

STUDIES ON THE COCCIDIA OF PHEASANTS AND ON STRAIN
VARIATION OF COCCIDIA IN GALLINACEOUS BIRDS

by

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ABSTRACT

A complete study of the life history and pathogenicity of Eimeria phasiani Tyzzer 1929, from the pheasant (Phasianus colchicus) was performed for the first time. This study was conducted on a strain of the parasite derived from a single oocyst infection and included investigations of the endogenous and exogenous phases.

E. phasiani was shown to exhibit three separate asexual cycles prior to the onset of the sexual cycle. The oocysts were ellipsoidal in shape, with a smooth, two-layered wall. The size of the oocysts varied significantly from infected chick to infected chick and there was a tendency for the oocysts to increase in size during the patent period. Oocyst production continued for ten to sixteen days and revealed a diurnal periodicity.

The species was pathogenic to young poults causing a loss of live-weight gain and even death depending on the size of the infective dose.

E. phasiani was host specific; both the common partridge and domestic fowl were not susceptible to the species, E. tenella was unable to infect the pheasant.

In a field survey of adult pheasants, E. phasiani was commonly found in Berkshire, Hampshire, Norfolk and Staffordshire but all infections were light.

E. phasiani was controlled completely by 0.006% w/w sulphaquinoxaline but was less susceptible to 0.006% w/w amprolium and 0.0125% w/w zoalene. A search for strain variance with respect to drug action in E. phasiani and E. maxima of the domestic fowl was unsuccessful.

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INTRODUCTION

The coccidia are protozoan parasites commonly found in the epithelial lining of the intestine and associated organs in many vertebrates and invertebrates. The life cycle comprises two phases, an endogenous or intracellular phase within the host and an exogenous phase or maturation of the oocyst, which passes from the host in the faeces. Work on the coccidia has largely been centered on the species in hosts of great economic importance, the domestic fowl for example, and little is known of coccidia from less important hosts including game birds.

Five species have been named from the pheasant Eimeria dispersa (Tyzzer, 1929), E. langeroni (Yakimoff and Matschoulsky, 1937), E. megalo-stomata (Ormsbee, 1939), E. pacifica (Ormsbee, 1939) and E. phasiani (Tyzzer, 1929); and four species from the grouse E. angusta (Allen, 1934), E. bonasae (Allen, 1934), E. lagopodi (Galli-Valerio, 1929) and Isospora mutus (Galli-Valerio, 1931). However, they were all established on incomplete descriptions of the oocysts with little or no account of the endogenous phases.

The object of this work was to study the species in the pheasant and grouse with emphasis on:

1. The complete life cycles of the parasites;
2. Their pathogenicity;
3. Natural incidence of the species; and
4. Cross infectivity of these species to other gallinaceous birds.

Initially it was hoped to study several species from the pheasant

and grouse. However, because of the necessity to isolate infected hosts, only a single species could be studied at any one time. Further, the hosts have a short breeding season and coccidia-free pheasant chicks were available only during the spring for experimental work. Grouse chicks were at no time available.

Thus, the work was centered on one species, Eimeria phasiani Tyzzer, 1929 from the pheasant (Phasianus colchicus) and included a search for drug resistant strains of this species and of Eimeria maxima Tyzzer, 1929 from the domestic fowl. A short description of a second species from the pheasant and one species from the red grouse is given in the Appendix.

HISTORICAL REVIEW

The history of coccidian parasites dates back to the 17th Century when von Leeuwenhoek (1674) described oval bodies in the bile of rabbits. But it was not until Rivolta (1869) that a coccidium was described from gallinaceous birds and this was an unnamed species from the domestic fowl.

The initial naming of a coccidium from gallinaceous birds has often been accredited to Rivolta and Silvestrini (1873) under the name Psorospermium avium, as quoted by Wenyon (1926) and many other authors; but as Tyzzer (1929) points out, the name does not appear in the paper. In fact, it was Rivolta (1878) who gave the first name, Gregarina avium intestinalis, to a coccidium from the fowl, blackbird, crow and several other birds. Although no mention is made of the hosts, Psorospermium avium is also named in this paper but refers to a parasite developing two spores and not the four, characteristic of the species from the fowl.

Railliet and Lucet (1891) published an account of an organism causing acute caecal coccidiosis in the fowl which was similar to Rivolta's description of Gregarina avium intestinalis. They named this parasite Coccidium tenellum and as Rivolta's name is a binomial, and therefore invalid, Coccidium tenellum takes precedence.

McFadyean (1894) gave the first description of coccidiosis in the pheasant, an acute disease in birds one month old. The cysts, most numerous in the caeca but also present in the small and large intestines, were 30 μ long and of similar appearance to Coccidium oviforme of the rabbit.

There was much destruction of the epithelium, the parasite being found in both the glands and the surface epithelium of the intestine. However, he was unable to decide whether this was the same or a separate species from that of the rabbit.

A similar disease was described by Sjöbring (1897) from young pheasants in Sweden. He observed the sporulation of the cysts, 28 x 16 μ in size, into four spores and went one stage further than McFadyean in stating that Coccidium oviforme and the species in the pheasant were identical.

Both Labbé (1899) and Minchin (1903) stated that Coccidium avium, which they considered to be the same parasite as Rivolta's Gregarina avium intestinalis, was probably also present in the pheasant (Phasianus sp. and Phasianus colchicus).

Morse (1908) reported C. tenellum from the pheasant and several other birds including the chicken, in which he considered it caused a disease called white diarrhoea. However, he did not describe any stages of the parasite.

Doflein (1909) noted the similarity between the two genera, Coccidium Leuckart, 1879 and Eimeria Schneider, 1875. And, therefore, under the rule of priority the genus Coccidium is synonymous with Eimeria. Several authors regretted this move, in spite of the rule of priority, and Minchin still used the generic name Coccidium as late as 1922.

Bassett (1909), Cole, Hadley and Kirkpatrick (1910) and Hadley (1910, 1911) all considered E. avium (= C. avium) to have a wide host range. Bassett reported it from pheasants, pigeons and chickens and Cole, Hadley

and Kirkpatrick from many gallinaceous birds including pheasants, grouse, passeriformes and even mammals. Hadley (1910) gave a preliminary study on the coccidium from sparrows, turkey and chicken and, in 1911, described the life cycle of the parasite, naming it E. avium and giving additional hosts as pheasant, grouse, quail and many other wild birds including passeriformes.

Fantham (1912) described the life cycle of E. avium from the duodenum, small intestine and caeca of the red grouse (Lagopus scoticus) and claimed to have transmitted this species to fowl and pigeon. However, as Reichenow (1921) and Wenyon (1926) point out, it differed morphologically from the forms described under the name by Hadley (1911) and other authors. Later in 1915, Fantham listed the hosts of E. avium as fowls, turkeys, pigeons, geese, ducks, pheasants, partridge and grouse, recording similar symptoms in all hosts and a prepatent period of 8 - 10 days.

Johnson (1923) also questioned the validity of E. avium as a single species. He considered the descriptions of Hadley (1910, 1911) incorrect as he, Johnson, was unable to transmit E. avium from fowl to sparrow and, thus, considered the species in sparrow to be separate from that of fowl but identical to that of blackbird. He also considered the species in turkey to be separate ones, as later confirmed and named E. meleagridis, E. dispersa and E. meleagritidis by Tyzzer (1927, 1929).

Brinkman (1927), describing E. avium from the willow grouse (Lagopus lagopus), found the ^{species} ~~Eimeria~~ in this host showed morphological differences as compared with the forms found in chickens, turkeys, and

British grouse. His reason for still calling this species E. avium was that he accepted the view of Verwey (1926) who in describing E. avium from the pheasant and partridge, stated that phenotypes of this species occur in different species of birds; and that the differences of form were to be explained by the development of this parasite in different hosts.

It was Tyzzer (1927, 1929) and Tyzzer, Theiler and Jones (1932) who ascertained the true position of E. avium. Using the criterion of pure line infections with single oocysts, E. avium of the domestic fowl was split into six species and the species in turkey and pheasant were identified. In his paper of 1929, Tyzzer described two species from the pheasant - E. phasiani from the small intestine and caeca and E. dispersa from the posterior part of the small intestine and terminal part of the caeca. He stated that E. phasiani had a prepatent period of 5 days and produced long-ellipsoidal oocysts, $23 \times 15.9 \mu$ in size, which sporulated in 24 hours at room temperature. However, there was no description of the endogenous cycle except for a drawing of a schizont and gametocytes. Tyzzer considered that there were two varieties of E. dispersa, one in quail and the other in pheasant, differing in size, shape and rate of development. He found that it was possible to transmit the pheasant strain to quail but not vice versa. No cross-infectivity was shown with E. phasiani.

Becker (1934) reported E. phasiani and E. dispersa from the pheasant and stated that the pathogenicity of both had yet to be evaluated. However, he considered E. phasiani to have only a mild effect on the host.

Yakimoff and Matschoulsky (1937) described a new species, E. langeroni

from the faeces of Phasianus colchicus chrysomelas. Only the sporulated oocysts, elongate oval in shape and $32.5 \times 18.4 \mu$ in size, were observed.

In 1939, Haase described coccidiosis from the common pheasant (P. colchicus colchicus) and the ring-necked pheasant (P. colchicus torquatus) in Germany. He identified two species, E. phasiani and E. tenella on the basis of oocyst characters. His description of the oocysts of E. phasiani, $23.2 \times 16.2 \mu$ in size, was in close agreement with Tyzzer (1929), although, when giving the sporulation time as 24 - 36 hours, he did not state at which temperature sporulation was observed. Haase's records of E. tenella from the pheasant and partridge (Perdix perdix) and of E. procera from both the partridge and capercaillie (Tetrao urogallus) are questionable, considering the now known almost complete host specificity of the genus Eimeria in gallinaceous birds. However, Clapham (1950) also considered that the pheasant might be infected by E. tenella.

Ormsbee (1939) studied coccidiosis in the adult ring-necked pheasants of Washington. He, also, confirmed the oocyst characters of E. phasiani, as described by Tyzzer, and gave the sporulation time of 24 - 36 hours at 20°C . but he did not describe any endogenous stages. In addition, he described two new species from the same host, E. pacifica and E. megalostomata and a further unnamed species, Type IV. The oocysts of E. megalostomata, $24 \times 19 \mu$ in size, were ovoidal with characteristic large micropyles; E. pacifica also had ovoidal oocysts, $22 \times 18 \mu$ in size, but with no micropyle. Both species sporulated in 48 hours at 20°C . No endogenous stages were described for E. megalostomata but Ormsbee did state

that in E. pacifica developmental stages were found in the upper intestine and caeca and that schizont development occurred above and below the host cell nuclei. However, he did not describe other characteristics of the schizonts. The fourth species, Type IV, was not named, as the oocyst characters were incompletely observed. The oocysts measured 18 x 15 μ and were ovoidal but no sporulation time was given.

Madsen (1941) in a survey of partridges and pheasants in Denmark found the incidence of coccidiosis in the pheasant to be 82% in the adults and 73% in the chicks, in contrast to the figure of 38%, in adult pheasants, by Ormsbee (1939). Ormsbee claimed a seasonal change in the rates of incidence, but the number of birds he examined each month was very low and the figures do not appear significant.

Muroma (1951) listed the coccidian parasites of game birds in Finland. Only six pheasants (P. colchicus) were infected out of 22 examined - the species surprisingly being given as E. avium in three birds, E. tenella in two and Eimeria sp. in one. He also recorded six cases in 57 partridges (Perdix perdix) of which he attributed four to E. tenella and the others to indeterminate species. In the willow grouse (Lagopus lagopus) five out of 22 birds were infected but no species were named.

Prasad (1960) was unable to show the presence of any coccidia in five species of game bird, the red grouse (Lagopus scoticus), the ring-necked pheasant (P. colchicus torquatus), the bob white quail (Colinus virginianus), the golden pheasant (Crysolophus pictus) and the quail (Colinus = Eupsychortyx cristatus) although only small numbers were examined.

Keymer, Rose, Beesley and Davies (1962) in a survey of wild and game birds in Britain reported 30 deaths due to coccidiosis in pheasant and a minimum number of carriers as 42 in 603 examined. Amongst several species present they only positively identified E. phasiani and E. megalostomata.

