of the caeca due to the presence of cellular débris and extravasated blood in the lumen.

Following the administration of the lower challenge dose of 32,000 oocysts per bird on day 22 no indication of infection was seen on day 29 in the chickens from groups R.2A (Figure 22) or R. 4A, R.5A and R.6A which represented birds inoculated with 1 dose of 256,000 oocysts or 2 doses of 64,000, 128,000 and 256,000 oocysts respectively, after exposure to 10,000R. Very slight evidence of infection was shown by the presence of a little blood in the caecal contents of group R.1A which was given a single dose of 128,000 oocysts per bird. Slight lesions were found in group R.7A which received a similar number of oocysts after exposure to 12,500R. The caecal mucosa was slightly thickened with evidence of very slight erosion of the epithelium, although no indication of haemorrhage was seen in these birds. Careful examination suggested that the mucosa was possibly very slightly thickened in the caeca of the birds from group R.8A and R.11A which were inoculated with 1 dose of 256,000 oocysts and 2 doses of oocysts respectively after exposure to 12,500 R. (figure 25). No indication of infection was present in the birds examined from group R.12A which were vaccinated with 2 doses of 256,000 oocysts.

After the administration of the higher challenge dose of 64,000 oocysts postmortem examination demonstrated the presence of slight thickening in the caecal mucosa of the birds from groups R.1B and R.7B (Figure 23). The changes were a little more pronounced in group R.7B where the mucosa was thrown into small ridges, giving a corrugated appearance. Evidence of infection in these groups was also emphasised by the presence of blood in the caecal contents. Quite severe lesions were found in the caeca of the chickens of groups R.2B and R.8B (Figures 23 and 26). The caecal mucosa was markedly thickened and raised int ridges presenting a corrugated appearance, the lesions being most severe in the latter group. No evidence of blood was seen in the contents of the caeca in either group. Careful examination suggested that the caecal mucosa was possibly very slightly thickened in the chickens from group R.4B, R.6B and R.11B. (Figure 24). No indication of infection was found in the caeca of chickens from group R.5B and R.10B. (Figure 24). Birds were not available for postmortem examination from groups R.3A, R.3B., R.9A, R.9B, R.10A and R.12B or E.1A - E.2B.

(ii). <u>Postmortem Examination of the Chicks which Died after Inoculation with Irradiated Oocysts between Day 1 and Day 3.</u>

Typical lesions associated with potassium dichromate toxicity were demonstrated in the chicks of groups R.6A, R.6B, R.12A and R.12B which died following administration of the inoculum on day 0. Severe necrotising lesions were present on the mucosa of the crop, the proventriculus and the gizzard, being most pronounced in the gizzard where there was also evidence of erosion of the mucosa. The lumen of this portion of the alimentary tract contained evil smelling, straw coloured fluid, together with a small amount of partially digested food. No lesions were demonstrated in any other part of the alimentary tract in these chicks.

FIGURE 22 - FIGURE 30.

Please note that experiment number and group number in text does not correspond with experiment number and group number in photographs.

FIGURE 22.

SINGLE VACCINATION IN CHICKS WITH OOCYSTS EXPOSED TO 10,000R AGAINST REINFECTION WITH 32,000 NORMAL OOCYSTS.

Note:-

Typical lesions of acute caecal coccidiosis in fully susceptible bird (Ch.1), in contrast to only slight evidence of haemorrhage in bird immunised with 128,000 X-irradiated oocysts (R.5) and complete absence of lesions in bird immunised with 256,000 X-irradiated oocysts (R.9).

FIGURE 23.

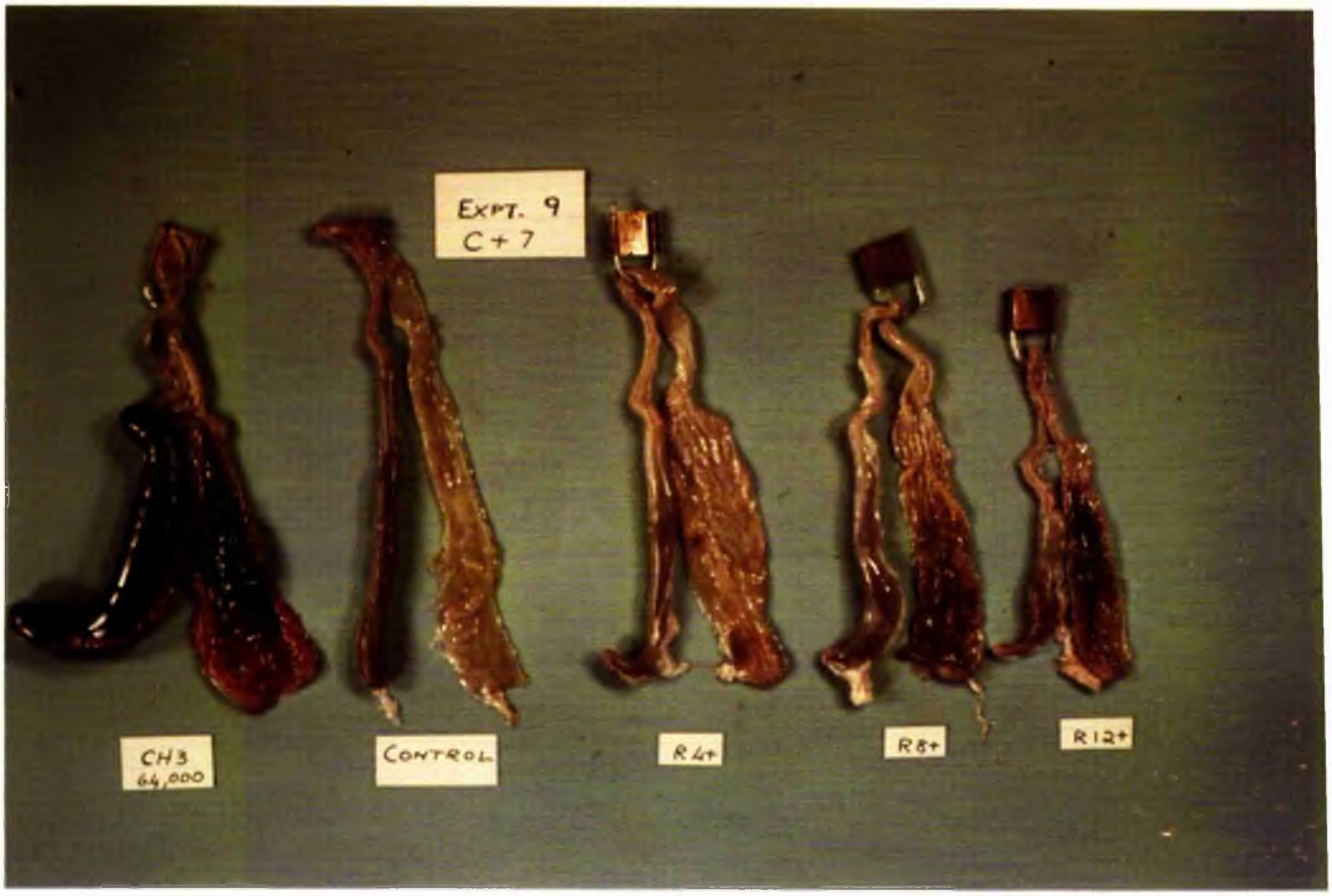
SINGLE VACCINATION IN CHICKS WITH OOCYSTS EXPOSED TO 10,000R AGAINST REINFECTION WITH 64,000 NORMAL OOCYSTS.

Note:-

(1) Typical signs of acute caecal coccidiosis in susceptible bird (Ch.3).

(2) Marked evidence of haemorrhage in caeca of birds immunised with one dose of 128,000 or 256,000 irradiated oocysts (R.6 and R.10), showing that a satisfactory immunity against a high challenge dose of normal oocysts is not conferred by a single vaccination with irradiated oocysts (compare with Figs.22 and 24).

FIGURE 24.



DOUBLE VACCINATION IN CHICKS WITH OOCYSTS EXPOSED TO 10,000R AGAINST REINFECTION WITH 64,000 NORMAL OOCYSTS.

Note:-

Typical lesions of acute caecal coccidiosis in susceptible chicken (Ch.3), in contrast to the absence of lesions in the vaccinated birds given two doses of either 64,000, 128,000 or 256,000 irradiated oocysts (R.4, R.8 and R.12). This indicates the significance of two immunising doses of oocysts. (Compare with Fig.23, i.e. R.6 v R.4 and R.10 v R.8).

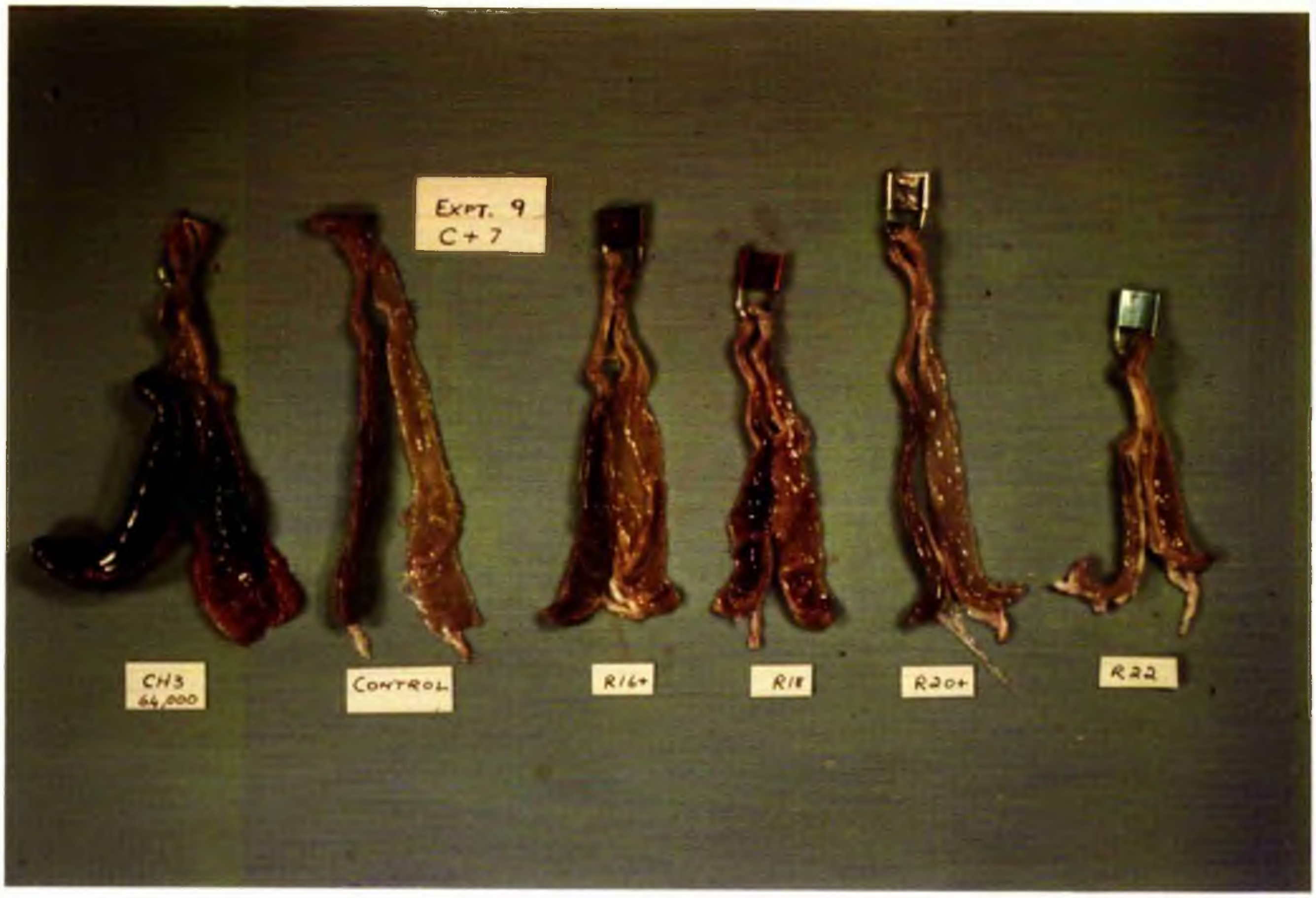
FIGURE 26.

COMPARISON BETWEEN THE RESISTANCE CONFERRED BY SINGLE AND DOUBLE VACCINATION IN CHICKS WITH OOCYSTS EXPOSED TO 12,500R AGAINST REINFECTION WITH 32,000 NORMAL OOCYSTS.

Note:-

Typical lesions of caecal coccidiosis in susceptible bird (Ch.1), in contrast to the absence of lesions in birds immunised with either one (R.17 & R.21) or two (R.19 & R.23) doses of 128,000 or 256,000 irradiated oocysts, indicating that satisfactory immunity was conferred against reinfection in the vaccinated birds.

FIGURE 26.



COMPARISON BETWEEN THE IMMUNITY CONFERRED BY SINGLE AND DOUBLE VACCINATION IN CHICKS WITH OOCYSTS EXPOSED TO 12,800R AGAINST REINFECTION WITH 64,000 NORMAL OOCYSTS.

Note:-

(1) Typical lesions of acute caecal coccidiosis in susceptible chicken (Ch.3).

(2) Absence of lesions in birds immunised with two doses of 64,000 (R.16) or 128,000 (R.20) irradiated oocysts, in contrast to the slight evidence of haemorrhage in the caeca of birds immunised with one dose of 128,000 (R.18) or 256,000 (R.22) irradiated oocysts. These differences emphasise the significance of two doses of vaccine when appropriate groups (i.e. R.16 v R.18 and R.20 v R.22), receiving the same total number of oocysts, are compared.

(iii) Post mortem Examination of the Chickens which Died on Day 6 and Day 7.

Examination of the chickens which died after inoculation with normal oocysts on day 0 in groups N.1 and N.1/2 demonstrated typical lesions of acute caecal coccidiosis.

(6) Oocyst Production.

The results (Table 4. 25) demonstrated a significant difference between the oocyst production of the birds given normal oocysts and those inoculated with irradiated oocysts. The total average production per bird was 28 and 51 million in group N.1 and N.2 respectively, compared with less than 2 millions per bird in groups R.1 and R.12. These results indicated that the production of oocysts was higher in birds aged 16 days compared with that of birds only 8 days old following inoculation with 32,000 normal oocysts. This variation was not seen so clearly when the chicks received irradiated oocysts, the difference recorded between the groups being of doubtful significance. The oocyst production after inoculation varied slightly between the groups given oocysts exposed to 10,000R and those receiving oocysts exposed to 12,500R, the output per bird ranging from 0.4 - 2 million and approximately 0.05 - 0.8 million respectively. There was no significant increase in the oocyst production of the vaccinated chickens when the inoculum was increased from 32,000 - 256,000 irradiated oocysts per bird. No significant increase occurred following administration of the second dose of vaccine in the appropriate groups on day 8

Table 4. 25.