A seventh species, but an unnamed one, was described by Davies, Joyner and Kendall (1963) from the caeca of a pheasant (P. colchicus). Only the broadly ovoidal oocysts, $28.9 \times 18.6 \mu$ in size, which sporulated in 72 hours at $19 - 21^{\circ}\text{C}$., were mentioned. They were unable to transmit this species to domestic fowl.

Since the work of Brinkman (1927) little has been achieved in the field of coccidiosis of the grouse. Galli-Valerio (1929) described E. lagopodi, pathogenic in the intestine of the young ptarmigan (Lagopus mutus) and in 1931, Isospora lyruri from the black grouse (Lyrurus tetrix). This is the only description of an Isospora from game birds but some authors, including Becker (1956), consider this to be the same species as E. lyruri described by Galli-Valerio (1927) from the red-legged partridge (Alectoris rufa = Perdix rubra).

Allen (1934) reported two new species, E. angusta from the caeca of the ruffed grouse (Bonasa umbellus) and the spruce grouse (Canachites canadensis) and E. bonasae from the caeca and small intestine of the ruffed grouse and the willow grouse.

Boughton (1937a) confirmed the presence of E. angusta in the ruffed grouse and also found it in the sharp-tailed grouse (Pediacetes phasianellus).

campestris). In addition he reported E. dispersa, which had only been previously described from pheasants, bob-white quail and turkey, also from the spruce grouse, the fowl and the sharp-tailed grouse.

It is difficult to assess the significance of these reports of species with a wide host range as their status is based only on incomplete descriptions of the oocysts. No sporulation times were given and there was no description of the endogenous phases or pathogenicity. However, it is known from the work of Fantham (1911), Brinkman (1927) and Jenkins (1957, 1959) with the red grouse, that coccidiosis may be fatal in young birds whilst being relatively harmless in adults. In contrast, Douglas Clarke (1936) found no coccidiosis in young ruffed grouse.

From this review of the literature it can be seen that, although several species (Table 1) have been described from the pheasant and the grouse, their status is only based on descriptions of the oocyst and little or no work has been performed on the endogenous phases of the parasite.

No work has been performed on the drug response in the coccidia of pheasants, although most gamekeepers use sulphonamides to control outbreaks. Most of the work has been centered on the most commercially important species - those in fowl and turkey, where occasional strain variance of coccidian species to drugs has been reported.

The importance of this strain variance or drug resistance, is summed up by Horton-Smith (1960) who stated "Recently we have found strains within species which do not respond to the same kind of medication and there is also evidence that one kind of coccidiostat may not control more than one

Table 1. Named Species of Coccidia from the Pheasant and Grouse.

<u>Species</u>	<u>Host</u>	<u>Author</u>
<u>E. angusta</u>	<u>Bonasa umbellus</u>	} Allen (1934)
	<u>Canachites canadensis</u>	
	<u>Pediocetes phasianellus campestris</u>	Boughton (1937a)
<u>E. bonasae</u>	<u>Bonasa umbellus</u>	} Allen (1934)
	<u>Lagopus lagopus</u>	
<u>E. dispersa</u>	<u>Phasianus colchicus</u>	} Tyzzer (1929)
	<u>Colinus virginianus</u>	
	<u>Lagopus lagopus</u>	} Boughton (1937a)
	<u>Pediocetes phasianellus campestris</u>	
<u>E. lagopodi</u>	<u>Lagopus mutus</u>	Galli-Valerio (1929)
<u>E. langeroni</u>	<u>Phasianus colchicus chrysomelas</u>	Yakimoff and Matschoulsky (1937)
<u>I. lyruri</u>	<u>Lyrurus mutus</u>	Galli-Valerio (1931)
<u>E. megalostomata</u>	<u>Phasianus colchicus torquatus</u>	Ormsbee (1939)
<u>E. pacifica</u>	<u>Phasianus colchicus torquatus</u>	" "
<u>E. phasiani</u>	<u>Phasianus colchicus torquatus</u>	" "
	<u>Phasianus colchicus</u>	Tyzzer (1929) Haase (1939)

species. How widespread these strain differences are, is still uncertain but their existence adds a further possible complication in selecting the form of medication to be used".

The search for drug resistant strains was started in the early 1950's. However, Harwood and Stunz (1953) failed to demonstrate the phenomenon in field strains of E. tenella collected from areas where sulphaquinoxaline and nitrofurazone had been in use. The first report is probably that of Waletzky, Neal and Hable (1954) who found a strain of E. tenella in a broiler flock which had failed to respond to sulphaquinoxaline or sulpha-dimidine treatment.

Cuckler and Malanga (1955), using the criterion of oocyst production, showed a high percentage of field strains of Eimeria were resistant to treatment with either nitrophenide (43% of strains resistant), sulphaquinoxaline (45% resistant) and nitrofurazone (57% resistant). They also demonstrated experimentally that resistance was induced in one strain of E. acervulina and two strains of E. tenella after 15 serial passages with exposure to suboptimal doses of sulphaquinoxaline.

A similar experimental result was obtained by Joyner (1957) when, after 9 passages through birds treated with nitrofurazone, E. tenella failed to respond to high doses of this drug. The nitrofurazone resistant strain responded normally to treatment with sulphonamides.

Horton-Smith (1958) obtained similar results with nitrofurazone and sulphaquinoxaline and confirmed that there was no evidence of cross-resistance between the drugs.

Kantor, Kennet and Waletsky (1959) reported one of two strains of E. acervulina to be four times as resistant to sulphaquinoxaline as the other.

Long (1963) showed strain variance in both E. necatrix and E. maxima. The two strains of E. necatrix employed responded equally well to amprolium but the Houghton strain was resistant to sulphaquinoxaline. As the resistant strain had only been passaged through unmedicated chicks, it was not known whether the resistance was acquired as a result of an exposure to sulphaquinoxaline before the strain was isolated. The U.S.A. strain of E. maxima showed a definite response to amprolium but with a lesser degree to sulphaquinoxaline, whereas the Houghton strain was controlled by sulphaquinoxaline but not by amprolium.

MATERIALS AND METHODS.1. Birds.(a) Wild birds.

Wild pheasants were captured in the field by using a simple cage trap made from 2" wire mesh. The trap measured 3' x 3' x 2½' with a door at one end and a funnel at the other through which the birds entered in search of bait. The bait used was wheat and chicken-mash with a supply of water provided. Although this method proved successful during spells of bad weather, insufficient birds were captured and supplies were augmented from the shoots of Lord Rank's Hampshire Estate. A few birds were obtained from other sources (p.106).

(b) Experimental birds.

Day-old coccidia-free pheasant chicks were obtained from the incubators of the Eley Game Advisory Service, Fordinbridge and the Cotswold Game Farm, Stroud. Day-old coccidia-free partridge chicks were obtained from the incubators of Lord Rank's Estate in Hampshire and day-old coccidia-free chickens from Stirling Poultry Products, Andover.

On collection, they were transferred to an incubator maintained at 32.5 - 35.0°C. (90 - 95°F.). After about seven days the temperature was gradually lowered, over a period of four weeks, to 20°C. (68°F.).

2. Strains of Parasite.

Strains of Eimeria maxima were obtained from Houghton Poultry

Research Station, Central Veterinary Laboratories, Weybridge and Bundesforschungsanstalt für Kleintierzucht, Celle, West Germany.

Two separate strains of Eimeria phasiani were isolated from two adult pheasants captured at Silwood Park, Berkshire.

3. Housing of Birds.

All wild birds were kept in a separate room from the experimental ones to prevent cross-infection.

Infected and uninfected experimental birds were also kept in separate, thermostatically-heated rooms which had filtered air supplies. The filters removed particles above $5\ \mu$ in diameter and, therefore, excluded all oocysts. It was not possible to have a separate isolation room for each species of coccidium and, at first, different strains were kept at separate parts of the isolation room. This proved to be unsatisfactory as on one occasion cross-infection of two species occurred. Afterwards, only one strain was used in the animal room at one time and the room was sterilised between experiments.

4. Cages.

All birds were kept in metal cages, measuring $2' \times 1\frac{1}{2}' \times 1\frac{1}{2}'$ with wire floors above metal trays (Fig. 1). The trays, which were changed daily, facilitated the collection of faecal droppings and were easily sterilised. Food and water were supplied by hoppers attached to the side of the cage. Each hopper could be filled through holes in the top, which allowed the birds to be fed without the handler coming into contact with them.

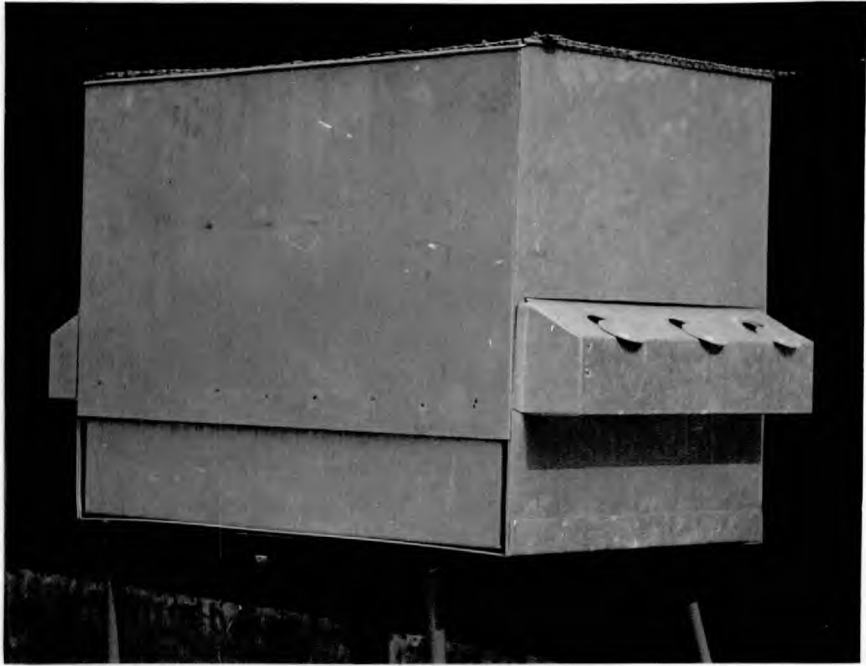


Fig. 1. Experimental Animal Cage.

5. Feeding of Birds.

Pheasant and partridge chicks were fed on Cotswold High Protein Starter (No. 1) Crumbs (22% Protein) for the first two weeks. This was gradually mixed with No. 2 crumbs in decreasing proportions during the third week until the diet was entirely of No. 2 crumbs. Chickens were fed on this diet during the summer but on normal chick starter crumbs in the winter as the other was not available. All birds above six weeks old were fed on 20% protein Growers Mash.

On two separate occasions wide-spread cannibalism broke out in the stocks of uninfected pheasants. These birds were approximately seven to nine days old and within two days three quarters of the batches were dead. Salt was subsequently added to the diet, one tablespoon of salt per 300 grammes of food, and no further outbreaks occurred.

6. Sterilisation of Equipment.

Before use all animal rooms were thoroughly washed down and fumigated with ammonia to kill any oocysts present (Horton-Smith, Taylor and Turtle, 1940). The cages, incubator and apparatus too large for the autoclave were washed with ammonia solution and burnt with a blow lamp (Horton-Smith and Taylor, 1939). Cages containing non-experimental birds were cleaned and sterilised every three to four days.

All food and water hoppers and other apparatus which were to be used in the animal rooms were sterilised in the autoclave at 15 lbs/sq.in. in pressure for 15 minutes. All food used was heated to 60°C. for 12 hours

before feeding to the birds and water taken boiling from the water heater was considered sterile (Tyzzer, 1929).

On entering the isolation rooms shoes were changed and laboratory coats were worn. A separate coat and pair of shoes being used for each room. Birds were handled with rubber gloves, a separate pair being used for each cage of birds. All faeces which were not examined were burnt directly in the incinerator.

During all experiments, uninfected birds were kept as controls in separate cages in the same room as the experimental birds.

7. Initiation of a Pure Line Infection.

In order to study the life histories of the coccidia, pure-line infections were produced by the infection of coccidia-free pheasant chicks with single oocysts as advocated by Tyzzer (1929). Oocysts were isolated with the aid of a micropipette from cultures obtained from infections in the wild bird. Many complex manipulators have been produced for the isolation of single oocysts (Chambers, 1923; Joyner, 1958), but the methods of Tyzzer (1929) - a capillary thin pasteur pipette - and of Goldacre (1954) - a simple micromanipulator made from microscope slides - proved effective and convenient.

A single sporulated oocyst of the required species was selected from a dilute suspension of oocysts and drawn into the pipette. It was, then, transferred to a microscope slide coated with 10% agar where it was checked that only a single oocyst was present. Agar has the advantage

over cellophane used by some workers (Clarkson, 1959) that it is moist and does not allow the oocyst to dry out. The small area of agar was cut away, checked again for the oocyst and placed in a gelatin capsule. The capsule was administered per os. to eight-day old pheasant chicks which had failed to show oocysts in the faeces since hatching.

8. Collection of Oocysts.

(a) Concentration method for diagnosis.

Infections in wild adult pheasants were light and oocysts were recovered after concentration by a modification of the method of Faust, Sawitz, Tobie, Odom, Peres and Lincicome (1939).

The faecal material was macerated with water in a mechanical blender, then filtered through a wire sieve (40 mesh/square inch) and centrifuged. The deposit was then recentrifuged with a solution of high specific gravity, to allow the oocysts to float up to the surface, where they were "skimmed" off with a platinum loop. This is the basis of the method of Faust et al. (1939) who used 33.1% Zinc Sulphate as the solution of high specific gravity. There are two other common solutions for floatation, namely saturated salt solution (Nöller and Otten, 1921) and saturated sugar solution (375 gm. sugar in 500 ml. water and 3.5 gm. phenol - Farr and Luttermoser, 1941). The comparative efficiency of these three solutions was tested to see which would be the most suitable for floatation of Eimeria phasiani oocysts.

For a comparison of the solutions, a modification of the method

of Gill (1954) was used. 0.5 ml. of an homogeneous suspension of oocysts was placed in a centrifuge tube and the solution under investigation was added to fill the tube completely. A coverslip was placed over the mouth of the tube and the tube centrifuged for three minutes at 2,000 r.p.m. The total number of oocysts which floated up on to the coverslip was counted, the tube was topped up with the respective solution and a coverslip was placed over the tube. The tube was recentrifuged and the number floating up the second time counted. This was repeated until two successive floats failed to reveal any oocysts. This process was applied to each of three solutions and three replicates of each reading were taken. As each equal sample was taken from the same homogeneous source, it was considered that they contained an approximately equal number of oocysts at the start of the experiment. The specific gravity of each replicate was tested by a standard hydrometer, the average being stated below in Table 2.

Table 2. Comparison of efficiency of floatation of three solutions of high specific gravity.

(i) Saturated sodium chloride. Specific gravity 1.202.

	1st Replicate	2nd Replicate	3rd Replicate	Mean
1st Count	1105	1129	1101	1112
2nd "	56	46	72	
3rd "	10	7	22	
4th "	4	-	1	
5th "	-	-	-	
6th "	-	-	-	
Total	1175	1182	1196	1184

(ii) 33.1% Zinc Sulphate. Specific Gravity 1.200.

	1st Replicate	2nd Replicate	3rd Replicate	Mean
1st Count	1037	1109	1120	1088
2nd "	73	59	56	
3rd "	25	12	3	
4th "	4	1	-	
5th "	-	-	-	
6th "	-	-	-	
Total	1139	1181	1179	1166

(iii) Saturated sugar solution. Specific Gravity 1.200.

	1st Replicate	2nd Replicate	3rd Replicate	Mean
1st Count	701	663	749	704
2nd "	292	293	201	
3rd "	65	102	63	
4th "	45	65	34	
5th "	16	18	15	
6th "	-	-	1	
7th "	-	-	-	
8th "	-	-	-	
Total	1119	1114	1063	1107

Table 3. Percentage first recovery of oocysts/total recovery.

	1st Replicate	2nd Replicate	3rd Replicate	Mean
Sat. NaCl	94.0%	95.5%	92.1%	93.9%
33.1% ZnSO ₄	91.0%	93.9%	95.0%	93.3%
Sat. sugar	62.6%	58.1%	70.5%	63.7%

A high percentage of oocysts on the first recovery was the most important factor in evaluating the solutions. There was no statistically significant difference between saturated NaCl and 33.1% ZnSO_4 in this respect or in total number of oocysts floated (Tables 2 and 3). The speed of floatation of the two solutions was also similar. Saturated salt solution was always used, however, because of cheapness and convenience. 33.1% ZnSO_4 had a disadvantage in that it floated more debris than the salt solution. Saturated sugar solutions suffered from defects of poor first recoveries and a tendency to be very sticky on handling.

(b) Method of collection of oocysts for sporulation.

Oocysts were extracted from the faeces by emulsifying the droppings in 2.5% potassium dichromate in a mechanical blender. A suitable proportion, as recommended by Davies, Joyner and Kendall (1963) is 20 ml. of solution to each gramme of faeces. After filtration through muslin and 40 mesh/sq. in. sieves, the filtrate was allowed to stand overnight to sediment. The supernatant was siphoned off and, after further filtrations and sedimentation, the sediment was resuspended in 2.5% potassium dichromate for sporulation.

(c) Collection of oocysts from infected tissues.

Some wild birds were only obtained for examination after death and with some gut decomposition. In these cases, the most satisfactory method of oocyst recovery was to emulsify the infected tissue with 2.5% potassium dichromate in a mechanical blender. The emulsion was then filtered, examined and the oocysts sporulated in potassium dichromate.

9. Sporulation of Oocysts.

After filtration and sedimentation, the residue containing the oocysts was spread evenly in a petri dish and 2.5% potassium dichromate added to make up one third of the volume of the dish. This allowed an ample supply of oxygen for development. For routine sporulation the petri dishes were placed in a C.T. Room at 20°C.

For accurate determinations of the times for sporulation, only faeces collected within an hour of defaecation were tested. Petri dishes containing equal volumes of faeces and dichromate were incubated at 6°C., 15°C., 20°C., 25°C., 30°C., 35°C. and 40°C.

10. Storage of Oocysts.

After sporulation, oocysts were kept in screw-top McCartney bottles at 6°C. for use in further experiments. The oocysts of E. phasiani remained viable for more than nine months.

11. Estimation of the Number of Oocysts passed in the Faeces.

(a) Estimation of the number of oocysts per gramme of faeces.

The number of oocysts per gramme of faeces was counted by the technique of Stoll (1923) and Gordon and Whitlock (1939), modified by Parfitt (1958) using a representative sample of 3 gm. of faeces. The faeces were mixed with 42 ml. of water in a mechanical blender. 15 ml. of this suspension were centrifuged and the sediment resuspended in 15 ml. of saturated NaCl. Samples of this suspension were then counted in a

McMaster slide.

The number of oocysts per gramme being estimated by the following formula:-

$$\text{No. of oocysts per gramme of faeces} = \frac{a}{2} \times 100.$$

a = Total number of oocysts in both chambers of the McMaster slide.

(b) Total oocyst production per day.

The total daily faeces output was collected and the volume made up to one litre with tap water. 500 ml. of saturated salt solution were added and the whole homogenised in a mechanical blender. The salt facilitates an even suspension of oocysts. A uniform suspension was obtained after 10 minutes and five samples of one ml. of suspension were withdrawn and added to 45 ml. of saturated salt solution, making a final dilution of 1 in 10. A dilution of 1 in 100 or even 1 in 1000 parts was used if the number of oocysts present was high. A sample of diluted suspension was then transferred to the counting chambers of the McMaster slide and the number present counted. The total number of oocysts present being given by the formula:-

$$\text{Total no. of oocysts per day} = \frac{10x}{3} \times a_1 \times 15,000$$

where x = total number of oocysts in both chambers of the McMaster slide.

a₁ = dilution factor (10, 100, 1000 etc.).

12. Infection of Birds per os.

(a) Standardization of dose.

The number of oocysts administered to a chick was determined by a haemocytometer as recommended by Long and Rowell (1958). The total number of oocysts in a suspension was estimated by counting the numbers present in 1 : 10 or 1 : 100 dilutions in the Neubauer haemocytometer. A dilution which gave approximately 50 oocysts per large square was the most convenient. The total number of oocysts per ml. was found by multiplying the number of sporulating oocysts in all 400 small squares, i.e. a volume of (1 x 1 x 0.1 cu. mm.) by 10 x 1000. Long and Rowell gave the accuracy of this method to within 5%.

After the number of oocysts in the fluid was ascertained, suitable dilutions were made to adjust the concentration of the oocysts to the required dose. A dose of 0.5 ml. of diluted fluid was normally given.

(b) Administration of oocysts to hosts.

Suspensions of oocysts were administered directly into the crop of the birds by means of a graduated syringe with a polythene tube attached to the nozzle. The oocysts were placed in the crop, care being taken not to pass the tube into the respiratory opening. The use of a 10 ml. graduated syringe enabled several inoculations of 0.5 ml. to be given in succession to different birds (Prasad, 1960 and Davies, Joyner and Kendall 1963). Care was taken, between each inoculation, to shake the syringe to get a uniform suspension and, therefore, an accurate dose.

13. Measurements of Oocysts.

All measurements were made with a micrometer eyepiece, each division of which represented 3.354μ . Readings were taken to the nearest 0.1 div. which was considered to be the limit of resolution. As all the measurements were made by the author, the personal bias in measuring was considered constant.

(a) Comparison of measurements of oocysts during the patent period.

(i) Pilot sample.

Using one bird, ten oocysts were measured each day of the patent period and the following values; length (L), width (W), shape (L/W) and volume (LW^2) were analysed. The term LW^2 was considered proportional to the exact volume.

An analysis of variance was performed to ascertain whether the variation between times was significantly greater than that within times. If this was so, the between times variation was further analysed by the method of Orthogonal Polynomials, which successively fits the linear, quadratic, cubic etc. terms to the data to see which contributes the greater part of the between times variation. From this the best fitting graph could be calculated by the least squares method.

(ii) Complete data.

Oocysts passed each day, during the patent period, were measured in four birds. The number measured each day varied between 10 and 20

depending on the bird, but the daily numbers were kept constant in any one bird.

From the results obtained in the pilot sample and the fact that LW^2 is the most logical factor to represent a change in size, LW^2 was used for the analyses. The analyses were carried out as in the pilot sample and the complete specimen calculation of an example is given in the Appendix Table A.3.

(b) Comparison of measurements of oocysts in individual birds.

The measurements of oocysts in individual birds were compared by taking the volume of ten oocysts from eight birds and performing an analysis of variance on these. In all eight birds a random sample of ten oocysts was taken on the same day of the patent period to limit the factor of difference in size due to time.

14. Staining of Oocysts.

Fresh oocysts were stained by the method of Crouch and Becker (1931). After washing, the oocysts were treated with warm glacial acetic acid, and were stained in a 0.1% solution of Janus Green for ten minutes. After washing again they were stained in a 10% solution of eosin for 5 minutes, washed a third time and mounted in Canada Balsam.

15. Estimation of Weight Gains of Host Birds.

To ascertain the effect of E. phasiani on the live-weight gains of pheasants, fifteen 3-week old pheasants from the same hatch, having been

kept under identical conditions from the day of hatching, were divided into three groups of five birds by random selection. This method ensured that each group contained light, medium and heavy birds. Group I were uninfected controls, Group II received 25,000 oocysts and Group III 100,000 oocysts each. Food was given, ad lib. throughout the experiment and all three groups were kept in the same room ensuring a constant environment. Daily weighings of each bird were taken for five days prior to and seventeen days after infection. The design of the cages used was such that an accurate estimate of food consumption was not possible.

The usual precautions against chance infection were taken throughout the experiment, and as the controls failed to show any oocysts in the faeces, they were considered adequate.

16. Haematology.

The same birds used in the weight experiment were subjected to blood counts one day prior to and for six days after infection.

Differential white blood cell counts were made on blood films, fixed in methanol and stained in Giemsa stain.

Total white and red blood cell counts were made using the technique of Sadek (1955). A red cell diluting pipette being used for both red and white cell counts. Blood was taken from the wing of the bird in the diluting pipette, and then diluted with the Sadek diluting fluid, which consists of the following:

Giemsa Stain	10 ml.	
Neutral Formalin	5 ml.	
Isotonic 0.85% NaCl	85 ml.	pH: 7.0

to give a dilution of 1 : 100.