The Average Total Daily Oocyst Production of the Chickens, Expressed in Millions of Oocysts per Bird, After Infection with Different Numbers of Sporulated Oocysts of E. tenella on Day 0 and Day 8, Groups R+ and on Day 8 alone Groups R. The Oocysts Administered to Groups R.1 - R.6. and R.7 - R.12 were Exposed to 10,000 and 12,500 Rontgen Doses of X Irradiation before Inoculation.

Day	E1	E2	R1	R2	R3+	R4+	R5+	R6+	R7	R8	R9+	R10+	R11+	R12+
7	6.5				0.1	0.1	*	*			*	*	-	-
8	7.7				0.6	0.9	0.4	0.1			0.1	0.1	*	0.1
9	1.3				0.4	0.3	0.1	0.1			*	*	*	0.1
10	5.6				0.1	*	0.1	0.1			*	*	*	0.5
11	1.4				*	*	*	*			-	*	*	0.1
12	0.2				-	*	*	*			-	-	*	*
13	0.2				*	-	-	-			-	-	*	-
14	0.2				-	*	-	-			-	-	-	-
15	0.1	17.7	0.1	-	-	-	-	-	-	-	-	-	-	-
16	0.1	15.0	1.2	0.1	*	*	*	*	*	*	*	-	-	-
17	*	1.1	0.2	0.1	-	*	-	-	*	*	-	-	-	-
18	*	1 .1	0.1	*	-	*	*	-	*	*	-	*	-	-
19	*	3.3	0.1	*	*	-	-	-	*	*	-	*	*	*
20	-	1.5	*	*	-	-	*	-	-	-	-	-	-	-
21	*	0.7	*	*	-	*	-	-	-	-	-	-	-	-
22	-	0.2	*	-	-	-	-	-	-	-	-	-	-	-
Total** per Bird	23	51	2	1	1	1	1	.4	*	.1	.1	.2	.1	.8

* Indicates that oocyst production was less than 50,000 per bird.

** The total production of oocysts per bird calculated to the nearest million oocysts.

The results (Table 4. 26) showed a marked difference in the total oocyst production of the fully susceptible chickens of group Ch.A and Ch.B and also between these birds and those of groups R.1A - R.12B. The total average oocyst production per bird being 79 and 109 million in the former groups compared with an oocyst output ranging from 0.1 to 65 million oocysts in the vaccinated chickens. The oocyst production of the chickens which received normal oocysts was slightly lower in the groups which were inoculated on day 8, being less than 50,000 per bird in group K.2B and 0.3 million in group E.2A compared with 1 and 4 million in group E.1A and E.1B respectively which were inoculated on day 0.

The results (Table 4. 27) clearly indicated a definite variation in the oocyst production of the vaccinated chickens, after reinfection between the birds given 32,000 oocysts and those given 64,000 oocysts on day 22. The oocyst production was considerably higher in the groups which received the latter challenge dose of oocysts. These results clearly demonstrated a marked difference in the total oocyst production after reinfection with 64,000 oocysts per bird between the birds given single and double vaccination, the number of oocysts being significantly lower in the groups which were inoculated with 2 doses of vaccine. There was little variation in the oocyst production after challenge in groups R.3A - R.5B, which were inoculated with doses of oocysts ranging from 32,000 to 128,000 per bird after exposure to 10,000R on day 0 and day 8. The oocyst output was lowest in group R.4A and R.4B which received 2 doses of irradiated oocysts each containing 64,000 oocysts per bird. The oocyst production was significantly higher in the corresponding groups R.9A - R.11B which were vaccinated with oocysts exposed to 12,500R.

Table 4. 26

The Average Total Daily Oocyst Production of the Chickens, Expressed in Millions of Oocysts per Bird, after Reinfection with a Standard Challenge Dose of 32,000 or 64,000 Sporulated Oocysts of E. tenella per Bird in Groups /A and /B Respectively on Day 22.

Day	ChA/2	ChB/2	E1A	E1B	E2A	E2B	R1A	R1B	R2A	R2B	R3A	R3B
7	27.9	42.2	0.1	1.4	0.3	*	*	5.9	1.5	3.6	0.1	0.2
8	31.0	33.0	0.5	1.9	*	*	0.5	8.3	3.0	5.0	0.3	0.8
9	6.9	7.4	0.1	0.7	*	*	0.9	5.4	2.6	7.2	0.3	1.2
10	1.3	2.4	*	*	*	-	0.1	3.2	2.0	5.8	0.2	1.5
11	2.8	3.4	-	*	*	-	0.1	1.5	1.6	4.0	0.1	0.7
12	3.1	3.6	-	-	-	-	*	0.6	0.7	1.9	*	0.1
13	1.9	2.4	-	-	-	-	*	0.9	0.5	0.7	*	*
14	1.7	3.6	-	-	-	-	-	0.2	0.3	0.4	-	*
15	0.7	1.7	-	-	*	-	-	0.1	*	0.2	-	*
16	1.0	1.7	-	-	-	-	-	*	*	0.1	-	-
17	0.2	3.4	-	-	-	-	-	-	-	0.1	-	-
18	0.1	2.3	-	-	-	-	-	*	-	0.1	-	-
19	0.1	0.5	-	-	-	-	-	*	-	0.1	-	-
20	*	0.5	-	-	-	*	-	-	-	*	-	-
21	-	0.4	*	-	-	-	-	-	-	0.1	-	-
Total** per Bird	79	109	1	4	.3	*	2	26	12	29	1	5

* Indicates that oocyst production was less than 50,000 per bird.

** The total production of oocysts per bird calculated to the nearest million oocysts.

R4A	R4B	R5A	R5B	R6A	R6B	R7A	R7B	R8A	R8B	R9A	R9B	R10A	R10B	R11A	R11B	R12A	R12B
-	0.3	*	1.9	0.9	3.3	0.2	5.7	1.1	1.6	0.2	0.3	0.1	0.6	1.6	1.6	0.2	4.2
*	1.5	0.4	4.4	4.4	16.8	3.4	18.5	12.9	7.3	1.6	2.9	0.8	4.6	3.8	9.9	1.7	7.1
*	1.8	0.7	3.6	3.0	8.9	3.7	15.9	9.0	9.5	2.2	3.6	0.8	4.9	4.4	8.0	1.5	2.8
*	0.7	0.5	2.1	1.5	4.6	1.9	15.6	4.2	9.7	1.2	3.3	0.4	2.3	3.6	3.7	0.4	3.4
*	0.3	0.1	0.9	0.9	2.8	1.5	4.0	3.6	4.7	0.7	1.3	0.2	0.9	1.9	3.2	0.4	2.2
*	0.1	*	0.3	0.3	1.1	0.4	2.3	1.7	1.5	0.2	1.0	0.4	0.5	0.5	2.0	0.6	1.3
*	*	*	0.1	0.1	1.0	0.2	1.9	0.9	1.0	0.1	*	*	0.2	0.4	0.1	0.5	0.3
-	-	-	*	0.1	0.5	0.1	1.0	0.2	0.3	0.1	-	*	*	0.2	*	*	0.6
-	-	-	*	0.1	0.1	*	0.2	0.1	0.1	0.1	*	*	*	*	*	*	1.2
-	-	-	-	*	0.1	-	0.1	0.1	0.1	*	-	-	*	*	-	*	1.1
-	-	-	-	*	*	*	*	*	*	-	-	-	*	-	-	*	0.5
-	-	-	*	*	-	*	*	-	0.1	*	*	-	*	-	-	-	0.1
-	-	-	-	-	*	*	-	-	*	*	-	*	-	-	-	-	*
*	-	-	-	0.1	-	*	-	-	0.3	-	-	-	-	-	-	-	*
-	-	-	-	*	*	*	-	-	0.1	-	-	-	-	-	-	-	*
.1	4	2	13	11	39	14	65	34	37	7	13	3	14	16	28	5	23

Table 4. 27

The Average Total Oocyst Production of the Chickens, Expressed in Millions of Oocysts per Bird, after Reinfection on Day 22 with a Standard Dose of 32,000 Oocysts or 64,000 Oocysts per Bird in Groups /A and /B Respectively.

Rontgen Dose	10,000R		12,500R	
Oocyst Dose	Single Vaccination*			
	A	B	A	B
128,000	2	26	14	65
256,000	12	29	34	37
Oocyst Dose	Double Vaccination**			
32,000	1	5	7	13
64,000	0.1	4	3	14
128,000	2	13	16	28
256,000	11	39	5	23

* Administration of the vaccine on day 8.

** Administration of the vaccine on day 0 and day 8.

Table 4. 28.

The Pathogenic Effects of E. tenella in Chickens Following Infection with Different Numbers of Sporulated Oocysts on Day 0 and Day 8 (Groups R+), on Day 8 Alone (Groups R) and Subsequently after Reinfection on Day 22, with a Standard Challenge Dose of 32,000 or 64,000 Sporulated Oocysts per Bird in Groups /A and /B respectively.

Group	Oocyst Dose	Rontgen Dose	Haemoglobin Decrease* 1	Haemoglobin Decrease* 2	Mortality 1	Mortality 2	Weight Gain** Day 10	Weight Gain** Day 31	Oocyst*** Output 1	Oocyst*** Output 2
Control	-	-	-	-	-	-	166	328	-	-
Ch.A	-	-	-	1.2	-	-	-	-	-	-
Ch.A/2	-	-	-	1.5	-	-	-	298	-	79
Ch.B	-	-	-	2.6	-	1	-	219	-	-
Ch.B/2	-	-	-	2.5	-	-	-	285	-	109
E.1/A	32,000	-	4.1	-	1	-	110	294	23	1
E.1/B	32,000	-	4.1	-	1	-	110	177	23	4
E.1/2	32,000	-	3.6	-	2	-	145	-	-	-
E.2A	32,000	-	3.5	-	-	-	-	337	51	.3
E.2B	32,000	-	3.5	-	-	-	-	326	51	.1
R.1A	128,000	10,000		-	-	-	-	373	2	2
R.1B	128,000	"		-	-	-	-	359	2	26
R.2A	256,000	"		-	-	-	-	354	1	12
R.2B	256,000	"		-	-	-	-	351	1	29
R.3A	32,000+	"		-	-	-	196	384	1	1
R.3B	32,000+	"		-	-	-	182	375	1	5
R.4A	64,000+	"		-	-	-	181	376	1	.1
R.4B	64,000+	"		-	-	-	173	353	1	4
R.5A	128,000+	"		-	-	-	131	363	1	2
R.5B	128,000+	"		-	-	-	105	287	1	13
R.6A	256,000+	"		-	-	-	65	333	.4	11
R.6B	256,000+	"		-	-	-	66	367	.4	39
R.7A	128,000	12,500		-	-	-	-	368	-	14
R.7B	128,000	"		-	-	-	-	315	-	65
R.8A	256,000	"		-	-	-	-	376	.1	34
R.8B	256,000	"		-	-	-	-	345	.1	37
R.9A	32,000+	"		-	-	-	166	355	.1	7
R.9B	32,000+	"		-	-	-	166	328	.1	13
R.10A	64,000+	"		-	-	-	172	383	.2	3
R.10B	64,000+	"		-	-	-	174	357	.2	14
R.11A	128,000+	"		-	-	-	106	345	.1	16
R.11B	128,000+	"		-	-	-	97	326	.1	28
R.12A	256,000+	"		-	-	-	93	359	.8	5
R.12B	256,000+	"		-	-	-	86	296	.8	23

* Haemoglobin expressed as grammes per 100 ml. blood. 1 and 2 represent the decrease recorded following the initial and challenge infections respectively.

** The gain in weight represents the difference between day 10 and day 0-1, day 31 and day 21.

*** The average total oocyst production per bird to the nearest million recorded aft the initial and challenge infections.

Following reinfection the total oocyst production was greater in the chickens of groups R.2A, R.2B, R.6A, R.6B and R.8A which received 256,000 oocysts per bird in each dose of vaccine compared with that of chickens in the corresponding group vaccinated with 128,000 oocysts per bird, represented by groups R.1A, R.1B., R.5A, R.5B and R.7A respectively. (Table 4.26).

The results showed that the average oocyst output per bird after challenge was significantly lower in the chickens which received the irradiated oocysts in 2 doses compared with the birds which were given the same total number of irradiated oocysts in 1 inoculation (Table 4.27).

DISCUSSION.

The results (Table 4. 28) demonstrated that resistance to reinfection with a high challenge dose of normal oocysts was conferred by vaccination with irradiated oocysts in young chickens. The only indication of infection after inoculation was shown by the presence of a relatively small number of oocysts in the faeces (Table 4. 25) during the patent phase of the disease. There was no significant variation in the oocyst production of the chickens when the inoculum was increased from 32,000 to 256,000 irradiated oocysts per bird, and no significant increase in oocyst output after administration of the second dose of vaccine in the appropriate groups. The oocyst production varied only very slightly between the birds given oocysts exposed to 10,000R and those receiving oocysts exposed to 12,500R in the vaccine, although a marked difference was recorded between these birds after reinfection. (Table 4. 26 and Table 4. 27).

The pathogenicity of the culture used for X irradiation was established by the administration of a standard dose of normal oocysts to susceptible chickens.

The results (Table 4. 22) of the haemoglobin estimation demonstrated a marked fall in the concentration of haemoglobin in these birds after infection which confirmed the clinical findings during the acute phase of the disease. The pathogenic effects of the normal oocysts were also emphasised by the death of three birds after infection on day 0 and by the high oocyst production of the surviving chickens. The results (Table 4. 25) indicated that the reproductive potential of the parasite is greater in a 16 day old chicken, compared with that in an 8 day old chicken, after inoculation with a standard dose of 32,000 sporulated oocysts.

Unfortunately evidence of potassium dichromate toxicity occurred in the chicks which received doses of 128,000 or 256,000 irradiated oocysts on day 0. This was most marked in groups R.6A, R.6B, R.12A and R.12B which received the latter dose of oocysts. The first sign of toxicity was indicated by the morbidity recorded in these chicks on day 0, day 1 and day 2, when the birds appeared slightly depressed. It was also illustrated by the less satisfactory weight gains of the chickens in groups R.5A, R.5B, R.6A, R.6B, R.11A, R.11B, R.12A and R.12B on day 10 (Table 4. 24), the retardation of growth being still evident in the total weight gain on day 42, although there was no difference in the weight gains of these groups when compared with that of the non-infected control chickens on day 31 after reinfection. The toxicity was confirmed on post mortem examination of the chickens which died on day 2 and day 3. These deleterious effects were not observed in the other groups of vaccinated chickens which made satisfactory weight gains after both administration of the vaccine and following reinfection with a high challenge dose of normal oocysts (Table 4. 24).