After shaking to give a thorough mixture of blood and fluid, the pipette was stored in the refrigerator to give maximum staining. Counts were taken, using a Neubauer haemocytometer, in the normal manner.

17. Life Cycle of the Parasite.

In the experiments on the life cycle, heavier doses of oocysts were given when tracing early asexual stages than when tracing sexual stages. Doses of three million oocysts were necessary in order to recover first generation schizonts but only 50,000 oocysts were necessary in order to find gametocytes. The birds were killed at 12 hourly intervals and usually two birds were killed at each time especially early in the life-cycle.

The life cycle was studied in tissue sections, cut at 5 or 6 μ and in fresh and stained smears of the intestinal mucosa; tissue being taken from the twelve sites as shown in Fig. 2.

Smears of the intestine were examined directly in saline, with the microscope stage warmed by the heat of the lamp. Smears were also dry fixed or methanol fixed and stained in Giemsa. Sporozoites and sporocysts were also methanol fixed and stained by the mercuric bromophenol blue method used by Mazia, Brewer and Alfert (1953) and modified by Bonhag (1955).

The intestine was fixed in formol sublimate (10% formol saline saturated with mercuric chloride) which proved an excellent fixative. After fixation, dehydration and clearing, the tissue was embedded in 52 - 54.5°C. M.P. paraffin wax.

REGIONS OF INTESTINE REMOVED FOR HISTOLOGY

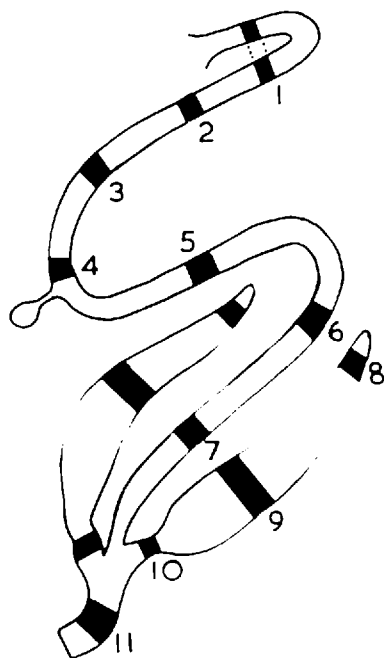


FIG. 2

Sections were stained either in Ehrlich's haematoxylin counter-stained with eosin, or in Heidenhain's haematoxylin. The Feulgen reaction (de Tomasi method) was used for nuclear detail.

Infected tissues were compared with the normal healthy intestines.

Drawings of the stages of the parasite were made with the aid of a camera lucida from fresh preparations in saline, from smears and from tissue sections.

18. Strain Variance in Relation to Drug Action.

Three strains of Eimeria maxima in fowl chicks and two strains of E. phasiani in pheasant chicks were tested for strain variance.

(a) Drugs.

The drugs used in this study were 0.006% w/w amprolium, 0.006% w/w sulphaquinoxaline and 0.0125% w/w zoalene. Each drug was obtained as a premix and was mixed with the food, in 5000 gm. lots, in a Kenwood Electric Mixer for 20 - 30 minutes. All food was made up at the start of each experiment. Treatment commenced 24 hours before infection and continued throughout the experiment.

(b) Experimental procedure.

Each strain was tested separately to eliminate the chance of cross-infection of strains. In each case, fifty two-week old chicks from the same hatch were divided into five groups of ten birds by random selection as in method 15.

The forty infected birds in each experiment were given the same dose, the fowl chicks 20,000 oocysts of E. maxima and pheasant chicks 25,000 oocysts of E. phasiani. Group I were uninfected controls fed on mash without coccidiostat; Group II, infected, were controls fed on mash without coccidiostat; Group III, infected, were given doses of 0.006% w/w amprolium; Group IV, infected, were given doses of 0.006% w/w sulphaquinoxaline; and Group V, infected, were given doses of 0.0125% w/w zoalene.

The birds were weighed each day, commencing three days before infection and throughout the experiment. Oocyst-outputs per group were calculated on 24 hour samples throughout the patent period of the parasites.

RESULTSEimeria phasiani Tyzzer, 1929.1. Studies on the Exogenous Stages.(a) Infection of pheasants with a single oocyst of E. phasiani.

Twelve 6-day old coccidia-free pheasant chicks were infected with single oocysts of E. phasiani isolated from infections in wild birds. Of these, seven became infected, passing oocysts early on the sixth day. The remainder failed to become infected.

The patent period of single-oocyst infections was measured accurately in four birds, being 126, 128, 130 and 132 hours respectively (mean 129 hours). Oocyst production in all infected chicks ceased after three or four days and only in two birds were oocysts passed in large numbers on the fourth day. A determination of the total oocyst production was made in six of the birds, the number produced per bird ranging from 138,400 to 182,900 (mean 151,440 oocysts/bird). Table 4 summarises the information from the single oocyst infections.

The oocysts from the seven separate infections were sporulated in 2.5% potassium dichromate and numbered as strains B1 - B7. All further experiments were performed with oocysts derived by passage from the culture of strain B1.

Table 4. Oocyst production in infections with single oocysts of *E. phasiani*.

<u>Chick No.</u>	<u>Strain No.</u>	<u>Prepatent Period</u>	<u>Patent Period</u>	<u>Total No. of Oocysts Produced</u>
67	B1	Early 6th day	4 days	182,900
69	B2	Early 6th day	4 days	155,100
92	B3	126 hrs.	3 days	138,400
94	B4	128 hrs.	3 days	168,100
95	B5	132 hrs.	3 days	155,300
101	B6	130 hrs.	3 days	108,800

(b) Size of oocysts.

Measurements were made of oocysts collected from the faeces of experimental birds infected with a "pure line" of *E. phasiani*. The number measured varied between 25 and 200 per bird.

Three samples, two of 100 and one of 50 oocysts from three different birds, were measured before and after sporulation to ascertain whether this process effected a change in oocyst dimensions. The results are summarised in Table 5.

In two cases, Chicks 104 and 106, the oocysts were larger after sporulation, the mean measurements being $24.10 \pm 0.15 \mu \times 16.70 \pm 0.10 \mu$ before and $24.76 \pm 0.15 \mu \times 16.99 \pm 0.11 \mu$ after sporulation, and $24.00 \pm 0.16 \mu \times 16.66 \pm 0.11 \mu$ and $24.37 \pm 0.19 \mu \times 16.83 \pm 0.12 \mu$ respectively. In one, Chick 67, the oocysts were smaller after sporulation, the mean measurements being $26.46 \pm 0.27 \mu \times 18.35 \pm 0.21 \mu$ before and $26.00 \pm 0.30 \mu \times 18.01 \pm 0.20 \mu$ after sporulation.

Table 5. Comparison of dimensions of unsporulated and sporulated oocysts.

	Chick No.	Days of Patent Period	No. Measured	Length (μ)			Width (μ)			Range S.I.	$\bar{m}$ S.I.
				Range	$\bar{m}$	S.E.	Range	$\bar{m}$	S.E.		
Unsporulated	67	4	50	22.1-30.9	26.46	± 0.27	15.4-19.8	18.35	± 0.21	1.36-1.54	1.44
Sporulated	67	4	50	21.8-28.8	26.00	± 0.30	15.4-19.8	18.01	± 0.20	1.38-1.52	1.44
Unsporulated	104	1.3.8	100	21.8-27.5	24.10	± 0.15	15.1-19.1	16.70	± 0.10	1.38-1.53	1.44
Sporulated	104	1.3.8	100	22.1-27.5	24.76	± 0.15	15.1-19.4	16.99	± 0.11	1.38-1.52	1.46
Unsporulated	106	1.3.8	100	21.8-27.5	24.00	± 0.16	15.1-19.4	16.66	± 0.11	1.39-1.55	1.44
Sporulated	106	1.3.8	100	21.8-27.5	24.37	± 0.19	15.1-19.1	16.83	± 0.12	1.38-1.52	1.45

There was no significant difference in the shape, given by the shape index (L/W), in any sample before or after sporulation.

Further, the differences in dimensions were not significant and it was concluded that there was no change in size or shape during sporulation.

All other measurements were made on oocysts sporulated in 2.5% potassium dichromate, so that they could be compared with oocysts from the original "pure line" infection. This was an additional check that they were of the same species.

(i) Comparison of measurements of oocysts during the patent period.

A variation in the size of oocysts passed on separate days of the patent period of the parasite was observed in four birds, given doses of 10,000 oocysts of E. phasiani. The following experiments and analyses were

performed to ascertain the nature and significance of this variation.

A. Pilot Sample.

A pilot sample was made on Chick 15 by taking ten oocysts each day of the patent period. Four measurements, namely length (L), width (W), shape (L/W) and volume (LW^2) were made so as to obtain the most suitable value to be used as a standard for showing variations in size of the oocysts.

Since the biometrical data were taken at equal (daily) intervals of the independent variate, time, it was possible to examine the regression with respect to time of each variate, using the method of orthogonal polynomials. The analysis of variance then shows (Table 6), firstly, whether the scatter from one time to another is significantly greater than at any one time (lines h and i) and secondly, if so, the extent to which the relationship between the variate (y) and time(x) is explicable in terms of successively increasing powers of x (lines a to e and g).

Table 6. Analysis of Variance of Length (L), Width (W) and Volume (LW^2) of Oocysts during the Patent Period of an Infection in Chick 15.

(i) Length (L).

	Source	S. of S.	D.F.	M.Sq.	F.	P.
a	Linear	4.49	1	2.88	$a+b/c+d+e$ = 48.0	< 1%
b	Quadratic	1.27	1			
c	Cubic	0.09	1			
d	Quartic	0.05	1	0.06		
e	Quintic	0.04	1			
f	Fitting	5.94	5			
g	Residual	3.64	7			
h	Between Times	9.58	12	0.798	$h/i = 3.63$	< 0.1%
i	Within Times	25.78	117	0.220		
j	Total	35.36	129			

$$b: 0.050 \pm 0.0115$$

$$c: -0.00796 \pm 0.00340$$

$$t_b: 4.35$$

$$t_c: 2.34$$

$$P: < 0.1\%$$

$$P: < 0.1\%$$

Table 6 Continued.(ii) Width (W).

	Source	S. of S.	D.F.	M.Sq.	F.	P.
a	Linear	1.53	1	1.28	$a+b/c+d+e$ = 9.6	< 5%
b	Quadratic	1.03	1			
c	Cubic	0.31	1			
d	Quartic	0.02	1			
e	Quintic	0.07	1			
f	Fitting	2.96	5			
g	Residual	1.66	7			
h	Between Times	4.62	12	0.385	$h/i = 4.23$	< 0.1%
i	Within Times	10.67	117	0.091		
j	Total	15.29	129			

b: 0.029 ± 0.0077

t_b : 3.77

P_b : < 0.1%

c: -0.00718 ± 0.00233

t_c : 3.08

P_c : < 0.1%

(iii) Volume (LW^2).

	Source	S. of S.	D.F.	M. Sq.	F.	P.
a	Linear	21704	1	16353	$a+b/c+d+e$ = 13.71	< 5%
b	Quadratic	11002	1			
c	Cubic	2461	1			
d	Quartic	57	1			
e	Quintic	1060	1			
f	Fitting	36284	5			
g	Residual	17885	7			
h	Between Times	54169	12	4514	$h/i = 3.67$	< 0.1%
i	Within Times	143914	117	1230		
j	Total	198083	129			

b: 3.45 ± 0.87

t_b : 3.97

P_b : < 0.1%

c: -0.741 ± 0.253

t_c : 2.92

P_c : < 0.1%

In the event, the shape variate (L/W) showed no significant change with time and so was not analysed further (Table 7).

Table 7. Analysis of Variance on the Shape Index (L/W) of Oocysts during the Patent Period.

Chick 15.

Source	S. of S.	D.F.	M. Sq.	F.	P.
Between Times	0.061	12	0.0051	1.31	not significant
Within Times	0.454	117	0.0039		
Total	0.515	129			

Oocysts were produced for 16 days and measured for the first 13 days.

There was a gradual but statistically significant increase in length, width and volume of the oocysts during the patent period which reached a peak around day 10 and then fell off slightly. The best fitting graph, as given by the least squares method, was a quadratic (Fig. 3). The results, as shown by the variance ratios (F) and their associated probabilities (P), show that the quadratic term of the variation between times is best fitted by the measurements of length; the associated probabilities being $< 1\%$ in the case of length and $< 5\%$ in the case of width and volume. However, as the difference in variation of L, W and LW^2 was only slight, it was considered that the most logical factor in measuring a variation was

VARIATION IN OOCYST VOLUME DURING PATENT PERIOD
 showing the least-squares fit for a straight line(x) and for
 a quadratic curve(x^2)

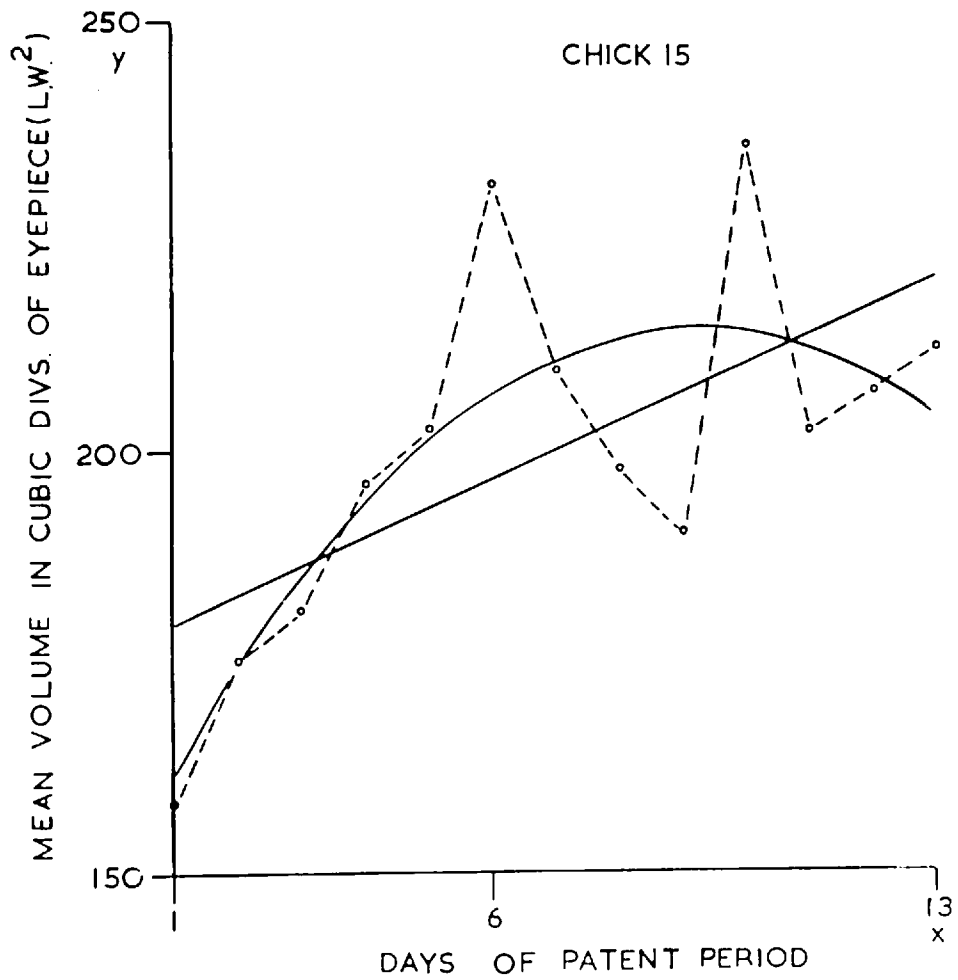


FIG. 3

volume (LW^2) and this was used in the other analyses.

The biological significance of these results is discussed in conjunction with the complete data.

B. Complete Data.

The variation in size of oocysts as given by the volume (LW^2) was measured in three infections in addition to that in Chick 15.

From Chick 42, fifteen oocysts were measured each day for the complete length of the patent period, i.e. 11 days. The variation (Fig. 4) was constant showing neither a trend to increase nor decrease during the patent period and so analyses were not performed on these measurements.

From both Chick 38 and 41, a trend to increase in size was noted (Fig. 5 and Fig. 6). In Chick 38, twenty oocysts were measured each day for 10 days, the complete patent period, and in Chick 41, fifteen oocysts each day for the complete period, also of 10 days.

The analyses of these measurements are given in Tables 8 and 9.

The increase in size in both these infections was best summarised by a straight line, the probabilities of the observed variance ratios being $< 0.1\%$ in Chick 38 and $< 1\%$ in Chick 41.

There was no variation in the daily mean values of shape (L/W) in any of the four infections (Table 10).

VARIATION IN OOCYST VOLUME DURING PATENT PERIOD

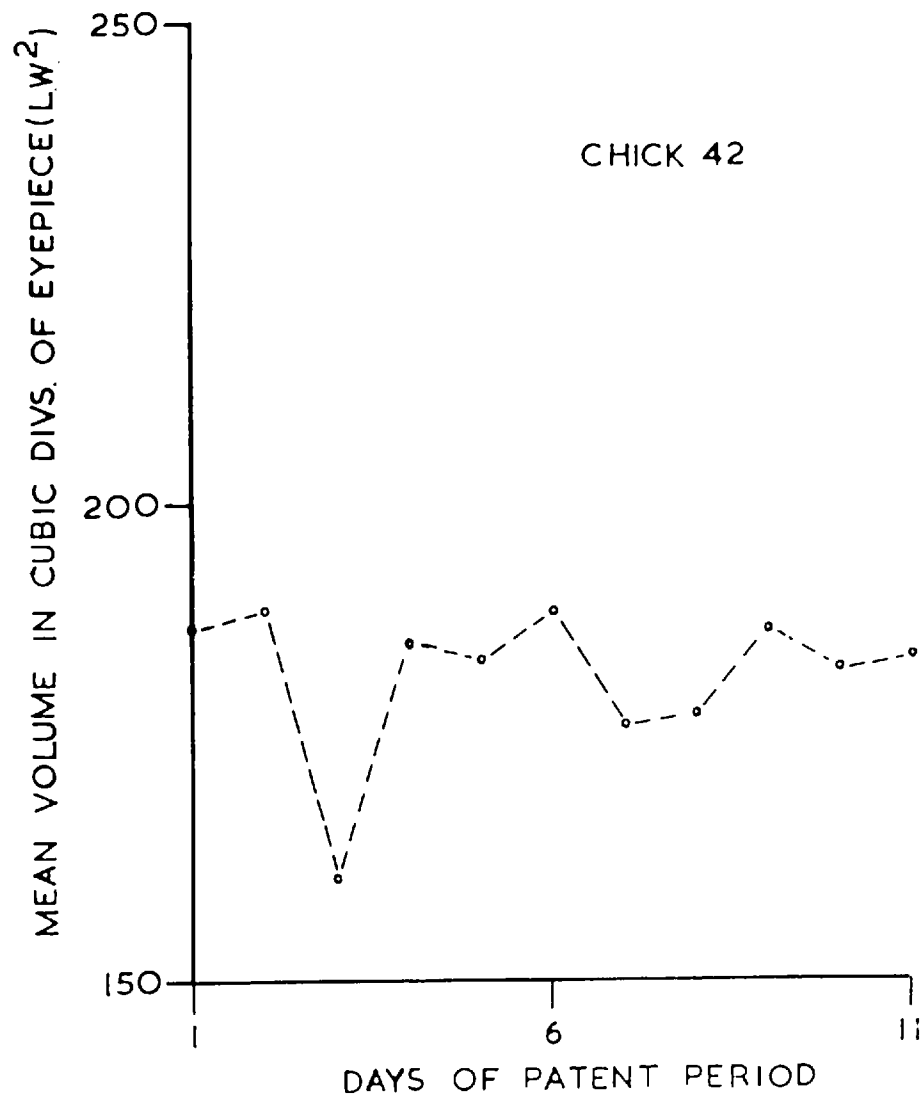


FIG. 4.

VARIATION IN OOCYST VOLUME DURING PATENT PERIOD
showing the least-squares fit for a straight line(x)

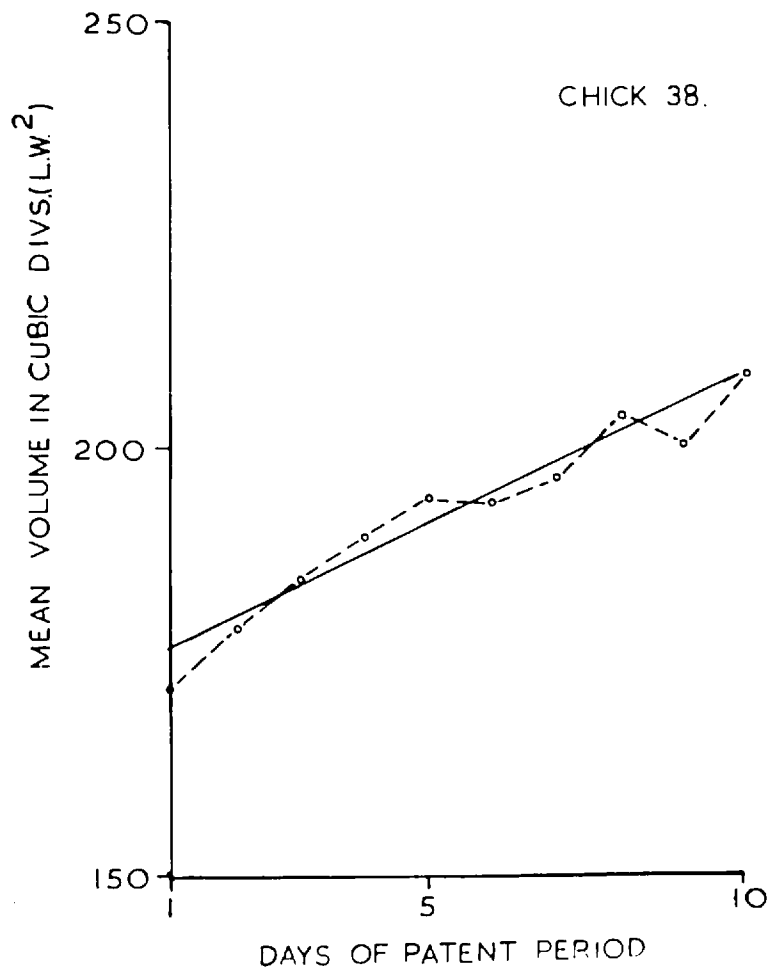


FIG. 5.

VARIATION IN OOCYST VOLUME DURING PATENT PERIOD
showing the least-squares fit for a straight line (x)

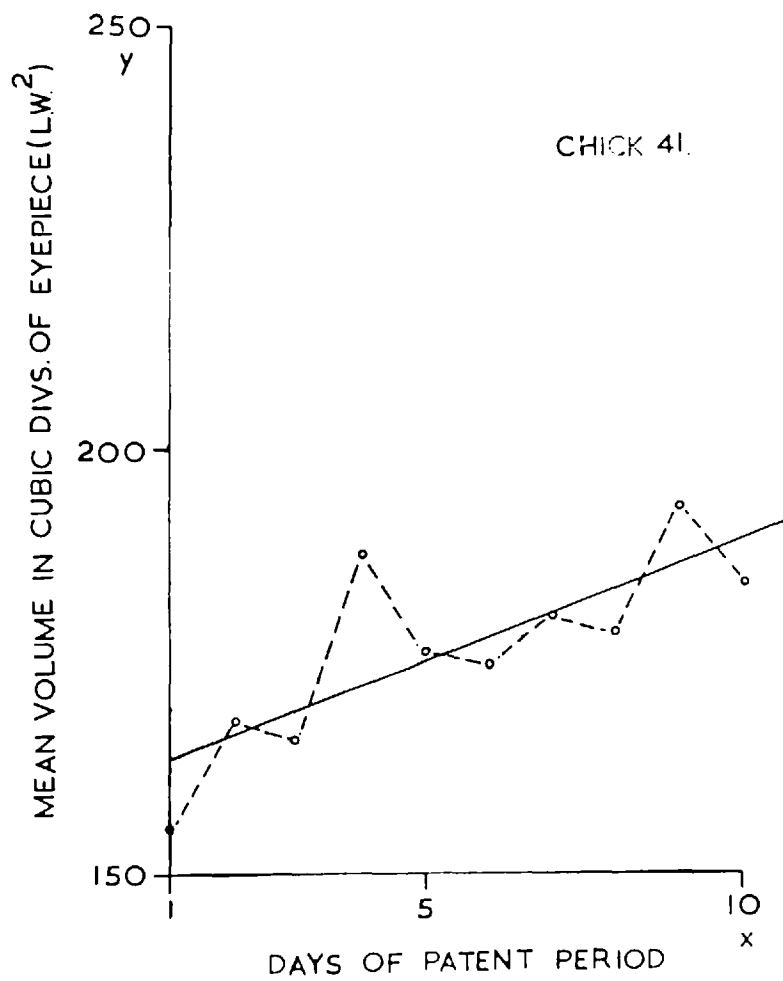


FIG. 6

Table 8. Analysis of Variance of the Volume (LW^2) of Oocysts during the Patent Period of an Infection.