The pathogenicity of the challenge doses of 32,000 and 64,000 normal oocysts,administered on day 22 to the birds in groups /A and /B respectively, was demonstrated by the typical signs of caecal coccidiosis which occurred in the fully susceptible chickens of groups Ch.A and Ch.B and the corresponding replicate groups Ch.A/2 and Ch.B/2 after infection. These findings were confirmed by the decrease in the concentration of haemoglobin recorded on day 28 (Table 4. 23) and by the death of one bird in group Ch.B. The pathogenic effects of the challenge infections were also demonstrated at post mortem examination on day 28 when severe lesions were found in the caecal of the chickens from group Ch.A and Ch.B, and by the high total oocyst production of 79 and 109 million per bird in the replicate groups Ch.A/2 and Ch.B/2 during the patent period of the disease (Table 4. 26). The pathogenicity of the higher challenge dose of oocysts was emphasised by the adverse effect on the weight gains of the chickens in group Ch.B on day 31 (Table 4. 24).

There was no evidence of clinical disease in the vaccinated chickens after reinfection with 32,000 normal oocysts. The only indications of infection were shown by the presence of very slight lesions in the caecal mucosa of some of the chickens which had been inoculated with oocysts exposed to 12,500R, and by the production of oocysts during the patent phase of the disease. No changes were found in the caeca of the birds which had been vaccinated with oocysts exposed to 10,000R, with the exception of group R.1A in which the caecal contents contained a very slight trace of blood. The oocyst production (Table 4. 26 and 4. 27) ranged from 0.1 to 34 million per bird, being significantly lower than that of the fully susceptible chickens in group Ch.A/2. There was quite a marked variation in the oocyst production between the corresponding groups vaccinated with oocysts exposed to 10,000R and 12,500R which confirmed the post mortem findings made on day 28. These observations suggested that a higher level of immunity to reinfection was conferred by vaccination with oocysts exposed to 10,000R.

Following the administration of the higher challenge dose of 64,000 normal oocysts on day 22, marked differences were clearly demonstrated in the level of resistance conferred by single and double vaccination with irradiated oocysts and also between the immunising effects of oocysts exposed to 10,000R and 12,500R respectively.

There was no indication of morbidity or any decrease in the level of haemoglobin (Table 4. 23) in the vaccinated chickens after reinfection. However slight evidence of haemorrhage was observed on day 28 in each group of birds which received only one dose of vaccine and in the birds which were inoculated with two doses of 256,000 oocysts exposed to 12,500R. These observations were confirmed at post mortem when quite severe lesions were found in the caeca of the birds in group R.1B, R.2B, R.7B and R.8B, the changes being more pronounced in the birds vaccinated with oocysts exposed to 12,500R. No indication of infection was seen in the caeca of the birds examined on day 28 from group R.5B and R.10B, and only very slight lesions were present in the birds representing groups R.4B, R.6B and R.11B, in which no sign of haemorrhage was observed during the acute phase of the disease after reinfection. These results emphasised the significance of two immunising doses of irradiated oocysts when the level of immunity was compared between the birds which received the same total number of oocysts given as one single infection in groups R.1B, R.2B, R.7B and R.8B, with the birds which received the oocysts in two doses of vaccine in groups R.4B, R.5B, R.10B and R.11B respectively.

The total oocyst production (Table 4. 26 and 4. 27) of the vaccinated chickens after reinfection was lowest in the groups which had received two doses of vaccine. There was also a marked difference in the oocyst production of the chickens between the groups vaccinated with oocysts exposed to 10,000R and 12,500R respectively, which confirmed the variation seen in the corresponding groups after administration of the lower challenge dose of 32,000 normal oocysts. The lowest oocyst production of 0.1 and 4 million per bird was recorded in the birds which were vaccinated with two doses of oocysts exposed to 10,000R. These results also confirmed the clinical and post mortem findings after reinfection and indicated conclusivley that the highest level of immunity is conferred by double vaccination with oocysts exposed to 10,000R.

There did not appear to be a close relationship between the number of irradiated oocysts administered to the birds and the subsequent oocyst production of the birds after reinfection (Table 4. 27). The results suggested that the level of immunity was slightly higher when the immunising dose of irradiated oocysts was increased from 32,000 to 64,000 per bird in each dose of vaccine. However, when the inoculum contained 128,000 or 256,000 irradiated oocysts, the oocyst production after challenge tended to increase, which suggested that the immunity of these birds was less satisfactory. This observation was most marked in the groups which received the high challenge dose of normal oocysts. It was possible that these results were due to the effect of the potassium dichromate toxicity which occurred in these birds after administration of the first dose of vaccine on day 0. Therefore, further investigations are necessary to determine the optimum number of irradiated oocysts for administration in each dose of vaccine.

Good immunity was demonstrated in the chickens which received normal oocysts on day 0 and day 8 respectively, although this method of vaccination was contraindicated by the deleterious effects which occurred during immunisation. After reinfection the oocyst production was slightly greater in the chickens which were inoculated on day 0 compared with that of the birds infected on day 8. This may indicate that the level of immunity is beginning to decrease on the twentyfirst day after inoculation with one dose of normal oocys

The work of Horton-Smith, Beattie and Long, (1961) showed that when chickens are reinfected with sporulated oocysts of E. tenella 14, 21 and 28 days after infection with a single dose of oocysts, macroscopic and microscopic lesions are present in the caeca of the birds challenged at 21 and 28 days, while evidence of reinfection is practically indetectable in the chickens challenged at 14 days.

These experimental observations demonstrated that 8 day old chicks develop a high level of immunity to reinfection with 64,000 normal sporulated oocysts of E. tenella after vaccination with two doses of oocysts exposed to 10,000R. Therefore, this method of immunisation could have practical implications in the field as it is possible to ensure that immunity is developed at an early age before a significant challenge infection is established in the litter under intensive conditions of management. The very low oocyst production of the immunised chickens after reinfection suggests that vaccination may have a beneficial effect on the epidemiology of the disease. This fact is illustrated by the significant difference between the oocyst production of the vaccinated and susceptible chickens after reinfection.

EXPERIMENT TWELVE.

IMMUNISATION AGAINST *EIMERIA TENELLA* INFECTION BY DOUBLE VACCINATION WITH IRRADIATED OOCYSTS IN 9 DAY OLD CHICKENS REARED ON DEEP LITTER

EXPERIMENTAL AIMS.

The experiment was designed to study the immunity conferred by double vaccination with irradiated oocysts of *E. tenella* in 9 day old chickens, reared intensively on deep litter, against reinfection with normal oocysts.

Earlier experimental observations clearly demonstrated that a high level of resistance to reinfection was developed after double vaccination with sporulated oocysts exposed to 10,000R in broiler type hybrid chickens when the challenge infection was given to the birds 14 days after the administration of the second dose of vaccine. It was important to establish that the immunity conferred by this method of vaccination gave adequate protection against the pathogenic effects of the parasite throughout the life of the chicken under intensive conditions of husbandry practised in the field. Broiler chickens are usually marketed when approximately 70 days old, so it was necessary to determine if the level of immunity was still significant at this age. Therefore the challenge infection of normal oocysts was administered to the vaccinated chickens when they were 11 weeks old. Chickens were also reared in cages with wire floors after inoculation to record the total oocyst production during the patent period following vaccination. These birds were challenged on the fourteenth day after administration of the second dose of vaccine to confirm the previous results and show that a high degree of immunity to reinfection could be reproduced consistently in young chickens.

Preliminary studies showed that oocysts could be recovered from the faeces during the patent period of the infection after vaccination with irradiated oocysts, although the oocyst production was significantly lower than that of chickens given normal oocysts. It was essential to determine the importance of this factor under intensive management when the birds had free access to faecal material on the litter. Susceptible chickens were therefore placed with the vaccinated birds on the litter during the patent period of the infection. These were transferred to cages for observation at appropriate intervals.

The immunising dose of 64,000 irradiated oocysts was selected for administration on both day 0 and day 13, as good immunity had been demonstrated previously following vaccination with a similar number of oocysts exposed to 10,000R in 8 day old chickens.

The challenge dose of normal oocysts was increased to determine whether resistance was adequate when the chickens were exposed to very heavy challenge infections. The birds were selected at random from the vaccinated groups and placed in two groups before reinfection when they received either 128,000 or 256,000 normal oocysts per bird on day 68. The chickens which were reinfected on day 27 received either 32,000, 64,000 or 128,000 normal oocysts per bird, the two former doses of oocysts representing the levels of infection given to the corresponding groups in the previous experiment.

The severity of the disease in each group of chickens was determined from observations made on mortality, on clinical signs, on growth rate and on oocyst production after both vaccination and reinfection. Haemoglobin estimations were carried out on all the chickens after reinfection and also on the chickens which received normal oocysts on day 0 and on day 13 to confirm the pathogenicity of the culture exposed to X-irradiation. The pathogenicity of the challenge dose of oocysts was also confirmed in fully susceptible chickens on day 27 and day 68 respectively. Birds were killed for post mortem examination after reinfection to determine if there was any variation in the severity of the caecal lesions between each group which might indicate a difference in the immunity of the chickens.

MATERIALS AND METHODS.

Experimental Birds.

Broiler type cockerel chickens were used in the experiment. They were reared in complete isolation and were transferred to the experimental units on the appropriate day before inoculation. The chicks given irradiated oocysts were housed in a separate room from those which received normal oocysts. Two groups of vaccinated chicks were placed on clean wood shavings in identical pens measuring approximately 7' x 8' after administration of the first dose of vaccine. One group of vaccinated birds was retained in the experimental unit where they were housed in metal cages with wire floors. The chicks were fed on a special high energy broiler crumb containing high levels of both minerals and the Vitamin B complex (B.O.C.M. Special Ration E.208). The food was available ad lib. except on day 0 and day 14 when it was withdrawn three hours before administration of the vaccine, and on day 28 when food was removed from the birds 18 hours before infection with the challenge dose of oocysts. The food was also withdrawn on day 68, approximately 12 hours before the birds were reinfected with normal oocysts.

The chicks were 9 days old on day 0.

Parasitology.

The culture used for infection of the chickens on day 0 was 18 days old with a total oocyst count of 888,500 per ml. and a sporulation count of 83%. Three 15 ml. aliquots of the culture were exposed to 10,000R two days before inoculation, i.e. day 0-2. Total oocyst counts were carried out on the culture after X-irradiation on the day before infection, i.e., day 0-1. Dilutions were made to give 64,000 sporulated oocysts per ml. by the addition of distilled water to the culture which was suspended in 2% potassium dichromate solution. An aliquot of the culture containing normal oocysts was also diluted with distilled water to give 32,000 sporulated oocysts per ml. for administration to group E.1 and E.1/2. The dilutions were carried out on day 0.

The culture used for vaccination on day 13 was 32 days old, with a total oocyst count of 937,500 per ml. and a sporulation count of 80%. Three 15 ml. aliquots of the culture were exposed to 10,000R two days before administration on day 13, i.e., day 11. Total oocyst counts were made on the culture after X-irradiation on day 12. The vaccine was diluted with distilled water on day 13 to give 32,000 sporulated oocysts per ml. An aliquot of the culture containing normal oocysts was also diluted to give 16,000 sporulated oocysts per ml. for administration to group E.2 and E.2/2.

The culture administered to the chickens on day 27 was 46 days old with a total oocyst count of 900,000 per ml. and a sporulation count of 75%. Dilutions were made on day 27 to give 16,000, 32,000 and 64,000 sporulated oocysts per ml. respectively, by the addition of distilled water to appropriate aliquots of the culture.

The culture administered to the chickens on day 68 was 18 days old with a total oocyst count of 1,355,000 per ml. and a sporulation count of 65%. Dilutions were made on day 68 to give 64,000 and 128,000 sporulated oocysts per ml. by the addition of distilled water.

<u>Administration of the Inoculum.</u>

The infecting dose of irradiated oocysts was given in 1 ml. of water on day 0 and in 2 ml. of water on day 13. The challenge dose of normal oocysts was administered in 2 ml. of water on days 27 and 68.

The inoculum was administered directly into the crop using an automatic dosing syringe. During inoculation the stock of oocysts from which the syringe was filled was kept in suspension by decanting the solution between two beakers.

The chickens were inoculated in the morning on day 0 and in the afternoon on day 15. The challenge dose of oocysts were administered to the birds in the afternoon on both day 27 and day 68.

Experimental Design.

One hundred chickens were selected at random and divided into two groups, L.1 and L.2 after administration of the first dose of vaccine. Each group of chickens was then transferred to a separate pen where they were reared in isolation for a period of 61 days on deep litter composed of clean "green" sawdust. The litter was approximately 3 inches deep on day 0. The litter was kept moist by the addition of water during the first few weeks of the experiment to ensure that optimum conditions were present for sporulation of the oocysts passed in the faeces of the vaccinated birds. Twenty birds were selected at random from these groups on day 61 and transferred to cages with wire floors. These birds were divided into groups of 10 and designated V.1 and V.2.

Twenty susceptible chickens were placed in each pen, L.1 and L.2, on day 10. These birds were transferred to metal cages with wire floors for observation in groups of five, ten and five birds, on day 21, day 30 and day 40. The groups were designated S.1/A, S.2/A, S.1/B, S.2/B, S.1/C and S.2/C respectively.

Ten chickens were selected at random for groups R.1, R.2 and R.3, E.1, E.1/2, E.2, E.2/2, Ch.1, Ch.1/2, Ch.2, Ch.2/2, Ch.3, Ch.3/2, Ch.4 and Ch.4/2, and the non-infected control group.

The chickens in groups L.1, L.2, R.1, R.2 and R.3 received an immunising dose of 64,000 sporulated oocysts after exposure to 10,000R on both day 0 and day 13. The pathogenicity of the culture was determined by the administration of 32,000 normal sporulated oocysts per bird in groups E.1 and E.1/2 on day 0, and in groups E.2 and E.2/2 on day 13. The birds given irradiated oocysts were inoculated before those which received normal oocysts.

The chickens in groups R.1, Ch.1 and Ch.1/2, R.2, Ch.2 and Ch.2/2, R.3, Ch.3 and Ch.3/2 received a challenge dose of 32,000, 64,000 and 128,000 normal sporulated oocysts per bird respectively on day 27.

The chickens in groups V.1 and Ch.4/1 and V.2 and Ch.4/2 received a challenge dose of 128,000 and 256,000 normal sporulated oocysts per bird respectively on day 68.

Chickens were also vaccinated with irradiated oocysts on day 0 and day 13 for post mortem examination after reinfection on day 27 and day 68 respectively. Birds were included to respresent each group and also for the administration of a challenge dose of 500,000 normal oocysts on day 68 which were designated V.3, together with a corresponding susceptible group, Ch.4/3.

Haemoglobin estimations were made on the chickens of groups E.1, E.1/2 and the non-infected control chickens after inoculation on day 3 and day 6. Haemoglobin estimations were made on groups E.2, E.2/2 and the non-infected control chickens on day 16 and day 19, i.e., day 3 and day 6 after inoculation.

Haemoglobin estimations were carried out on the chickens of groups R.1, R.2, R.3, Ch.1, Ch.1/2, Ch.2, Ch.2/2, Ch.3 and Ch.3/2 and the non-infected control group on day 29 and day 33, i.e., day 2 and day 6 after reinfection. They were also made on the chickens of groups V.1, V.2, Ch.4/1, Ch.4/2 and the non-infected control chickens on day 70 and day 74, i.e. day 2 and day 6 after reinfection.

The birds in groups L.1 and L.2, and the non-infected control group, together with the corresponding chickens of groups Ch.4/1 and Ch.4/2 were weighed initially on day 1. The chickens taken from groups L.1 and L.2 for groups V.1 and V.2 were re-weighed on day 70 (i.e. C + 2) and on day 87 (C + 19), together with the corresponding birds of the non-infected control pen and the fully susceptible birds of group Ch.4/1 and Ch.4/2.

The birds in groups R.1, R.2 and R.3 were weighed initially on day 0-1, together with the chickens of group E.1 and E.1/2, while the fully susceptible chickens of the corresponding groups Ch.1, Ch.2, Ch.3 and the birds of E.2 and E.2/2 were weighed on day 0. The chickens of groups R.1, R.2, R.3, Ch.1, Ch.1/2, Ch.2, Ch.2/2, Ch.3 and Ch.3/2 plus the non-infected control group were reweighed on day 28 (i.e. C + 1), day 37 (i.e. C + 10) and on day 47 (i.e. C + 20).

The total oocyst production of the vaccinated birds of groups R.1, R.2 and R.3 was recorded after vaccination, together with that of the chickens which received normal oocysts in the corresponding groups E.1 and E.2 on day 0 and day 13 respectively.

The total oocyst production of the susceptible chickens reared with the birds of groups L.1 and L.2 was recorded after transfer to cages on day 21, day 30 and day 40, in groups S.1/a and S.2/a, S.1/B and S.2/B, S.1/C and S.2/C, on day 22 to day 37, on day 31 to day 44 and on day 41 to day 49 respectively.

Following reinfection on day 68 the total oocyst production was recorded during the patent period of the infection from day 75 to day 88 in groups V.1, V.2, Ch.4/1 and Ch.4/2.

Clinical signs and mortality were also recorded.

RESULTS.

(1) Haematology.

The results (Table 4. 29) of the haemoglobin estimations did not show any significant variation in the concentration of the haemoglobin in the non-infected control chickens on day 3 and day 6 or on day 16 and day 19. The results demonstrated a marked fall of 4g. and 5g. in the level of haemoglobin in groups R.1 and R.1/2 respectively on day 6. There was no significant difference between the concentration of haemoglobin recorded on day 16 and day 19 in group R.2 and R.2/2.

The results (Table 4. 30) showed no significant variation in the level of the haemoglobin recorded on day 29 and day 33 in groups R.1, R.2 and R.3 after reinfection on day 27. The results demonstrated a small decrease of approximately 1g. to 2g. of haemoglobin in groups Ch.1, Ch.1/2, Ch.2 and Ch.2/2 and a marked fall of approximately 4g. in groups Ch.3 and Ch.3/2 on day 33.

The results (Table 4. 31) demonstrated no significant difference in the concentration of haemoglobin of the non-infected control birds and the vaccinated chickens in group V.1 and V.2 on day 70 and day 74. There was a decrease of 0.7g. and 2.3g. of haemoglobin in group Ch.4/1 and Ch.4/2 respectively on day 74.

(2) Mortality.

Two deaths occurred in group R.1 on day 5 and a third bird died in this group on day 6. One bird was found dead in group R.1/2 on both day 5 and day 11.

Following the administration of the challenge dose of normal oocysts on day 27, one chicken died in both groups Ch.2 and Ch.2/2 on day 32. Five and six birds died in groups Ch.3 and Ch.3/2 respectively on day 32. One death occurred in both group Ch.1/2 and group Ch.2/2 on day 33.

Table 4. 29.

The Mean Haemoglobin Concentration of the Chickens Infected with a Standard Dose of 32,000 Sporulated Oocysts of *E. tenella* in Groups E.1, E.1/2, E.2 and E.2/2 on Day 0 and Day 13 respectively, to Confirm the Pathogenicity of the Culture which was Exposed to X-Irradiation on Day 0-2 and Day 11.

Group	Day 3		Day 6		Haemoglobin Decrease	"P"
Control	8.0*	±0.7**	8.7	±0.3	nil	
E.1.	7.8	±0.6	3.8	±1.1	4.0	0.001***
E.1/2.	8.1	±0.3	3.0	±1.0	5.0	0.001
	Day 16		Day 19			
Control	8.6	±0.5	9.1	±0.7	nil	
E.2.	8.2	±0.5	7.8	±0.7	0.4	0.25
E.2/2	7.9	±0.7	7.7	±0.9	0.2	0.25

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

** The Standard Deviation.

*** The probability calculated by the "t - test".

Table 4. 30.

The Mean Haemoglobin Concentration in the Blood of the Vaccinated Chickens and the Fully Susceptible Chickens after Reinfection with a Standard Challenge Dose of Sporulated Oocysts on Day 27.

Group	Oocyst Dose Day 27	Day 29	Day 33	Haemoglobin Decrease.
Control	nil	8.0* ±0.4**	7.7 ±0.9	nil
Ch. 1	32,000	7.3 ±0.6	6.2 ±1.5	0.9
Ch. 1/2	"	7.3 ±0.5	5.3 ±1.6	2.0
R. 1.	"	7.6 ±0.7	8.1 ±0.8	nil
Ch. 2	64,000	7.4 ±0.7	6.2 ±1.4	1.2
Ch. 2/2	"	7.2 ±0.6	4.9 ±1.1	2.3
R. 2	"	7.5 ±0.7	7.6 ±0.5	nil
Ch. 3	128,000	7.0 ±0.6	2.8 ±0.7	4.2
Ch. 3/2	"	7.4 ±0.6	3.8 ±1.4	3.6
R. 3.	"	7.0 ±0.6	7.6 ±0.5	nil

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

** The standard deviation.

Table 4. 31.

The Mean Haemoglobin Concentration in the Blood of the Vaccinated Chickens and the Fully Susceptible Chickens after Reinfection with a Standard Challenge Dose of Sporulated oocysts on Day 68.

Group	Oocyst Dose Day 68	Day 70	Day 74	Haemoglobin Decrease.
Control	nil	8.4* ±0.8**	8.8 ±0.8	nil
Ch.4/1	128,000	8.4 ±0.8	7.7 ±0.9	0.7
V.1.	"	8.1 ±0.6	8.6 ±0.6	nil
Ch.4/2	256,000	8.7 ±1.0	6.4 ±0.7	2.3
V.2.	"	7.8 ±0.7	8.2 ±0.6	nil

* Haemoglobin concentration expressed as grammes per 100 ml. of blood.

** The standard deviation.

After reinfection on day 68, two deaths occurred in group Ch.4/2 on day 74.

(3) Clinical Findings.

No evidence of morbidity or haemorrhage was seen in the birds which received irradiated oocysts on day 0 and day 13. Typical signs of acute caecal coccidiosis occurred in groups S.1 and S.1/2 which received normal oocysts on day 0. Slight evidence of haemorrhage was also seen in group S.2 and S.2/2 after administration of the normal oocysts on day 13, although morbidity was not marked.

No indication of haemorrhage or morbidity was recorded in the vaccinated chickens of groups R.1, R.2 and R.3 after reinfection on day 27, in contrast to the typical symptoms of caecal coccidiosis seen in the corresponding groups of susceptible chickens on day 32, day 33 and day 34. The haemorrhage was most marked in groups Ch.3 and Ch.3/2 and was emphasised by the high morbidity and mortality recorded in these groups on day 32. The evidence of haemorrhage was less pronounced in group Ch.2 and Ch.2/2, although these birds appeared quite depressed on day 32 and day 33. The haemorrhage was comparatively slight in group Ch.1 and Ch.1/2 in which no marked indication of morbidity was seen during the acute phase of the disease.

After reinfection on day 68 no evidence of haemorrhage or morbidity was recorded in the vaccinated birds of group V.1 and V.2, in contrast to the marked clinical symptoms found in susceptible chickens of the corresponding groups Ch.4/1 and Ch.4/2. The first indication of haemorrhage occurred on day 73 when it was most severe in group Ch.4/2. On day 74 the haemorrhage appeared very pronounced in both groups Ch.4/1 and Ch.4/2. Morbidity was less marked in group Ch.4/1 compared with that in group Ch.4/2 where the birds appeared depressed on day 73 and day 74.

(4) Weight Gains.

The results (Table 4. 32) showed no significant variation in the mean weight of the chickens of the experimental groups on day 0-1, day 0 and day 1 respectively.

There was no significant difference in the mean weight gains on day 28, day 37 and day 47 between the non-infected controls and the vaccinated chickens in groups R.1, R.2 and R.3. On day 47 the mean weight of the birds in groups R.1, R.2 and R.3 was slightly greater than that of the chickens in the non-infected control group.

The results did not show any significant difference in the mean weight of the chickens in the fully susceptible challenge groups on day 28, but the values were slightly lower than those recorded in the vaccinated and non-infected control groups. This difference was also reflected in the weight gains of the birds on day 28 (Table 4. 36).

The mean weights of the fully susceptible challenge groups Ch.1, Ch.1/2, Ch.2 and Ch.2/2 were slightly lower than those of the vaccinated and non-infected control chickens on day 37. The mean weight gain recorded was less than that attained by the vaccinated and non-infected control chickens over the same period of time between day 28 and day 37 (Table 4. 36). There was no significant difference between the weight gain of these susceptible birds which ranged from 248g. to 286g., compared with 310g. in the non-infected control group. There was a significant difference between the mean weight gain of groups Ch.1, Ch.1/2, Ch.2 and Ch.2/2 on the one hand and Ch.3 on the other where the growth rate was severely depressed, the mean weight gain of the latter groups ranging from 60g. to 151g. respectively on day 37.

The results (Table 4. 32) indicated that satisfactory weight gains were made by the surviving chickens in groups Ch.1 to Ch.3/2 between day 37 and day 47.

Table 4. 32.

The Mean Weight of the Vaccinated Chickens in Groups R.1, R.2, and R.3, and the Fully Susceptible Chickens in the Corresponding Groups Ch.1, Ch.1/2, Ch.2, Ch.2/2, Ch.3 and Ch.3/2 on Day 0-1, Day 28, Day 37 and Day 47.

Group	Day 0-1[x]	Day 28[xxx]	Day 37	Day 47
Control	163[xx]	894	1204	1523
Ch.1	126	824	1072	1432
Ch.1/2	127	828	1096	1477
R.1	112	860	1191	1626
Ch.2	116	781	1066	1459
Ch.2/2	123	800	1064	1458
R.2.	112	838	1191	1691
Ch.3	124	790	860	1245
Ch.3/2	117	793	944	1425
R.3.	109	846	1187	1586

x The mean weight expressed in grammes.

xx The non-infected control chickens were weighed initially on day 1, and the birds in groups Ch.1 - Ch.3/2 were weighed on day 0.

xxx The standard challenge dose of normal oocysts was administered to the chickens on day 27. The inoculum contained 32,000, 64,000 and 128,000 sporulated oocysts per bird in groups R.1, R.2 and R.3 respectively. A similar dose of oocysts was given to each corresponding group of susceptible chickens in Ch.1, Ch.1/2, Ch.2, Ch.2/2, Ch.3 and Ch.3/2.

Table 4. 33.

The Mean Weight Gain of the Vaccinated Chickens in Groups V.1 and V.2 and the Fully Susceptible Chickens in the Corresponding Groups Ch.4/1 and Ch.4/2 on Day 1, Day 70 and Day 87.

Group.	Day 1*	Day 70***	Day 87	Total Gain****
Control	153**	2239	2667	2514
Ch.4/1	130	2382	2694	2564
V.1	154	2355	2890	2766
Ch.4/2	130	2209	2447	2317
V.2	134	2544	3107	2973

* The weight of groups V.1 and V.2 on day 1 is represented by the mean weight recorded in group L.1 and L.2 on day 1.

** The mean weight expressed in grammes.

*** The standard challenge dose of normal oocysts was administered to the chickens on day 68. The birds in group V.1 and V.2 and each corresponding challenge group received 128,000 and 256,000 sporulated oocysts per bird respectively.

**** The total weight gain represents the difference between the value recorded on day 1 and day 87.

The mean weights of the chickens in groups Ch.1, Ch.1/2, Ch.2 and Ch.2/2 were slightly lower than than of the non-infected control group on day 47. However, this variation was less than that recorded on day 28. The mean weight of the chickens in groups Ch.3 and Ch.3/2 on day 47 appeared less satisfactory, the retardation of growth being marked in group Ch.3 in which the total weight gain was approximately 260g. less than that of the non-infected control group.

The results (Table 4. 33) showed no significant variation in the mean weight of the chickens of group V.1, V.2, Ch.4/1 and Ch.4/2 on day 1. A slight variation was recorded in the mean weight of these groups on day 70.

The results (Table 4. 37) showed no evidence of an adverse effect on the mean weight gains of the chickens in group V.1 and V.2 after reinfection, in contrast to the less satisfactory weight gains which were recorded in group Ch.4/1 and Ch.4/2 on day 87. The mean weight gain was approximately 100g. and 200g. less in groups Ch.4/1 and Ch.4/2 respectively than that attained by the non-infected control group.

(5) Pathology.

(i) Post mortem Examination of the Chickens Killed on Day 33 after Reinfection on Day 27.

Typical lesions of caecal coccidiosis were found in the caeca of the fully susceptible chickens of group Ch.1, Ch.2 and Ch.3. The lesions were very severe in both groups Ch.2 and Ch.3 and were slightly less pronounced in the birds from groups Ch.1 (Figures 27, 28 and 29).