(i) Chick 15.

Table 6 (iii).

(ii) Chick 38.

	Source	S. of S.	D.F.	M. Sq.	F.	P.
a	Linear	21420	1	21420 195	$a/b+c+d+e$ = 109.8	< 0.1%
b	Quadratic	433	1			
c	Cubic	337	1			
d	Quartic	7	1			
e	Quintic	5	1			
f	Fitting	22202	5			
g	Residual	444	4			
h	Between Times	22646	9	2516	$h/i = 4.63$	< 0.1%
i	Within Times	103202	190	543		
j	Total	125848	199			

b: 3.60 ± 0.28

t_b : 12.85

P_b : < 0.1%

(iii) Chick 41.

	Source	S. of S.	D.F.	M. Sq.	F.	P.
a	Linear	10522	1	10522	$a/b+c+d+e$ = 13.81	< 5%
b	Quadratic	981	1	762		
c	Cubic	792	1			
d	Quartic	56	1			
e	Quintic	1219	1			
f	Fitting	13570	5			
g	Residual	3976	4			
h	Between Times	17546	9	1950	$h/i = 4.34$	< 0.1%
i	Within Times	62877	140	449		
j	Total	80423	149			

b: 2.92 ± 0.98

t_b : 2.97

P_b : < 1%

Table 9. Test of Significance of Fitting b.(i) Chick 15.

Source	S. of S.	D.F.	M.Sq.	F.	P.
Effect of fitting b	21704	1	21704	7.35	< 5%
Quadratic and higher	32465	11	2951		
Between Times	54169	12			

(ii) Chick 38.

Source	S. of S.	D.F.	M.Sq.	F.	P.
Effect of fitting b	21420	1	21420	140	< 0.1%
Quadratic and higher	1226	8	153		
Between Times	22646	9			

(iii) Chick 41.

Source	S. of S.	D.F.	M.Sq.	F.	P.
Effect of fitting b	10522	1	10522	11.98	< 1.0%
Quadratic and higher	7024	8	878		
Between Times	17546	9			

Table 10. Mean Daily Shape Indices (L/W) of Oocysts during the Patent Period.

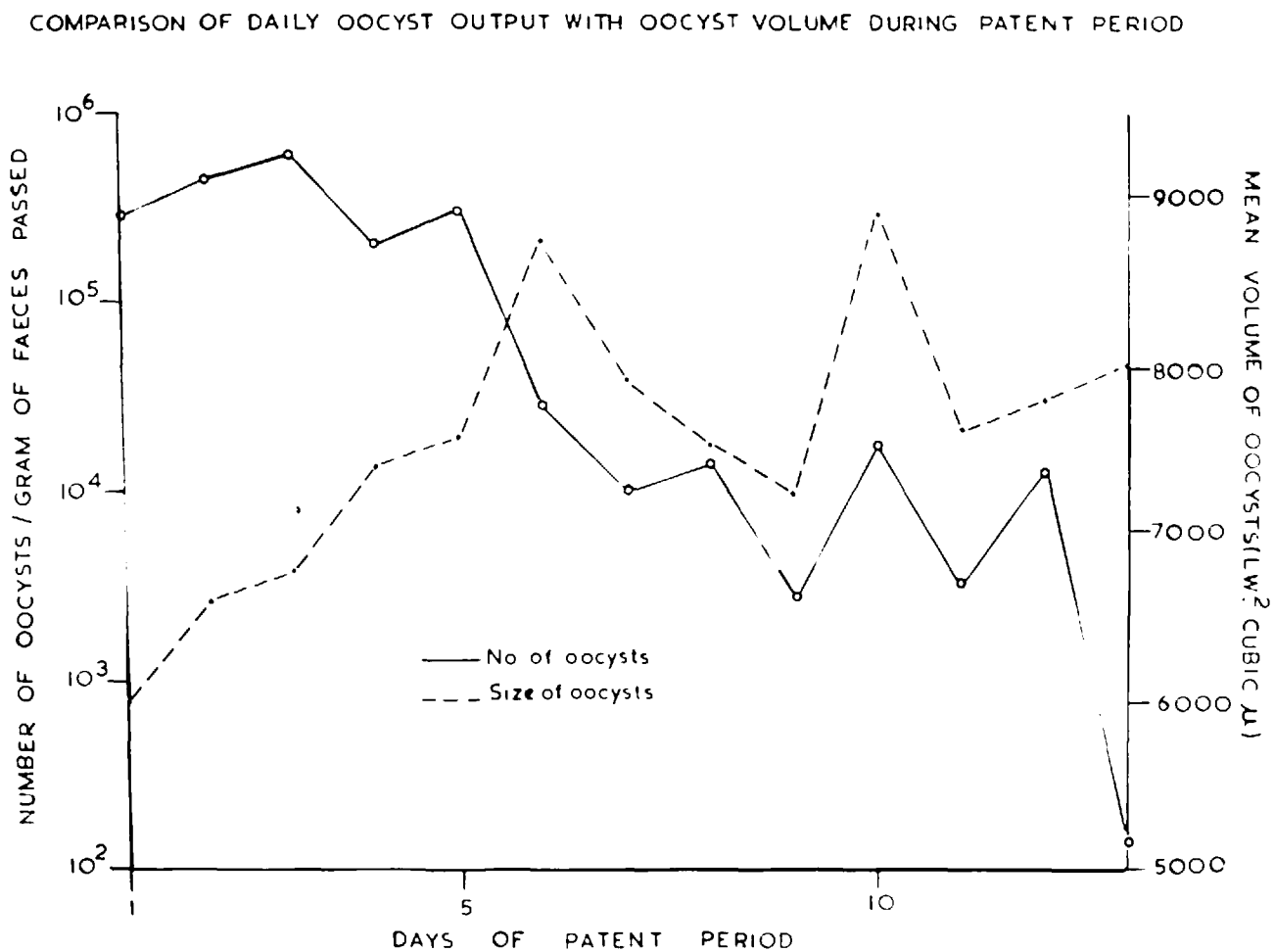
<u>Day of</u> <u>Patent Period</u>	<u>Chick 15</u>	<u>Chick 38</u>	<u>Chick 41</u>	<u>Chick 42</u>
1	1.45	1.42	1.42	1.45
2	1.44	1.43	1.44	1.44
3	1.44	1.43	1.44	1.44
4	1.44	1.45	1.43	1.43
5	1.42	1.43	1.44	1.44
6	1.43	1.43	1.45	1.46
7	1.39	1.46	1.44	1.43
8	1.46	1.44	1.44	1.44
9	1.47	1.43	1.43	1.45
10	1.44	1.42	1.45	1.45
11	1.47			1.44
12	1.45			
13	1.44			

The biological significance of the variation of oocyst size during the patent period is difficult to explain completely, as a similar result was not obtained in all four cases of birds given similar infective doses. It is possible that there are several factors affecting the size of oocysts, one of which is probably a 'crowding' factor: the smaller oocysts being produced when the oocyst output is high and the larger ones when the production is low. There is some evidence for this in Fig. 7, where the change in volume is compared with the oocyst output in Chick 15. Other factors may be the physiology and diet of the host.

(ii) Comparison of measurements of oocysts in individual birds.

The variation in size of oocysts from infections in eight different birds, given the same dose of 10,000 sporulated oocysts, was measured

FIG. 7



by taking a random sample of ten oocysts from each bird. All the samples were collected on the same day, the first of the patent period, in order to eliminate variation due to time. Volume (LW^2) was used as a measure of variation and the analysis of variance is given in Table 11 and Table A.5 (Appendix p. 161).

Table 11. Analysis of Variance of the Volume of Oocysts from Different Infected Chicks.

Source	S. of S.	D.F.	M.Sq.	F.	P.
Between Chicks	33131	7	4733	8.35	< 0.1%
Within Chicks	40801	72	567		
Total	73932	79			

There was a significant difference at the < 0.1% level in the measurements between birds.

The frequency distributions of measurements taken of 1439 oocysts of E. phasiani, with respect to length, width and shape index are given as histograms in Fig. 8. These frequencies are compared with normal curves having the same mean and variance (Table A.6, Appendix pp. 162-5). The expected values (E), given by the curve, were compared with those observed (O) by means of a chi-squared test.

$$\chi^2 = \frac{\sum (O - E)^2}{E}$$

where $\sum$ = summation.

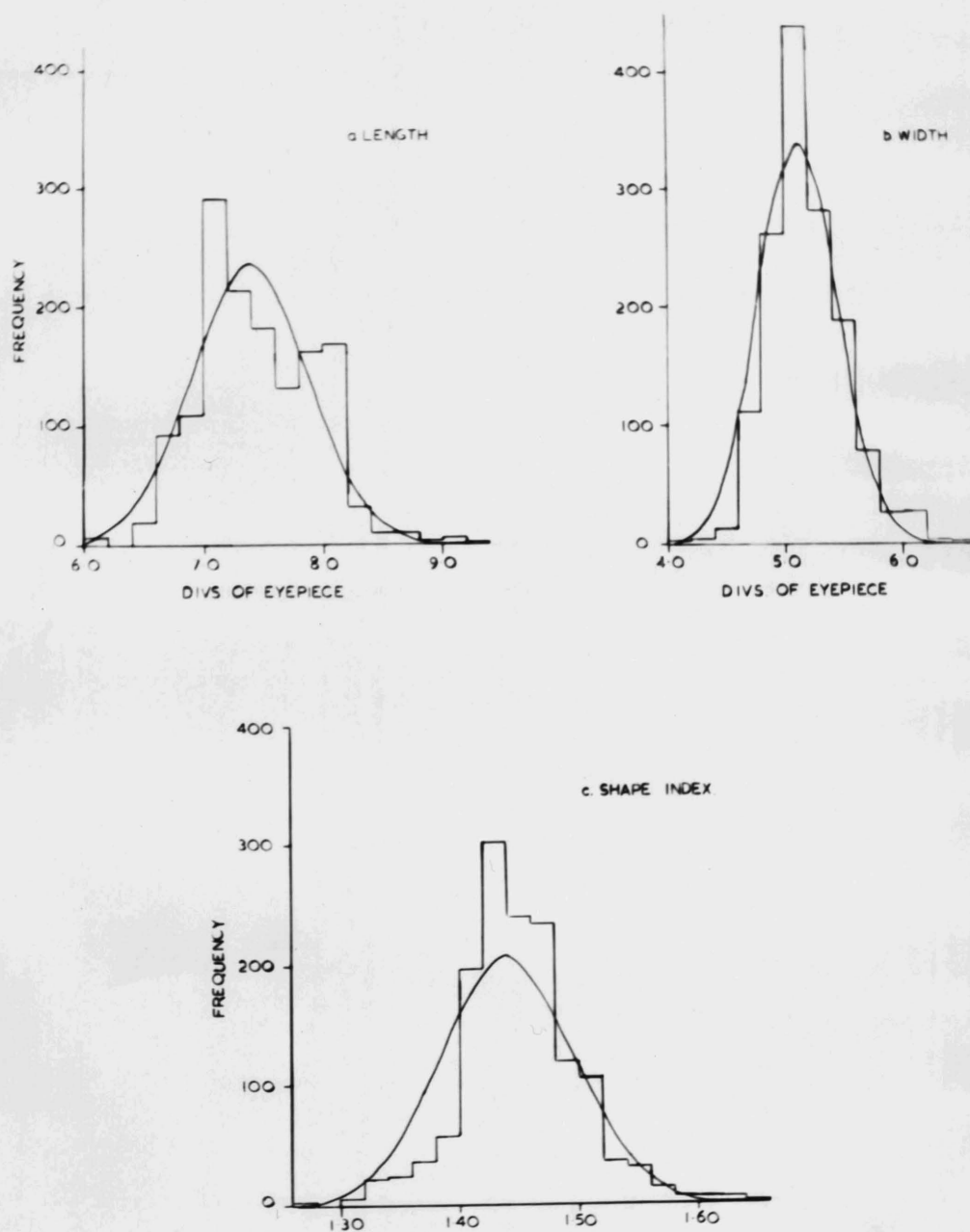
DIMENSIONS OF OOCYSTS OF *EIMERIA PHASIANI* COMPARED WITH NORMAL DISTRIBUTIONS

FIG. 8.