No evidence of infection was demonstrated in the chickens of group R.1 and R.3. Careful examination of the caecal mucosa in the chickens from group R.2 suggested that it was possibly very slightly thickened, although no evidence of haemorrhage was present (Figures 27, 28 and 29).

(ii) Post mortem Examination of the Birds Killed on Day 74 after Reinfection on Day 68.

Quite severe lesions were present in the caeca of the fully susceptible chickens from groups Ch.4/1 and Ch.4/2. The caecal walls were markedly thickened and showed evidence of erosion of the mucosa which was emphasised by the presence of numerous small haemorrhages on the mucosa. Extravasated blood and cellular debris was present in the lumen of the caeca.

No indication of infection was demonstrated in the caeca of the birds from groups V.1 and V.2 (Figure 30).

Chickens were also examined on day 74 after reinfection with a challenge dose of 500,000 sporulated oocysts on day 68. Very severe lesions were demonstrated in the fully susceptible chickens, in marked contrast to the very slight changes found in the corresponding birds of the vaccinated groups which were designated V.3 (Figure 30). The only evidence of infection in the chickens from V.3 was suggested by a very slight indication of thickening in the caecal mucosa. The caecal contents appeared quite normal, in contrast to the susceptible birds in which the lumen was filled with cellular debris and extravasated blood.

(iii) Post mortem Examination of the Birds which Died on Day 5 and Day 6 after Reinfection on Day 27 and Day 68 respectively.

Typical lesions of acute caecal coccidiosis were demonstrated in the birds of group E.1 and E.1/2 which died after receiving a standard dose of normal oocysts on day 0. Similar changes were also found in the caeca of the fully susceptible chickens from groups Ch.1/2, Ch.2, Ch.2/2, Ch.3, Ch.3/2 and Ch.4/2 which died after the administration of a standard challenge dose of normal oocysts on day 27 and day 68 respectively.

FIGURE 27.

THE IMMUNITY CONFERRED BY DOUBLE VACCINATION WITH 64,000 OOCYSTS EXPOSED TO 10,000R AGAINST REINFECTION WITH 32,000 NORMAL OOCYSTS.

Note:-

Absence of lesions in the vaccinated bird, in contrast to typical lesions of caecal coccidiosis in susceptible bird. (See also Figs. 28 and 29).

FIGURE 28.

THE IMMUNITY CONFERRED BY DOUBLE VACCINATION WITH 64,000 OOCYSTS, EXPOSED TO 10,000R AGAINST REINFECTION WITH 64,000 NORMAL OOCYSTS.

Note complete absence of lesions in vaccinated bird (R.20) after reinfection with high challenge dose of normal oocysts, in contrast to typical lesions of acute caecal coccidiosis in susceptible chicken (Ch.)

FIGURE 29.

THE IMMUNITY CONFERRED BY DOUBLE VACCINATION WITH 64,000 OOCYSTS, EXPOSED TO 10,000R, AGAINST REINFECTION WITH 128,000 NORMAL OOCYSTS.

Note complete absence of lesions in caeca of vaccinated bird (R.21), in contrast to typical lesions of acute caecal coccidiosis in susceptible bird.
This demonstrates conclusively that a high level of immunity is conferred by double vaccination with X-irradiated oocysts.

FIGURE 30.

IMMUNITY CONFERRED BY DOUBLE VACCINATION WITH 64,000 X-IRRADIATED OOCYSTS IN CHICKS REARED ON DEEP LITTER AGAINST REINFECTION WITH EITHER 256,000 or 500,000 NORMAL OOCYSTS.

Note complete absence of lesions in caeca of vaccinated birds challenged with 256,000 (V.2) or 500,000 (V.3) normal oocysts at 11 weeks of age, in contrast to the typical signs of acute caecal coccidiosis in susceptible birds (G.2 and G.3).

(6) Oocyst Production.

The results (Table 4. 34) demonstrated a significant difference between the oocyst production of the birds given normal oocysts and those given irradiate oocysts on day 0 and day 13. The total average production per bird was 11 and 7 million in group N.1 and N.2 respectively, compared with 0.7 million per bird in groups R.1 - R.3. No increase in oocyst production occurred after the administration of the second dose of vaccine on day 13.

There was a marked difference in the oocyst production of the susceptible chickens which were transferred to cages for observation from the replicate groups of vaccinated chickens on deep litter. The oocyst production appeared significantly greater in the birds which were removed from group L.1 on both day 21 and day 31, although no variation was found on day 41 when the final group of susceptible birds were taken from the deep litter pens. The total oocyst production per bird was 11 and 6 million in group S.1A and S.1B, compared with 2 and 0.1 million in the corresponding groups S.2A and S.2B respectively. No oocysts were recovered from the faeces of the chickens which were placed under observation in cages on day 41 in groups S.1C and S.2C.

After reinfection on day 68 a significant variation was recorded between the oocyst production of the susceptible chickens and the vaccinated chickens, the average total oocyst production per bird ranging from 71 to 87 and 5 to 9 million respectively (Table 4. 35).

Table 4. 34.

The Average Total Daily Oocyst Production, Expressed in Millions of Oocysts per Bird, of the Vaccinated Chickens in Groups R.1 to R.3 which received 64,000 Irradiated Oocysts on Day 0 and on Day 15, and Group N.1 and N.2 which received 32,000 Normal Oocysts per Bird on Day 0 and Day 15 respectively, and of the Fully Susceptible Chickens Transferred to Cages on Day 21, Day 30 and Day 40, from the Pens of Vaccinated Chickens reared on Deep Litter.

Day	N.1	N.2	R.1**	Day	S1A	S2A	Day	S1B	S2B	Day	S1C	S2C
7	2.7		*	22	0.8	0.4	31	4.1	0.2	41	-	-
8	5.4		0.6	23	0.7	0.5	32	1.3	*	42	-	-
9	1.5		*	24	0.5	0.5	33	0.3	*	43	-	-
10	0.3		0.1	25	0.1	0.1	34	0.2	*	44	-	-
11	0.2		*	26	0.3	*	35	0.1	-	45	-	-
12	*		*	27	0.1	-	36	-	-	46	-	-
13	0.1		*	28	4.3	*	37	*	-	47	-	-
14	0.1		-	29	3.2	0.1	38	-	-	48	-	-
15	0.3		-	30	0.8	0.1	39	*	-	49	-	-
16	0.2		*	31	0.1	*	40	-	*			
17	0.1		-	32	0.2	*	41	-	-			
18	*		-	33	*	-	42	-	-			
19	-		-	34	*	-	43	-	-			
20	*		-	35	-	-	44	-	-			
21	*	0.3	-	36	*	-						
22	*	2.9	-	37	-	-						
23	-	0.2	-									
24	-	0.5	-									
25	-	1.3	-									
26	*	0.8	-									
27	-	0.8	-									
Total per Bird	11	7	0.7		11	2		6	0.1		0	0

* Indicates that oocyst production was less than 50,000 per bird.

** i.e. Total daily oocyst production per bird recorded in R.1 represents mean value for R.1, R.2 or R.3.

Table 4. 36.

The Average Total Daily Oocyst Production, Expressed in Millions of Oocysts per Bird, of the Vaccinated Chickens in Groups V.1 and V.2 and the Fully Susceptible Chickens in the Corresponding Groups Ch.4/1 and Ch.4/2, after Reinfection with a Standard Challenge dose of 128,000 and 256,000 Normal Sporulated Oocysts per Bird on Day 68.

Day	Ch.4/1	V.1.	Ch.4/2	V.2.
75	24.2	*	18.9	-
75	36.0	1.3	42.1	0.9
76	6.1	2.6	5.3	3.6
77	0.4	0.7	0.6	3.1
78	1.5	0.1	0.4	0.7
79	4.8	0.1	0.6	0.4
80	3.6	*	0.1	0.1
81	1.5	*	0.5	*
82	2.0	*	0.1	*
83	1.2	*	0.2	*
84	1.2	-	0.2	-
85	2.5	-	0.3	-
86	0.3	-	0.1	*
87	0.7	-	0.1	-
Total per Bird	87	5	[illegible]	9

* Indicates that oocyst production was less than 50,000 oocysts per bird.

Table 4. 36.

The Effect of Vaccination with Two Doses of 64,000 Oocyst Exposed to 10,000R before Inoculation in Young Chickens against Reinfection with a Standard Challenge Dose of Normal Oocyst administered 14 Days after Administration of the Second Dose of Vaccine.

Group	Challenge Dose Day 27	Haemoglobin** Decrease		Mortality		Weight Gain***		Oocyst**** Output
		(1)	(2)	(1)	(2)	Day 28	Day 37	
Control	-	-	-	-	-	741	310	-
E.1*	-	4.0	-	3	-	-	-	11
E.1/2	-	5.0	-	2	-	-	-	-
E.2.	-	0.4	-	-	-	-	-	7
E.2/2	-	0.2	-	-	-	-	-	-
Ch.1	32,000	-	0.9	-	-	699	248	-
Ch.1/2	"	-	2.0	-	1	701	268	-
R.1.	"	-	-	-	-	738	341	0.7
Ch.2	64,000	-	1.2	-	1	666	295	-
Ch.2/2	"	-	2.3	-	2	677	254	-
R.2.	"	-	-	-	-	723	363	0.7
Ch.3	128,000	-	4.2	-	5	666	60	-
Ch.3/2	"	-	3.6	-	6	676	151	-
R.3.	"	-	-	-	-	737	321	0.7

* Groups E.1 to E.2/2 represent chickens given 32,000 normal oocysts on Day 0 or Day 13 to confirm the pathogenicity of the culture exposed to X-irradiation before vaccination.

** (1) and (2) represent the results after the initial and challenge inoculations respectively. Haemoglobin expressed as grammes per 100 ml. of blood.

*** The gain in weight represents the difference between day 28 and day 0 and between day 37 and day 28 respectively.

**** The average total oocyst production per bird to the nearest million recorded after vaccination only.

Table 4. 37.

The Effect of Vaccination with Two Doses of 64,000 Irradiated Oocysts of *E. tenella* in Young Chickens against Reinfection with a Standard Challenge Dose of Normal Oocysts on day 68, when the Chickens were 11 Weeks Old

Group	Challenge Dose Day 68	Haemoglobin* Decrease	Mortality	Weight Gain** Day 87	Oocyst*** Output
Control	-	-	-	428	-
Ch. 4/1	128,000	0.7	-	312	87
V. 1.	"	-	-	535	5
Ch. 4/2	256,000	2.3	2	238	71
V. 2.	"	-	-	563	9

* Haemoglobin expressed as grammes per 100 ml. of blood.

** The weight gain represents the difference between the mean weight recorded on day 70 and day 87

*** The average total oocyst production per bird to the nearest million recorded between day 75 and day 87, i.e., C+7 to C+20

DISCUSSION.

The results (Table 4.36 and Table 4. 37) demonstrated that inoculation with two doses of 84,000 sporulated oocysts exposed to 10,000R in young broiler chickens conferred a high level of resistance to infection with a heavy challenge dose of normal oocysts administered two and eight weeks after vaccination. No evidence of any detrimental effect was observed in the vaccinated chickens during immunisation or after reinfection. Satisfactory weight gains were recorded (Table 4. 32)and Table 4. 33) in the birds after administration of the vaccine and also following each challenge infection on day 27 and day 68 respectively.

The only indication of infection after vaccination was shown by the presence of a relatively small number of oocysts in the faeces (Table 4. 34). The observations made on the fully susceptible birds which were placed in the deep litter pens with the vaccinated chickens during the patent period after inoculation indicated that these oocysts could be responsible for infection under field conditions. There was no evidence of clinical disease in these birds, infection being demonstrated only by the presence of oocysts in the faeces of birds when they were transferred to cages for observation (Table 4. 34). The total oocyst production recorded in these chickens was quite low, the production being lower in the birds removed from the pens on day 30 compared with that of the chickens transferred from the pens on day 21, while no oocysts were demonstrated in the faeces of the groups removed from the pens on day 40. These results appeared to indicate that the number of oocysts was not building up in the litter as it would be anticipated that if the oocysts were increasing in the pen then the oocyst production of the susceptible chickens would also tend to increase initially, being reflected in the oocyst production of the second or third group of birds transferred to cages. This did not occur.

although precautions were taken to provide optimum conditions for the sporulation of oocysts in the deep litter pens. Therefore the oocyst production of the vaccinated chickens would not appear to constitute an important factor in the development of a heavy challenge infection under intensive methods of husbandry. The explanation of this observation may be due to both the low number of oocysts passed after infection with oocysts exposed to 10,000R and also to the ingestion of these oocysts by the vaccinated chickens which could be a significant factor, limiting the increase of the oocyst population in the litter. Recent experimental studies have shown that the life cycle of the parasite is suppressed when sporulated oocysts are administered to immune chickens (Pierce, Long and Horton-Smith, 1962). These observations suggested that only a very few of the sporozoites had developed to complete the life cycle. Thus the ingestion of oocysts by resistant chickens should tend to reduce the number of oocysts reaching the litter compared with the number which would be produced by susceptible chickens under similar circumstances. This conclusion is supported by the present series of observations on the oocyst production of the vaccinated chickens after reinfection with a high challenge dose of normal oocysts when the relatively low total oocyst production of the immunised birds suggested that the oocyst output would be negligible after reinfection with a low challenge dose of oocysts. The results (Table 4. 34) also demonstrated quite a marked difference in the oocyst production of the chickens taken from each identical deep litter pen which indicated the possible variation in the incidence of the disease under field conditions and emphasised the complexity of the epidemiological aspect of infection with <u>E. tenella</u>.

The evidence of slight infection in the susceptible birds is of interest in relation to the practical aspects of vaccination with irradiated oocysts in the field. The present trend in the poultry industry is to increase the size of the individual flock which indicates that it would be necessary to administer the vaccine in the drinking water. This would result in a certain variation in the number of irradiated oocysts ingested by the individual bird due to the difference in the amount of water consumed by each bird. It is possible that some chickens will receive either a low or a high dose of vaccine. No problem is presented by the chicken given a high number of irradiated oocysts, since no deleterious effects have been observed following the administration of large numbers of oocysts after exposure to 10,000R. However, the immunity conferred by a low dose of vaccine might be less satisfactory. It is probable, therefore, that the presence of a relatively small number of oocysts on the litter could be beneficial by providing an opportunity for these chickens to acquire a slight infection which would enhance their resistance to reinfection. The results (Table 4. 34) do not suggest that this infection would be associated with any significant increase in the number of oocysts in the litter but further investigations should be made to confirm this observation as the number of chickens studied in the present experiment was limited.

The pathogenicity of the culture which was later exposed to X-irradiation was established by the administration of a standard dose of normal oocysts to susceptible chickens. The results (Table 4. 29) of the haemoglobin estimation demonstrated a marked decrease in the concentration of the haemoglobin in the chickens on day 6, which was confirmed by the death of five birds during the acute phase of the disease. The pathogenic effects of the normal oocysts appeared less severe on day 16 when no significant change occurred in the level of haemoglobin of the susceptible chickens although slight evidence of haemorrhage was recorded in the birds on the fifth and sixth day after infection. However, the pathogenicity of the culture was demonstrated after the administration of the challenge doses of normal oocysts to the susceptible chickens on day 27. The results of the haemoglobin estimation (Table 4. 30) showed a fall of 1g. to 2g. of haemoglobin in the birds which received 32,000 or 64,000 oocysts, and a decrease of approximately 4g. of haemoglobin in the birds given the highest challenge dose of 128,000 oocysts. These changes were confirmed by the clinical findings and by the deaths which occurred on day 32 and day 33. (Table 4. 36). The adverse effects of the normal oocysts were also reflected in the weight gains of the susceptible birds on day 37 (Table 4. 36) when the marked depression of growth in the birds in group Ch.3 and Ch.3/2 emphasised the very high pathogenicity of the high challenge dose of 128,000 oocysts. The results (Table 4. 36) indicated a marked difference in the pathogenic effects of 64,000 and 128,000 normal oocysts respectively. However, there was also a variation in the severity of the disease between the groups given32,000 and 64,000 oocysts per bird and this was shown by the greater morbidity and higher mortality recorded in the birds given the latter challenge dose of oocysts.

There was no sign of clinical disease in the vaccinated chickens after reinfection on day 27. The only evidence of infection was found at post mortem examination on day 33 in the chickens challenged with 64,000 oocysts when very slight lesions were seen in the caecal mucosa. Unfortunately it was not possible to study the oocyst production of the vaccinated chickens during the patent period after reinfection. However, the very high level of immunity conferred by vaccination with irradiated oocysts was demonstrated conclusively by the comparison of the results after reinfection between group R.3 and the corresponding groups of susceptible chickens in group Ch.3 and

Ch. 3/2 which received the high challenge dose of 128,000 oocysts (Table 4. 36). The absence of any adverse effect associated with reinfection in the vaccinated chickens when the challenge dose of oocysts was increased from 64,000 to 128,000 oocysts clearly indicated that the immunity was adequate to protect the birds against a very heavy challenge infection.

The previous experimental observations were confirmed by the satisfactory resistance of the vaccinated chickens on day 27 against reinfection with 32,000 and 64,000 oocysts respectively. Close agreement was demonstrated between the results of the haemoglobin estimations, the weight gains and the clinical and post mortem findings after challenge in the corresponding groups of immunised and susceptible birds. This showed that a high degree of immunity to reinfection could be reproduced consistently in young chickens by the administration of two doses of oocysts after exposure to 10,000R.

The pathogenicity of the challenge doses of 128,000 and 256,000 normal oocysts administered to the vaccinated birds on day 68 was confirmed by the typical clinical and post mortem findings of caecal coccidiosis in the corresponding groups of susceptible chickens. The high pathogenicity of the latter challenge dose of oocysts was emphasised by the decrease of haemoglobin on day 74 (Table 4. 31) and by the death of two birds during the acute phase of the disease (Table 4. 37). The severe pathogenic effect of the challenge infections was still evident in the susceptible birds on day 87 when the weight gain of the birds was significantly lower than that of the non-infected control birds (Table 4. 33 and Table 4. 37).

No evidence of clinical disease was observed in the vaccinated birds after reinfection on day 68. This was confirmed by the satisfactory weight gains which were recorded on day 87 (Tables 4. 33 and 4. 37) and at post mortem on day 74 when no lesions were found in the caeca of the birds selected at random for examination from each group. The only indication of infection in the vaccinated birds was shown by the presence of oocysts in the faeces during the patent period, the total oocyst production being 5 and 9 million per bird, in contrast to 87 and 71 million per bird in the corresponding groups of susceptible chickens (Table 4. 56).

These results demonstrated a very significant degree of resistance to reinfection in the vaccinated chickens on day 68. The high level of immunity was emphasised at post mortem on day 74 by the presence of only very slight lesions in the caeca of the vaccinated chickens, in contrast to the very severe changes found in the caeca of the susceptible chickens after a challenge infection of 500,000 oocysts on day 68. Therefore vaccination with two doses of irradiated oocysts in young chickens gives satisfactory resistance against reinfection when the birds are exposed to a heavy challenge infection at 11 weeks old.

These observations indicate that this method of immunisation should provide adequate protection against the pathogenic effects of the parasite in the broiler chicken. It is also probable that the level of resistance would be satisfactory in replacement pullets and laying hens. However, further studies are necessary to determine the duration of immunity after vaccination under field conditions.

SUMMARY OF PART II.

SUMMARY - PART 2.

A series of six experiments were performed to investigate the effects of X-irradiation on the protozoan parasite *Eimeria tenella* with particular reference to (i) the pathogenicity of the parasite and (ii) the resistance to reinfection conferred by inoculation with irradiated oocysts.

The initial selection of the range of Rontgen doses was made purely on an arbitary basis as it was felt that there was unlikely to be any accurate correlation with the method of X-irradiation carried out by Albanese and Smetana (1937) or with Waxler (1941).

The pathogenicity of the cultures was confirmed on administration to fully susceptible chickens both before exposure to X-irradiation and also at challenge when immunity to reinfection was determined. Typical signs of acute caecal coccidiosis were recorded in the susceptible chickens following infection with a standard dose of normal oocysts. These were illustrated by the marked fall in the level of haemoglobin, the high morbidity and the mortality during the acute phase of the disease. They were confirmed by the poor mean weight gains and the high total oocyst production of the surviving chickens and by the presence of lesions in the caeca of the birds examined at post-mortem.

Observations were made over a wide range of Rontgen doses in the first experiment to establish the levels of X-irradiation where changes in the pathogenicity of the parasite are first recognised and where the parasite is completely inactivated.

The detrimental effects of the parasite were compared between 24 day old chickens given a standard dose of 32,000 irradiated oocysts exposed to doses ranging from 5,000R to 80,000R and chickens given a similar number of normal oocysts. The severity of the disease in each group was determined from

observations made on the changes in the concentration of haemoglobin, the clinical findings, the mortality, the weight gains and the oocyst production during the patent phase of the infection. Birds were also selected at random from each group for post mortem examination during the acute phase of the disease to determine whethere there was any variation in the severity of the caecal lesions between chickens given normal oocysts and those given irradiated oocysts. The degree of immunity conferred by the initial inoculation with irradiated oocysts was compared with that of the survivors from the group given normal oocysts after reinfection on day 21 with a standard dose of 32,000 normal oocysts.

The experimental results demonstrated a significant difference between the pathogenicity of the normal oocysts of E. tenella and those which had been exposed to X-irradiation before infection.

The detrimental effects of the sporulated oocysts were reduced after exposure to 5,000R, the lowest level of X-irradiation studied. The lower pathogenicity of the parasite was demonstrated by the absence of morbidity and by the small degree of haemorrhage during the acute phase of the infection, the decrease in the level of haemoglobin being only 2g. compared with a fall of 5g. in the group given normal oocysts. The beneficial effect of X-irradiation was also emphasised by the satisfactory mean weight gains recorded in the birds receiving irradiated oocysts, in contrast to the poor weight gain observed in the chickens given a similar number of normal oocysts. However, the deleterious effects of the parasite were indicated at post mortem when quite marked lesions were found in the caeca on the fifth day after inoculation and also during the patent phase of the disease when the total oocyst production of the chickens approached that of the bird given normal oocysts.

The pathogenicity of the sporulated oocysts was negligible after exposure to 10,000R. The only sign of infection was shown by the production of a relatively small number of oocysts during the patent phase of the disease. No indication of infection was observed after inoculation with oocysts exposed to doses ranging from 20,000R to 80,000R before administration.

A significant degree of resistance to infection was conferred by irradiated oocysts after exposure to 5,000R, 10,000R, 20,000R or 30,000R. This was shown by the absence of morbidity and mortality after challenge with normal oocysts during the acute phase of the infection. It was confirmed by the results of the haemoglobin estimation on the sixth day after reinfection when no decrease occurred in the concentration of the haemoglobin, in contrast to a fall of approximately 2g. in the birds of both the susceptible groups and the groups inoculated initially with oocysts exposed to 40,000R, 50,000R, 60,000R or 80,000R on day 0. The variation in the degree of immunity conferred by sporulated oocysts exposed to different levels of X-irradiation was emphasised further by the results of post mortem examination on the seventh day after challenge.

The immunity conferred by oocysts exposed to 5,000R appeared only slightly less than that demonstrated in the survivors from an infection with a similar number of normal oocysts. However, the high total oocyst production associated with vaccination contraindicated immunisation with oocysts exposed to this level of X-irradiation. The high level of resistance stimulated by inoculation with oocysts subjected to 10,000R or 20,000R before administration was not associated with this disadvantage. These observations suggested that the vaccination with oocysts exposed to appropriate doses of X-irradiation might have practical implications in the field.

Resistance appeared significantly less in the chickens inoculated with oocysts exposed to 30,000R as quite pronounced lesions were present in the caeca at post mortem, although no fall had been recorded in the concentration of haemoglobin after challenge.

Following reinfection the lesions appeared slightly less severe in the chickens vaccinated with oocysts exposed to 40,000R, 50,000R or 60,000R, compared with the changes in the fully susceptible birds. This suggested that a small degree of immunity was conferred by the oocysts exposed to these levels of X-irradiation.

No significant difference was observed between the birds given oocysts exposed to 80,000R on day 0 and the fully susceptible birds after administration of the challenge dose of normal oocysts. The susceptibility of the former birds was also emphasised by the death of one bird during the acute phase of the disease. These findings indicate that sporulated oocysts of E. tenella are completely inactivated by exposure to 80,000R.

The development of resistance to reinfection after inoculation with irradiated oocysts, without evidence of oocyst production during the immunising infection, indicates that certain levels of X-irradiation only partially inhibit the development of the parasite. The absence of lesions in the caeca after inoculation suggest that the life cycle is interrupted before completion of second stage schizogony, the phase responsible for the pathogenic effects of the parasite.

The development of immunity in the birds inoculated with oocysts exposed to 30,000R confirms that the decrease in the pathogenicity of the parasite following X-irradiation is not due to a simple reduction in the number of visible oocysts in the inoculum alone, since no oocysts were found in the faeces of this group after the initial infection.

These results determined the selection of the levels of X-irradiation ranging from 5,000R to 40,000R for further investigation in the second experiment. The interval between each Rontgen dose was halved so that the effect of X-irradiation on the parasite could be studied in greater detail while confirming the initial experimental findings. The challenge dose of oocysts was increased to 64,000 as it was important to establish the degree of immunity to a high level of infection and also show if there was a significant variation in the resistance correlated with the level of irradiation of the initial inoculum.

The experimental findings confirmed the observations made in the first experiment on the beneficial effect of X-irradiation on the pathogenicity of sporulated oocysts of E. tenella. There was close agreement between the results of the corresponding groups after inoculation with oocysts exposed to 5,000R, 10,000R, 20,000R, 30,000R and 40,000R before infection.

There was quite a marked variation between the effects of 5,000R and 7,500R on the subsequent pathogenicity of the parasite which was reflected in the clinical findings and confirmed by the results of the haemoglobin estimations and also by the oocyst production of the birds during the patent period of the disease.

The results showed conclusively that sporulated oocysts of E. tenella must be exposed to a minimum dose of 10,000R before administration to fully susceptible chickens to ensure that no detrimental effects occur after inoculation.

Slight differences were demonstrated between the effect of 10,000R on the one hand and 15,000R and 20,000R on the other. These were illustrated by the oocyst production after inoculation and by differences in the degree of immunity to reinfection with normal oocysts recorded after challenge in the appropriate groups of chickens. Small differences were also indicated between the effect of 20,000R and 25,000R when examination of the faeces during the patent period of the disease failed to demonstrate the presence of any oocysts in the samples from the chickens given oocysts exposed to 25,000R. The results did not suggest any variation in the effect of levels of X-irradiation ranging from 25,000R to 40,000R on the pathogenicity of the parasite and no evidence of infection was recorded in birds which received oocysts exposed to 25,000R, 30,000R, 35,000R or 40,000R.

Following reinfection with 64,000 normal oocysts, a high level of immunity was demonstrated in the birds vaccinated with oocysts exposed to 5,000R or 7,500R. Resistance to the high challenge dose of oocysts was significantly lower in birds inoculated with oocysts exposed to 10,000R while the level of immunity appeared negligible in the chickens vaccinated with oocysts exposed to levels of X-irradiation ranging from 15,000R to 40,000R. The administration of oocysts exposed to 5,000R or 7,500R was associated with adverse effects during immunisation which indicated conclusively that a dose of 32,000 irradiated oocysts was too low to confer satisfactory protection against a heavy challenge infection.

Therefore observations were made on the immunising effect of three additional levels of infection of 64,000, 128,000 and 256,000 sporulated oocysts per bird after exposure to 10,000R, 15,000R, 20,000R or 25,000R in Experiment 3. The challenge dose of 32,000 normal oocysts was selected to avoid an overwhelming infection which might mask differences in the resistance conferred by different numbers of oocysts at each level of X-irradiation.

No evidence of clinical disease was seen when the immunising dose of irradiated oocysts was increased from 32,000 to 256,000 oocysts per bird. The only indication of infection was shown by the production of relatively small numbers of oocysts in each group of birds receiving oocysts exposed to 10,000R. Oocysts were recovered from only one or two groups of chickens after inoculation with oocysts exposed to 15,000R, 20,000R or 25,000R.

A significant difference was demonstrated in the degree of resistance to reinfection between the chickens vaccinated with oocysts exposed to 10,000R or 15,000R on the one hand and oocysts exposed to 20,000R or 25,000R on the other. The difference was indicated by the clinical findings and confirmed by the results of the haemoglobin estimation on the sixth day after challenge and by the post mortem findings and oocyst production on the seventh day after reinfection.

Satisfactory immunity was conferred by administration of 128,000 or 256,000 oocysts exposed to 10,000R. The presence of slight lesions in the caeca of some of the birds inoculated with 32,000 or 64,000 irradiated oocysts suggested that the level of resistance increased when the dose of vaccine was raised from 64,000 to 128,000 oocysts per bird.