High values of the chi-squared discredit the hypothesis that the observed data form a single normal distribution. The values of chi-squared are all high, showing a heterogeneity in the measurements, although all the oocysts are of the same 'pure line' of E. phasiani, derived from a single oocyst. This heterogeneity, which cannot be due to the presence of more than one species, is probably due to the variation in measurements during the patent period and the variation from infection to infection, as shown above and in Tables 12 and 13.

Tables 12 and 13 summarise the complete data of measurements of oocysts from 16 birds, infected with doses varying between 1,000 and 10,000 sporulated oocysts of E. phasiani. Table 12 gives the range, mean, standard deviation, standard error of mean and coefficient of variation of length and width and the range and mean of the shape indices of oocysts from each bird. There is a wide spread of mean values of size from 23.07 ± 0.16 to $26.46 \pm 0.27 \mu$ in length and $16.17 \pm 0.15 \mu$ to $18.35 \pm 0.21 \mu$ in width.

The mean shape indices of oocysts from individual birds vary only slightly between 1.42 and 1.46, which shows that the shape of the oocysts stays fairly constant throughout.

Table 13 also summarises the data, arranged as a correlation table relating length (L) and width (W) by frequency groups. This shows the strong positive correlation required by a constant shape.

It can be concluded that the size of oocysts of E. phasiani varies greatly depending on several factors listed above. This shows that biometrical data obtained from oocysts taken from the host, at any one

Table 12. Variation in Size of Oocysts of *Eimeria phasiani* from Individual Birds.

Bird No.	Day of Patent Period	No. Measured	Length (μ)				Width (μ)				Range	
			Range	$\bar{m} \pm S.E.$	S.	CV %	Range	$\bar{m} \pm S.E.$	S.	CV %	S.I.	$\bar{m}$ S.I.
Chick 7	Complete Period	145	21.8-30.2	25.39 $\pm$ 0.14	1.64	6.46	15.4-20.1	17.46 $\pm$ 0.09	1.06	6.07	1.33-1.60	1.45
Chick 9	Complete Period	175	20.1-30.2	25.37 $\pm$ 0.10	1.31	5.16	13.4-20.1	17.37 $\pm$ 0.08	1.07	6.16	1.32-1.60	1.46
Chick 15	Complete Period	144	20.1-29.5	25.05 $\pm$ 0.14	1.74	6.95	14.1-20.1	17.37 $\pm$ 0.10	1.18	6.79	1.26-1.57	1.44
Chick 26	4	25	23.5-28.8	25.99 $\pm$ 0.26	1.31	5.04	16.1-20.1	18.31 $\pm$ 0.25	1.23	6.72	1.33-1.52	1.42
Chick 32	10	25	23.5-28.5	26.16 $\pm$ 0.29	1.46	5.58	16.8-20.5	18.18 $\pm$ 0.19	0.97	5.33	1.27-1.54	1.44
Chick 33	10	25	23.5-30.2	26.22 $\pm$ 0.29	1.46	5.57	16.8-20.1	18.14 $\pm$ 0.17	0.86	4.74	1.33-1.56	1.45
Chick 38	Complete Period	200	21.8-26.8	24.55 $\pm$ 0.09	1.30	5.29	15.4-18.8	17.11 $\pm$ 0.05	0.77	4.49	1.33-1.52	1.43
Chick 41	Complete Period	150	20.1-26.8	23.95 $\pm$ 0.09	1.09	4.55	15.4-18.8	16.67 $\pm$ 0.08	0.93	5.58	1.30-1.59	1.44
Chick 42	Complete Period	165	21.8-26.8	24.20 $\pm$ 0.09	1.12	4.63	15.4-18.8	16.77 $\pm$ 0.06	0.74	4.41	1.31-1.56	1.44
Chick 67	4	50	22.1-30.9	26.46 $\pm$ 0.27	1.89	7.14	15.4-19.8	18.35 $\pm$ 0.21	1.50	8.17	1.36-1.54	1.44
Chick 71	4	60	21.8-28.8	25.42 $\pm$ 0.24	1.84	7.24	15.4-17.8	17.71 $\pm$ 0.17	1.32	7.45	1.34-1.50	1.44
Chick 96	2	25	22.1-24.8	23.68 $\pm$ 0.16	0.79	3.34	15.4-17.8	16.57 $\pm$ 0.12	0.60	3.62	1.38-1.47	1.43
Chick 97	2	25	21.8-24.5	23.07 $\pm$ 0.16	0.79	3.42	15.1-17.8	16.17 $\pm$ 0.15	0.74	4.58	1.37-1.46	1.43
Chick 100	2	25	22.1-24.8	23.33 $\pm$ 0.19	0.93	3.99	15.4-17.1	16.13 $\pm$ 0.13	0.64	3.97	1.41-1.50	1.45
Chick 104	1.3.8	100	21.8-27.5	24.10 $\pm$ 0.15	1.54	6.39	15.1-19.1	16.70 $\pm$ 0.10	1.05	6.29	1.38-1.53	1.44
Chick 106	1.3.8	100	21.8-27.5	24.00 $\pm$ 0.16	1.57	6.54	15.1-19.4	16.66 $\pm$ 0.11	1.08	6.48	1.39-1.55	1.44

Table 13. A Correlation of Length (L) and Width (W) of Oocysts of
E. phasiani by Frequency Groups. (All measurements in
divisions of the micrometer eyepiece).

L \ W	6.00	6.20	6.40	6.60	6.80	7.00	7.20	7.40	7.60	7.80	8.00	8.20	8.40	8.60	8.80	9.00	9.20	Σ W.
	6.10	6.30	6.50	6.70	6.90	7.10	7.30	7.50	7.70	7.90	8.10	8.30	8.50	8.70	8.90	9.10	9.30	
4.00																		
4.10	1.		1.															2.
4.20																		
4.30	3.		1.															4.
4.40																		
4.50			8.	3.	1.	1.												13.
4.60																		
4.70	2.		6.	75.	18.	4.	6.	1.										112.
4.80																		
4.90			2.	10.	69.	121.	48.	10.	2.									262.
5.00																		
5.10			1.	4.	20.	148.	129.	89.	29.	12.	6.	1.						439.
5.20																		
5.30					1.	17.	24.	68.	76.	53.	40.	3.						282.
5.40																		
5.50							7.	12.	22.	80.	61.	7.						189.
5.60																		
5.70							2.	2.	16.	45.	10.	4.						79.
5.80										1.	5.	8.	4.	7.		1.		26.
5.90																		
6.00																		
6.10								1.	1.	12.	3.	2.	2.	1.	5.			27.
6.20																		
6.30													1.	2.				3.
6.40																		
6.50																1.		1.
Σ L.	6.		19.	92.	109.	291.	214.	182.	132.	163.	169.	32.	10.	10.	3.	6.	1.	1439.

time during an infection, cannot be relied upon as constant for the species. Conversely, one cannot use size as one factor in the diagnosis of a species, if it has been derived from only one sample.

From this study the mean size of oocysts of E. phasiani was $24.74 \pm 0.043 \mu \times 17.14 \pm 0.028 \mu$ with a shape index of 1.44 ± 0.002 .

(c) Sporulation of oocysts.

The sporulation time of oocysts is one of the criteria used in the identification of species of coccidia. This is only valid if standard conditions are applied to enable a comparison of sporulation times to be made. Unfortunately, often, this has not been the case.

In some cases, the sporulation time has been taken as the minimum time for oocystic development, i.e. when a single oocyst has sporulated. In other cases, it has been taken as the time when the maximum number of oocysts have completed development. However, when a pure line coccidial infection is available the most accurate estimate of this time is that at which 50% of the oocysts have sporulated. The oocyst is regarded as having completed sporulation when the sporozoites are visible as separate bodies within the sporocysts.

No cases were observed in which sporulated oocysts were passed in the faeces of the bird. In the freshly passed oocyst the zygote completely filled the cyst (Fig. 9) but it quickly shrank to form a fairly smooth, round ball of cytoplasm, which filled just over half the volume of the oocyst (Fig. 11: 1). Cytokinesis followed, furrows appearing in the



Fig. 9. An Unsporulated Oocyst of E. phasiani (X 1800)



Fig. 10. A Sporulated Oocyst of E. phasiani (X 2000)

Explanation of Figure 11.

Sporulation of E. phasiani at 20°C., fresh preparation. (Camera lucida drawing).

1. Oocyst with zygote filling half the cyst.
2. Oocyst containing zygote with rhombohedral sporoblasts.
3. Oocyst with sporoblasts rounded off.
4. Oocyst with elongate sporoblasts.
5. Oocyst containing four sporocysts.
6. Single sporocyst with two sporozoites.

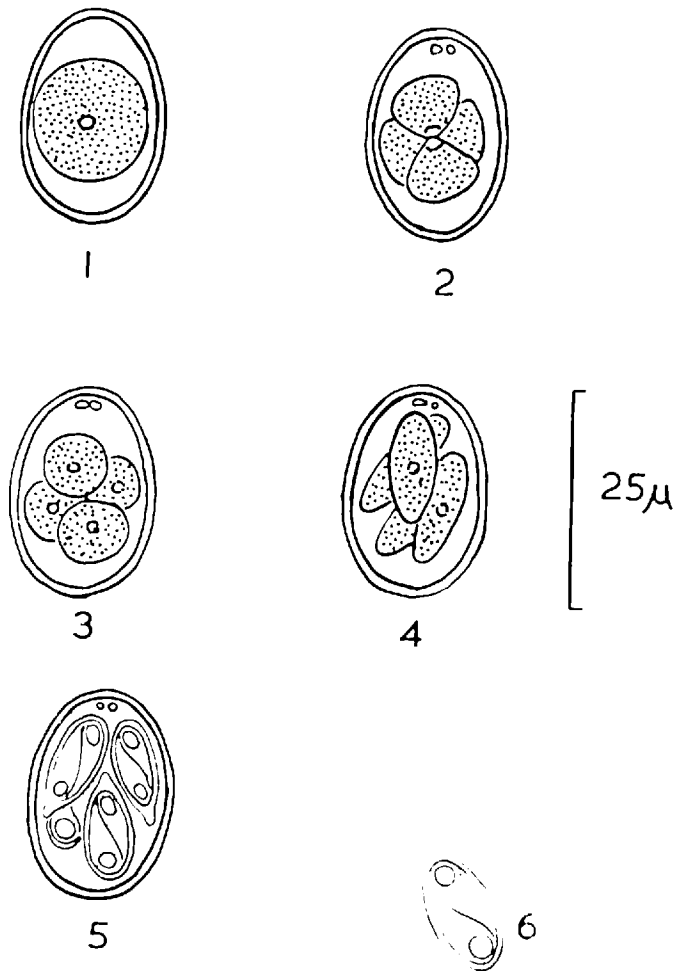


FIG. II.

zygote which eventually divided into four rhombohedral sporoblasts with clear nuclear areas at their apices (Fig. 11: 2). At this point 1 - 3 refractile polar granules appeared at the narrower pole of the oocyst. Very quickly the rhombohedrons rounded off to form four globular sporoblasts (Fig. 11: 3). These then elongated and secreted the sporocyst wall inside of which developed the sporozoites, two to each sporocyst (Fig. 10 and Fig. 11: 5).