A significant degree of resistance was also conferred by vaccination with oocysts exposed to 15,000R. The level of immunity appeared slightly lower than that conferred by oocysts exposed to 10,000R as slight lesions were found in the caeca of some of the birds after challenge from each appropriate group. This variation was also reflected by the higher oocyst production recorded on the seventh day after reinfection.

The degree of protection conferred by oocysts exposed to 20,000R or 25,000R was significantly lower, although the detrimental effects of the challenge infection were less severe than those recorded in the fully susceptible chickens. The initial conclusion that the beneficial effect of X-irradiation on the pathogenicity of the parasite was not due to a lethal effect alone on the viability of the oocysts, leading to a simple reduction in the number of oocysts in the infective dose, was confirmed by the presence of immunity in these birds. Resistance was demonstrated in chickens in which no oocyst production was recorded during the patent period of the immunising infection. Thus the development of the parasite can have been only partially inhibited by X-irradiation, as it is generally accepted that when the early phases of the life cycle are completely inhibited the birds are fully susceptible to re-infection

The fourth experiment was designed to compare the degree of immunity conferred by single and double vaccination with oocysts exposed to 10,000R, 12,500R and 15,000R. Several doses of oocysts ranging from 32,000 to 500,000 per bird were selected for comparison.

No detrimental effects were associated with immunisation. The only indication of infection was shown by the presence of a relatively small number of oocysts in the faeces during the patent phase of the disease. Following vaccination with oocysts exposed to 10,000R the production of oocysts increased slightly when the immunising dose was increased from 64,000 to 128,000 oocysts per bird but no further increase occurred when the dose was raised to 256,000 or 500,000 oocysts. No significant variation was recorded in the oocyst production between birds inoculated with oocysts exposed to 12,500R or 15,000R, although a marked difference was recorded between these birds after challenge. There was no significant difference in the total oocyst production after vaccination between the chickens receiving single or double vaccination and no increase occurred in the production of oocysts after administration of the second dose of irradiated oocysts.

The results demonstrated conclusively that the highest degree of resistance was conferred by double vaccination. This was indicated by the clinical and post mortem findings after challenge and confirmed by the marked differences in the total oocyst production of the chickens during the patent phase of the challenge infection. The difference was most marked in the birds immunised with oocysts exposed to 12,500R or 15,000R. The highest level of immunity was conferred by double vaccination with oocysts exposed to 10,000R. Resistance also appeared satisfactory after administration of two doses of oocysts exposed to 12,500R. However, resistance was significantly lower in birds immunised with oocysts exposed to 15,000R.

The beneficial effects of double vaccination were emphasised when the level of immunity conferred by the same number of oocysts was compared between groups given one large dose of oocysts and those receiving oocysts divided into two smaller immunising doses. The greater resistance of the latter birds indicated the significance of the two doses of vaccine rather than the total number of oocysts administered in the inoculum to the chickens. However, the results suggested a definite relationship between the number of oocysts in the vaccine and the total oocyst production of the birds after reinfection. The total oocyst production after challenge tended to decrease as the number of oocysts was increased in the vaccine. This observation suggested a difference in the level of immunity conferred by the different doses of irradiated oocysts and was further emphasised when the oocyst production of the corresponding groups was compared at each level of X-irradiation after challenge.

The results suggested that the resistance of the chickens vaccinated with two doses of 256,000 oocysts exposed to 10,000R equalled that of the surviving chickens immunised with normal oocysts. This observation emphasised the practical implications of immunisation with X-irradiated oocysts as the resistance in these groups appeared high, only negligible numbers of oocysts being recovered from the faeces of these birds after challenge. This fact also indicated an important effect on the epidemiology of the disease in the field, suggesting a significant difference in the number of oocysts which would be passed on to the litter under intensive management after infection of fully susceptible and immune birds.

The fifth experiment was designed to study the effects of single and double vaccination with irradiated oocysts in 8 day old chicks as it was important to show that similar levels of immunity could be produced in young chicks before a significant challenge infection was established in the litter under intensive methods of husbandry.

Several doses of oocysts ranging from 32,000 to 256,000 per bird were selected for vaccination after exposure to 10,000R or 12,500R. The challenge doses of 32,000 and 64,000 normal oocysts were slected to represent low and high levels of infection as it was necessary to determine if there was a significant variation in the resistance associated with different methods of vaccination and also establish the degree of immunity to a heavy challenge infection.

The results demonstrated conclusively that resistance to reinfection with a high challenge dose of oocysts was conferred by vaccination with irradiated oocysts in young chicks.

The only indication of infection after inoculation was shown by the presence of a relatively small number of oocysts in the faeces during the patent period of the disease. There was no significant variation in the oocyst production of the chickens when the inoculum was increased from 32,000 to 256,000 irradiated oocysts per bird and no significant increase in oocyst output after administration of the second dose of vaccine. The oocyst production varied only very slightly between birds given oocysts exposed to 10,000R and those given oocysts exposed to 12,500R in the vaccine, although a marked difference was recorded between these birds after reinfection.

There was no indication of morbidity or any decrease in the level of haemoglobin in the vaccinated chickens after reinfection, although slight evidence of haemorrhage was recorded in the birds given only one dose of vaccine. This observation, together with the post mortem findings on the seventh day after challenge and the results of the oocyst production during the patent phase of the challenge infection, confirmed the significance of double vaccination. The importance of two immunising doses of vaccine was also emphasised when the level of resistance was compared after administration of the same total number of irradiated oocysts in chickens given either one large dose or two small doses of vaccine.

There was a marked difference in the oocyst production after reinfection between birds vaccinated with oocysts exposed to 10,000R and 12,500R respectively, which confirmed that the highest level of immunity is conferred by double vaccination with oocysts exposed to 10,000R.

The absence of a definite relationship between the number of irradiated oocysts in the vaccine and the degree of immunity to reinfection was associated with the effect of potassium dichromate toxicity following administration of the first dose of vaccine. Therefore, further investigations were necessary to determine the optimum number of irradiated oocysts for vaccination.

The important practical implications of the attenuated vaccine were indicated by the high level of resistance to reinfection, demonstrated in young birds without evidence of any pathogenic effect during immunisation, and by the development of resistance at an early age which suggested that adequate protection would be present before a challenge infection was established under intensive methods of poultry hysbandry. The advantages of vaccination were also shown by the low oocyst production of the immunised chickens after reinfection compared with the high oocyst output of the susceptible chickens, indicating a significant effect of vaccination on the epidemiology of the disease.

It was necessary to establish that vaccination with irradiated oocysts gave satisfactory protection against disease throughout the life of the bird under intensive conditions of husbandy practised in the field. Therefore the sixth experiment was designed to study the duration of resistance to reinfection. Observations were also made to determine the significance of the oocyst production associated with vaccination as it was important to ensure that the vaccine did not introduce a continuous cycle of reinfection in the immunised chickens, leading to the "build up" of a heavy challenge infection in the litter.

The immunising dose of 64,000 irradiated oocysts was selected for administration on day 0 and day 13 as good resistance had been demonstrated previously with this number of oocysts. The challenge dose was increased to 128,000 and 256,000 normal oocysts per bird as it was important to determine if immunity was adequate when the chickens were exposed to a very heavy challenge. The vaccinated birds were reared on deep litter until 10 weeks of age when birds were selected at random for reinfection and transferred to cages for observation. Susceptible chickens were reared with the vaccinated birds during the patent phase of the infection after vaccination. These were transferred to cages for observation at appropriate intervals. Chickens were also reared in cages with wire floors after vaccination to record the oocyst production during the patent period after immunisation. These chickens were challenged with normal oocysts on the fourteenth day after administration of the second dose of vaccine to confirm previous results and to show that a high degree of immunity to reinfection could be reproduced consistently in young chickens.

The results demonstrated conclusively that vaccination with two doses of 64,000 sporulated oocysts exposed to 10,000R in young broiler chickens conferred a high level of resistance to reinfection with a heavy challenge dose of normal oocysts administered two and eight weeks after immunisation. No evidence of any pathogenic effect was observed in the vaccinated chickens during immunisation or after reinfection. Satisfactory weight gains were recorded in the vaccinated birds both after administration of the vaccine and after reinfection.

The previous experimental observations were confirmed by the satisfactory resistance of the vaccinated chickens on day 27 against reinfection with 32,000 and 64,000 normal oocysts respectively. Close agreement was demonstrated between the results of the haemoglobin estimations, the weight gains and the clinical and post mortem findings, after challenge in the corresponding groups of vaccinated and susceptible chickens. This showed that a high degree of immunity to reinfection could be reproduced consistently in young chickens by the administration of two doses of oocysts exposed to 10,000R.

No evidence of clinical disease was recorded in the susceptible birds transferred to cages from the deep litter pen for observation. The only indication of infection was shown by the presence of a relatively small number of oocysts in the faeces of the birds. The oocyst production was lower in the birds removed from the pen on day 30, compared with that of the group placed in cages on day 21, while no oocysts were demonstrated in samples from the group transferred to cages on day 40. It would be reasonable to anticipate that if the infective population of oocysts was increasing in the pen it should be reflected by an increase in the oocyst output of the birds removed from the litter on day 30 or day 40. This did not occur although precautions were taken to provide optimum conditions for sporulation of the oocysts in the deep litter pens. Therefore these results suggest that the oocyst production associated with vaccination does not introduce a continuous cycle of reinfection in the chickens, leading to the "build up" of a heavy challenge infection in the litter. However, further investigations should be made to confirm this observation as the number of birds studied in the experiment was limited. The explanation of this finding may be associated with three factors, (i) the comparatively low oocyst production of the vaccinated chickens, (ii) the failure of these oocysts to complete sporogony successfully and (iii) the ingestion of the oocysts by the vaccinated chickens leading to the suppression of the life cycle. The results also demonstrated quite a marked difference in the oocyst of the chickens taken from identical deep litter pens, showing the possible variation which can occur in the incidence of the disease under field conditions and emphasising the complexity of the epidemiological aspect of infection with Eimeria tenella.

These observations indicate that this method of immunisation should provide adequate protection against the pathogenic effects of Eimeria teneall in the BROILER CHICKEN. It is also probable that the level of resistanse would be satisfactory in replacement pullets and laying hens, although further studies are necessary to determine the duration of immunity under field conditions.

GENERAL SUMMARY AND CONCLUSIONS.

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The pathogenic effects of the protozoan parasite *Eimeria tenella* were studied in the domestic fowl (*Gallus domesticus*). Observations were then made on the effect of X-irradiation on the pathogenic effect and the immunogenic potential of sporulated oocysts of *Eimeria tenella*.

The initial findings established that the severity of an infection can be determined satisfactorily by consideration of the haemoglobin concentration, the clinical signs and the mortality during the acute stage of the disease, together with the results of the weight gains, the post mortem findings and the total oocyst production of the chickens during the patent phase of the disease. The pathogenicity of the parasite cannot be assessed accurately from consideration of only one aspect of the infection.

The disease was reproduced with consistent pathogenicity by the administration of a standard dose of sporulated oocysts.

A definite relationship was suggested between the age of the bird, the number of oocysts inoculated and the severity of the disease, although no significant difference in susceptibility to infection was shown which could be directly attributed to the age of the experimental chicken. It would appear reasonable to suggest that the degree of haemorrhage and tissue damage resulting from a specified dose of oocysts might constitute an overwhelming infection for a young bird while proving non-fatal for an older bird.

The results suggested that after a standard dose of oocysts retardation of growth increased in severity with the age of the chicken between four and six weeks of age.

Marked differences were recorded in the severity of the disease after administration of doses ranging from 1,000 to 500,000 oocysts per bird. The deleterious effects of relatively low levels of infection were reflected in the less satisfactory weight gains and the high total oocyst production during the patent period of the disease. No significant difference in haemoglobin concentration or mortality was recorded between birds given doses ranging from 32,000 to 500,000 oocysts, the increasing severity of infection being shown by the higher morbidity, the lower weight gains and the marked fall in oocyst production. The reproductive potential of the parasite decreased significantly as the dose of oocysts was increased. The total oocyst production per bird was relatively similar when the doses ranged from 1,000 to 8,000 oocysts, increasing significantly when the inoculum contained 16,000 to 64,000 oocysts and decreasing markedly when the dose was raised to 128,000 to 500,000 oocysts. It is suggested that the relatively low oocyst production following infection with high doses of oocysts may be correlated with the severity of the caecal lesions.

A significant relationship between the feeding regime before infection and the subsequent pathogenicity of the parasite was demonstrated, the most severe pathogenic effects occurring in birds given free access to food before infection. The pathogenicity of the parasite appeared significantly less when the food was removed from the chickens twelve hours before administration of the inoculum. The most significant variation occurred at low levels of infection.

A significant difference in susceptibility to infection was demonstrated between Broiler and Leghorn Type Hybrid chicks, the severity of the disease being greater in the Leghorn Hybrid chickens.

The pathogenic effects of a standard dose of oocysts was influenced by the diet of the experimental chickens. The effect of the ration on the severity of the infection also varied significantly in Broiler and Leghorn Hybrid chickens.

The pathogenic effects of a standard dose of oocysts was influenced by the diet of the experimental chickens. The effect of the ration on the severity of the infection also varied significantly in Broiler and Leghorn Hybrid chickens.

Resistance to reinfection was conferred by a single dose of sporulated oocysts but this method of immunisation was contraindicated by the pathogenic effects of the parasite during vaccination. The disadvantages of immunisation with normal oocysts were emphasised by the high total oocyst production of the chickens given relatively small numbers of oocysts as this factor could be important in the epidemiology of the disease under intensive poultry management in the field.

In the second series of experiments the pathogenicity of the cultures was confirmed on administration to fully susceptible chickens, both before exposure to X-irradiation and at challenge when immunity to reinfection was determined.

Observations made on the effect of levels of X-irradiation, selected on an arbitrary basis, ranging from 5,000R to 80,000R demonstrated significant differences between the pathogenicity of normal and irradiated oocysts.

Exposure to 5,000R reduced morbidity and haemorrhage during the acute phase of the disease, but evidence of infection was shown by the presence of lesions in the caeca at post mortem, and by the large number of oocysts recovered from the faeces during the patent period after inoculation. The attenuating effect was significantly greater after exposure to 7,500R.

However, the results demonstrated conclusively that sporulated oocysts must be exposed to a minimum dose of 10,000R to avoid pathogenic effects during immunisation, the only evidence of infection being shown by the production of a relatively small number of oocysts. The number increased slightly when the immunising dose was raised from 64,000 to 128,000 oocysts but no further increase occurred when the dose was raised to 256,000 or 500,000 irradiated oocysts. No significant variation was recorded in the oocyst production after vaccination between chickens given one or two doses of vaccine. Only very small numbers of oocysts were recovered from chickens given oocysts exposed to 12,500R, 15,000R or 20,000R. No indication of infection was indicated after administration of oocysts exposed to levels of X-irradiation ranging from 25,000R to 80,000R.

Observations on susceptible chickens reared with vaccinated chickens on deep litter suggested that the oocyst production associated with immunisation does not introduce a cycle of reinfection leading to the build up of a heavy challenge infection under intensive poultry management.

Significant differences were demonstrated in the effect of certain levels of X-irradiation on the immunogenic potential of the oocysts.

Following inoculation with oocysts exposed to either 5,000R or 7,500R, good resistance was demonstrated aginst reinfection with either 32,000 or 64,000 normal oocysts, but vaccination with oocysts exposed to these Roentgen levels was contraindicated due to the pathogenic effects of the parasite during immunisation.

Satisfactory immunity was conferred by oocysts exposed to 10,000R against reinfection with 32,000 normal oocysts. Good immunity was also developed after administration of oocysts exposed to 12,500R or 15,000R. A significant difference was demonstrated between the immunogenicity of oocysts exposed to 15,000R and 20,000R, resistance being significantly lower in birds vaccinated with oocysts exposed to 20,000R, 25,000R or 30,000R. A marked difference was also shown between the effect of 30,000R and 40,000R, immunity being negligible after exposure of oocysts to 40,000R, 50,000R or 60,000R. Chickens inoculated with oocysts exposed to 80,000R appeared fully susceptible on challenge, indicating that the oocysts were completely inactivated by exposure to 30,000R.

Single vaccination failed to confer satisfactory immunity against a high challenge infection. Resistance increased slightly as the immunising dose was raised from 64,000 to 256,000 irradiated oocysts, but the results demonstrated conclusively that the highest degree of immunity was conferred by double vaccination. The greater resistance of birds given two doses of irradiated oocysts compared with birds given the same total number of oocysts in one large dose confirmed the significance of two doses of vaccine rather than the total number of oocysts administered in the vaccine.

Satisfactory immunity was demonstrated against a high challenge dose of normal oocysts in eleven week old broiler chickens. This emphasised the practical implications of the X-irradiated vaccine which were indicated by the high degree of resistance to reinfection consistently reproduced in chicks without evidence of any pathogenic effect during immunisation and by the development of immunity at an early age, suggesting the presence of adequate protection before infection would be established under field conditions.

A significant effect of vaccination was also indicated on the epidemiology of the disease by the low oocyst production of the immunised chickens after challenge, compared with the high production of oocysts recorded in the surviving susceptible chickens.

The demonstration of resistance in birds given oocysts exposed to 25,000R and 30,000R which passed no oocysts during the patent period after vaccination suggested that gametogony is not necessary for the development of immunity. Since it is accepted that schisogony is essential for the stimulation of acquired immunity it would appear that certain levels of X-irradiation only partially inhibit the development of the parasite. The absence of lesions in the caeca after vaccination suggests that the life cycle is interrupted before completion of second stage schizogony, the phase responsible for the pathogenic effect of Eimeria tenella. This observation demonstrated that the decrease in the pathogenicity of the parasite after X-irradiation is not due to a simple reduction in the number of viable oocysts in the inoculum alone.

APPENDIX I

Table Al. 1.

The Haemoglobin Concentration in the Blood of Individual Birds, Aged 3 Weeks On Day 0. Group C.1, non-infected Control Birds and Groups E.1, Inoculated with 128,000 Sporulated Oocysts of *E. tenella* on Day 0.

Group C.1. — Day After Inoculation.

Bird No:	(1)	(3)	(4)	(5)	(6)	(7)	(12)	(14)
1.	8.85*	8.85	9.0	10.05	8.85	9.0	9.45	10.45
2.	8.85	9.45	8.3	8.55	8.85	9.15	8.55	7.5
3.	8.85	9.45	7.7	8.3	8.25	8.85	10.3	9.9
4.	9.75	7.7	7.4	6.95	7.25	8.25	8.55	9.0
5.	7.4	7.8	7.4	9.15	6.8	7.7	6.8	8.0
Mean	8.54	8.65	7.96	8.6	7.88	8.59	8.42	8.97
	±0.85	±0.86	±0.69	±1.1	±0.76	±0.6	±1.3	±1.1

Group E.1.

Bird No:	(1)	(3)	(4)	(5)	(6)	(7)	(12)	(14)
1.	7.7	9.45	6.95	5.6	4.15	4.15	7.1	7.4
2.	8.3	8.3	7.4	6.5	4.75	3.55	6.8	5.9
3.	9.9	12.3	11.2	10.6	7.4	5.6	10.2	10.3
4.	10.3	10.45	8.4	11.05	8.1	3.25	10.05	7.4
5.	7.5	9.9	2.8	3.0	died			
6.	7.1	8.7	9.75	10.05	7.7	7.4	8.0	6.95
7.	9.75	10.6	8.3	8.65	7.1	5.2	7.1	6.8
8.	10.3	9.45	8.3	8.65	7.1	5.2	7.1	7.5
9.	8.0	9.75	7.4	4.45	2.4	3.55	7.4	7.7
10.	8.3	9.15	9.0	4.45	2.65	4.15	7.25	5.5
Mean	8.69	9.79	7.95	7.23	5.4	4.61	7.89	7.27
	±1.2	±1.15	±2.2	±2.86	±2.18	±1.3	±1.31	±1.13
	p=.25	p=.25	p=.25	p=.25	p=.01	p=.001	p=.25	p=.02

Table A1.2.

The Individual Results of the Haemoglobin Concentration in Birds Aged Four Weeks on Day 0. Group C.2, Non-infected Control Birds, Group E.2 Inoculated with 128,000 Sporulated Oocysts of *E. tenella* on Day 0.

Group C.2. — Day After Inoculation.

Bird No:	(1)	(3)	(4)	(5)	(6)	(7)	(12)	(14)
1.	9.15	8.15	8.3	10.3	9.15	8.55	10.05	9.45
2.	8.85	9.15	7.7	7.4	7.7	9.45	10.2	9.0
3.	9.0	8.7	7.7	7.7	8.0	8.1	8.0	8.25
4.	9.15	9.6	9.15	9.45	9.15	9.6	9.3	9.3
5.	10.05	10.2	9.15	9.75	8.0	8.0	8.55	9.0
Mean	9.24 ± 0.47	9.24 ± 0.68	8.4 ± 0.73	8.92 ± 1.21	8.4 ± 0.66	8.74 ± 0.75	9.12 ± 0.95	9.0 ± 0.44

Group E.2

Bird No:	(1)	(3)	(4)	(5)	(6)	(7)	(12)	(14)
1.	8.55	8.4	9.0	died				
2.	7.4	8.4	7.25	5.8	4.9	4.75	6.5	8.25
3.	8.85	9.9	8.3	8.3	7.1	5.6	8.0	8.7
4.	8.55	9.0	9.3	-	died			
5.	6.95	9.15	8.85	6.5	4.0	4.45	7.4	7.4
6.	8.85	9.75	8.0	7.1	2.5	2.95	7.5	8.0
7.	8.44	9.15	7.5	7.5	3.0	3.0	8.0	8.0
8.	8.55	10.9	9.3	7.8	6.65	4.45	7.4	8.25
9.	8.55	8.7	9.0	4.75	died			
10.	10.05	9.15	10.0	9.45	9.45	6.95	8.1	7.4
Mean	8.47 ± 0.85	9.25 ± 0.74	9.65 ± 0.98	6.15 ± 1.38	5.52 ± 2.42	4.73 ± 1.49	7.55 ± 0.56	7.85 ± 0.37
	p=.25	p=.25	p=.25	p=.25	p=0.1	p=.001	p=.02	p=.02

Table A1. 3.

The Individual Results of the Haemoglobin Concentration in Birds Aged 5 Weeks on Day 0. Group C.3, Non-infected Control Birds, and Group E.3 Inoculated with 128,000 Sporulated Oocysts of E. tenella on Day 0.

Group C.3 — Day After Inoculation.

Bird No:	(1)	(3)	(4)	(5)	(6)	(7)	(12)	(14)
1.	9.0	8.85	8.85	8.3	9.3	9.15	8.85	9.15
2.	8.85	8.85	8.4	8.3	9.0	8.7	9.45	9.75
3.	7.7	8.7	8.3	8.0	8.85	9.45	9.9	9.15
4.	9.15	8.7	8.85	8.0	8.25	8.0	8.0	8.25
5.	8.55	8.4	7.4	8.0	8.0	9.3	8.25	10.05
Mean	8.64 $\pm$0.58	8.7 $\pm$0.18	8.3 $\pm$0.54	8.12 $\pm$0.16	8.68 $\pm$0.5	8.92 $\pm$0.59	8.89 $\pm$0.8	9.23 $\pm$0.7

Group E.3

Bird No:	(1)	(3)	(4)	(5)	(6)	(7)	(12)	(14)
1.	8.3	8.3	8.55	6.5	3.1	4.15	5.8	6.65
2.	9.75	10.3	8.55	7.4	3.4	died		
3.	6.8	8.3	8.3	5.5	4.45	3.85	5.2	6.2
4.	7.4	7.7	7.7	5.3	died			
5.	7.4	8.0	8.85	5.8	4.9	5.6	9.15	8.85
6.	7.7	8.85	9.0	7.4	died			
7.	7.7	8.55	8.7	6.2	3.25	4.0	6.05	7.1
8.	9.15	8.3	8.1	6.2	4.45	4.45	8.4	9.15
9.	7.1	7.25	7.4	6.5	3.85	2.8	5.6	6.05
10.	9.75	8.85	8.85	8.65	4.85	5.2	7.4	8.55
Mean	8.105 $\pm$1.09	8.44 $\pm$0.82	8.4 $\pm$0.53	6.55 $\pm$1.44	4.04 $\pm$1.22	4.29 $\pm$0.92	6.8 $\pm$1.51	7.51 $\pm$1.13
	p=.25	p=.25	p=.25	p=.05	p=.001	p=.001	p=.02	p=.05

Table A1. 3/2

The Individual Results of the Haemoglobin Concentration in Birds Aged 5 Weeks on Day 0. Group C.3/2, Non-infected Control Birds, and Group E.3/2 Inoculated with 128,000 Sporulated Oocysts of E. tenella on Day 0.

Group C.3/2 — Day After Inoculation.

Bird No:	(1)	(3)	(4)	(5)	(6)	(7)	(12)	(14)
1.	10.45	9.2	10.3	9.55	8.1	9.5	-	8.55
2.	7.5	8.2	8.2	9.8	7.25	8.4	8.55	8.1
3.	9.9	8.65	9.5	10.1	8.65	9.55	9.75	9.15
4.	9.0	8.2	9.2	10.1	8.4	9.55	9.15	8.85
5.	8.0	8.1	8.4	8.65	7.5	8.0	8.26	6.2
Mean	8.97 ±1.12	8.47 ±0.47	9.12 ±0.85	9.64 ±0.6	7.98 ±0.6	9.0 ±0.74	8.93 ±0.67	8.17 ±1.12

Group E.3/2

Bird No:	(1)	(3)	(4)	(5)	(6)	(7)	(12)	(14)
1.	7.7	8.0	8.5	5.85	3.75	4.45	6.95	7.5
2.	9.15	8.0	8.4	3.6	died			
3.	9.65	7.4	8.9	3.6	5.3	5.55	7.7	7.1
4.	8.1	8.4	6.4	6.55	died			
5.	8.7	9.8	7.25	3.35	4.6	died		
6.	9.75	8.9	9.5	9.4	5.0	4.45	7.5	7.5
7.	9.0	7.8	8.5	5.15	2.8	3.5	4.15	6.35
8.	7.5	8.5	7.8	3.05	died			
9.	9.0	7.7	8.9	4.7	3.05	5.4	7.1	8.1
10.	8.0	7.25	7.5	7.4	4.3	4.15	7.4	7.1
Mean	8.64 ±0.79	8.18 ±0.24	8.17 ±0.92	5.27 ±2.06	4.11 ±0.95	4.59 ±0.77	6.8 ±1.33	7.28 ±0.58
	p=.25	p=.25	p=.25	p=.001	p=.001	p=.001	p=.05	p=.25

Table A1. 4.

The Individual Results of the Haemoglobin Concentration in Birds Aged 6 Weeks on Day 0. Group C.4, Non-infected Control Birds, and Group E.4 Inoculated with 128,000 Sporulated Oocysts of E. tenella on Day 0.

Group C.4. Day After Inoculation.

Bird No:	(1)	(3)	(4)	(5)	(6)	(7)	(12)	(14)
1.	9.45	9.1	9.4	9.5	8.9	9.8	8.0	7.7
2.	9.0	9.1	9.2	8.9	8.65	8.8	8.55	7.8
3.	8.25	8.2	8.9	8.9	8.4	8.6	8.85	8.0
4.	9.3	8.1	8.5	9.5	9.2	9.8	9.75	8.55
5.	9.0	8.65	9.2	9.2	8.5	8.1	8.25	8.0
Mean	9.0 ±0.44	8.63 ±0.15	9.04 ±0.35	9.2 ±0.3	8.73 ±0.32	8.9 ±0.88	8.68 ±0.68	8.41 ±0.33

Group E.4

Bird No:	(1)	(3)	(4)	(5)	(6)	(7)	(12)	(14)
1.	7.5	7.4	8.0	3.6	3.35	3.5	7.4	9.75
2.	8.55	7.7	7.7	4.3	3.6	3.9	7.4	6.5
3.	8.1	7.5	7.5	3.9	3.05	3.9	7.1	7.1
4.	8.55	7.8	8.4	died				
5.	7.1	7.8	8.1	5.0	3.05	6.3	8.0	9.15
6.	8.4	8.0	8.65	4.15	3.5	4.0	7.7	7.4
7.	8.85	8.8	9.2	8.65	5.55	3.6	7.5	8.25
8.	7.8	8.4	8.4	8.5	5.3	4.45	8.25	8.1
9.	6.95	7.25	7.8	3.9	3.35	3.6	7.7	7.1
10.	8.25	7.8	7.0	7.25	6.15	6.4	8.25	7.4
Mean	8.04 ±0.96	7.85 ±0.42	8.05 ±0.62	5.47 ±2.07	4.1 ±1.21	4.41 ±1.14	7.68 ±0.4	7.86 ±1.05
	p=.1	p=.05	p=.05	p=.001	p=.001	p=.001	p=.05	p=.25

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