For a strict comparison of sporulation times equal amounts of faeces from three separate birds infected with E. phasiani were mixed with equal volumes of 2.5% potassium dichromate solution. The samples were incubated at 6°C., 15°C., 20°C., 25°C., 30°C., 35°C. and 40°C.

At 6°C., no sporulation had occurred after seven days, all the oocysts containing rounded zygotes. After seven days, these oocysts were placed at 20°C. and they developed normally, with the maximum sporulation of 94% of the total oocysts occurring between 54 and 60 hours. This shows that at 6°C. the oocysts were not damaged but remained dormant. The results (Fig. 12) show that the sporulation rate **increased with the** rise in temperature and also that there was a definite linear relationship between the probit of percentage sporulation and log. time at the lower temperatures. Above 25°C. a lethal factor was introduced and the linear relationship lost, as indicated by the scattered points for 30°C. Judging from the fitted line, the optimum temperature for unaffected sporulation was 25°C. As 20°C. is the temperature at which most figures for sporulation are given, the 50% sporulation time, i.e. 52 hours, was taken from this

RELATIONSHIP BETWEEN PERCENTAGE SPORULATION AND
TIME AT DIFFERENT TEMPERATURES

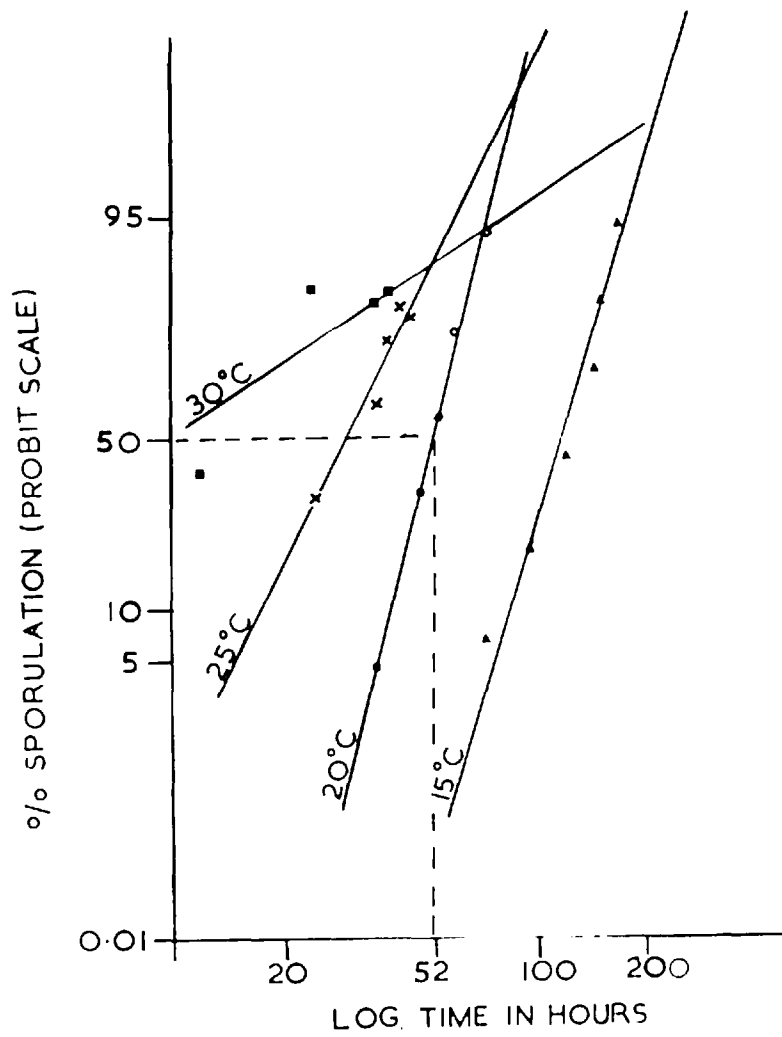


FIG.12.

fitted line.

The majority of oocysts kept at 35°C. for 36 hours were not viable. 5.3% of the oocysts completed sporulation and an average of 34.5% produced sporocysts but not sporozoites. Cytokinesis had begun in an average of 44% but after 36 hours slight degeneration was observed. The remainder of the oocysts were in the undivided zygote stage but these did not develop further when placed at 20°C.

The viability of the oocysts, which had completed sporulation at 35°C., was tested by administering, to each of two 30 day-old coccidia-free poults, 1,000 sporulated oocysts. The oocyst production of these chicks was compared with that of two chicks infected with an equal dose of oocysts sporulated at 20°C. One chick, which received oocysts from the 35°C. culture, failed to become infected and the other showed only a slight infection, as compared with that produced by a similar dose from the 20°C. culture (Fig. 13). This is indicative of a reduced viability.

Unsporulated oocysts kept at 40°C. for 24 hours were completely killed. Development, if at all, only progressed as far as cytokinesis, where abnormalities were observed. A few oocysts exhibited one extra large sporoblast and three smaller ones. Another abnormality was the formation of two large and two smaller sporoblasts.

Oocysts from each of the three birds behaved in the same way (Table 14).

COMPARISON OF OOCYST OUTPUT IN INFECTIONS PRODUCED BY
EQUAL DOSES OF OOCYSTS SPORULATED AT 20°C AND 35°C

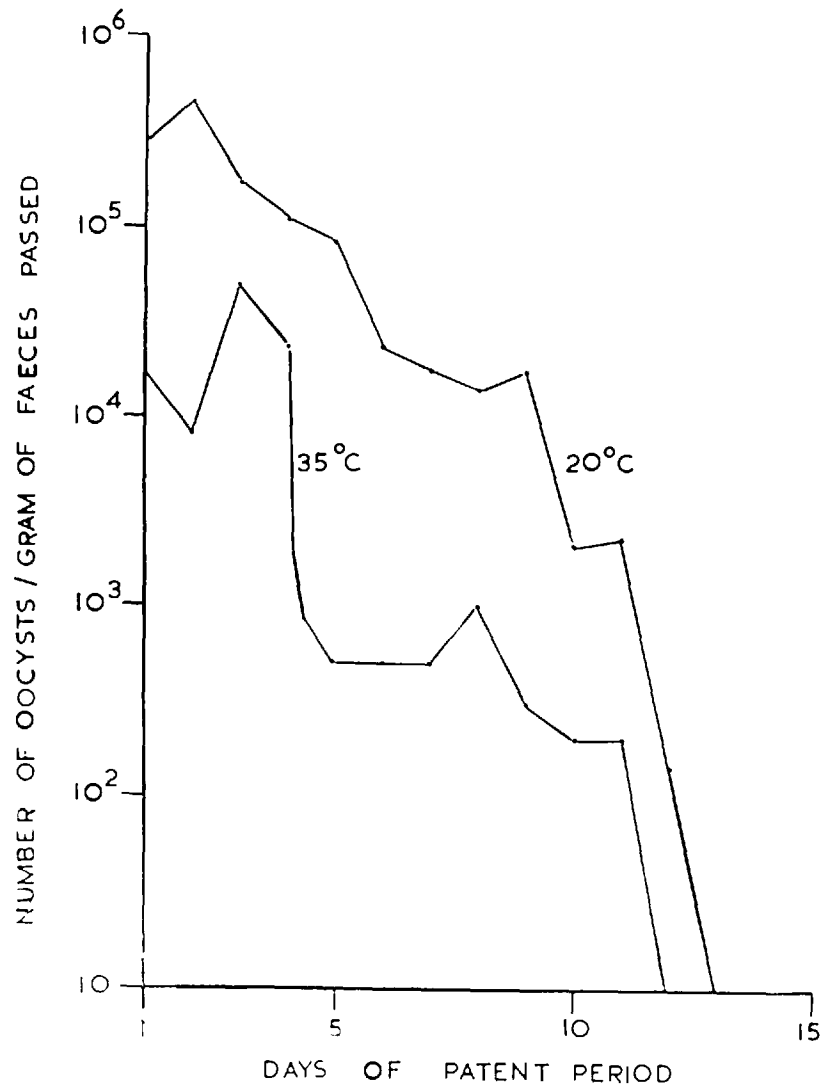


FIG 13

Table 14. To Show Variation in the Rate of Sporulation with Time.

Temp.	Chick No.	12 hours	24 hours	36 hours	39 hours	42 hours	45 hours	48 hours	54 hours	60 hours	72 hours	96 hours	120 hours	144 hours	150 hours	168 hours	174 hours
15°C.	35										11.0%	30.0%	58.0%	72.0%	86.0%	95.4%	
	36										3.6%	18.0%	36.0%	74.0%	85.0%	95.2%	
	37										5.3%	16.3%	41.0%	68.0%	88.0%	94.3%	95.2%
20°C.	35		1%	5.0%				20.3%	53.0%	76.6%	93.4%						
	36		-	4.6%				26.0%	58.9%	70.1%	96.3%						
	37		-	4.6%				30.0%	56.0%	88.0%	92.0%						
25°C.	35		31.5%	62.0%	69.3%	72.4%	74.7%	75.0%	75.3%								
	36		36.0%	63.1%	87.6%	90.0%	89.0%										
	37		29.9%	60.0%	74.0%	92.0%	87.0%										
30°C.	35	42.6%	88.6%	85.0%	86.1%												
	36	43.8%	87.8%	86.0%	86.2%												
	37	44.0%	86.0%	85.0%	88.3%												

Ormsbee (1939) reported that 2.5% potassium dichromate was harmful to oocysts of E. phasiani but this did not appear to be so in this study.

(d) Oocyst morphology.

The oocysts of E. phasiani were ellipsoidal in shape. The cyst wall was smooth in outline and was composed of two cyst membranes, an outer thicker ectocyst and a thinner endocyst. The thickness of the wall, 1.59μ , did not vary within the oocyst. When viewed in transmitted light the wall gave the oocyst a light brownish colour. One to three polar granules were present but a micropyle/^{and} oocystic and sporocystic residua were absent. The sporocysts, each with a well-developed Steida body, were elongate pyriform in shape, measuring $12.9 - 15.9 \mu \times 5.6 - 7.4 \mu$ (mean $14.26 \pm 0.06 \mu \times 6.69 \pm 0.04 \mu$). Each sporocyst contained two elongate sporozoites. These were pointed at the anterior and blunt at the posterior end and contained a central nucleus and a large posterior globule.

(e) Determination of the prepatent period.

The prepatent period, i.e. the time between the ingestion of the oocysts and the production of the first new generation of oocysts, is an important criterion in the identification of coccidial parasites.

An accurate determination of the prepatent period was made on eighteen coccidia-free pheasant chicks infected with doses, varying between 50 and 100,000 oocysts of E. phasiani. Faeces were examined every two hours during the fourth and fifth days after infection and the time was recorded when the first oocyst was passed. The results are summarised in Table 15.

p. 61.

Oocyst morphology.

What are your views on the means of hatching of oocysts in birds

- 1) Do oocysts have to be damaged
- 2) Are sporocysts released by enzyme action
- 3) Are sporozoites released from sporocysts by enzyme action.

Determination of pre-patent period para 2

doses of 50, $\rightarrow$ 100,000 oocysts
but on p. 62 no chicks
apparently received more than
60,000 oocysts!

Table 15. Variation of the Prepatent Period in *E. phasiani* infections.

<u>Chick No.</u>	<u>Infective Dose</u>	<u>Prepatent Period</u>
7	1,000	116 hrs
8	1,000	114 "
15	10,000	120 "
36	20,000	124 "
37	20,000	116 "
38	10,000	122 "
39	10,000	120 "
50	1,000	112 "
51	1,000	114 "
52	5,000	114 "
104	50	116 "
105	50	118 "
106	2,500	118 "
107	2,500	118 "
125	5,000	116 "
126	5,000	116 "
127	10,000	116 "
128	10,000	120 "

The prepatent period varied between 112 and 124 hours with a mean of 117 hours (Fig. 14).

(f) Oocyst production.

(i) Total oocyst output and the length of the patent period of *E. phasiani*.

Six 10 day-old coccidia-free pheasant chicks were infected with doses of 50; 5,000 and 10,000 sporulated oocysts of *E. phasiani* to determine the total oocyst output and patent period of the parasite. At each level of infection two chicks were infected with the same dose. The total output of oocysts for each dose level was estimated as the mean number produced between the two birds.

VARIATION IN PREPATENT PERIOD IN
E. PHASIANI

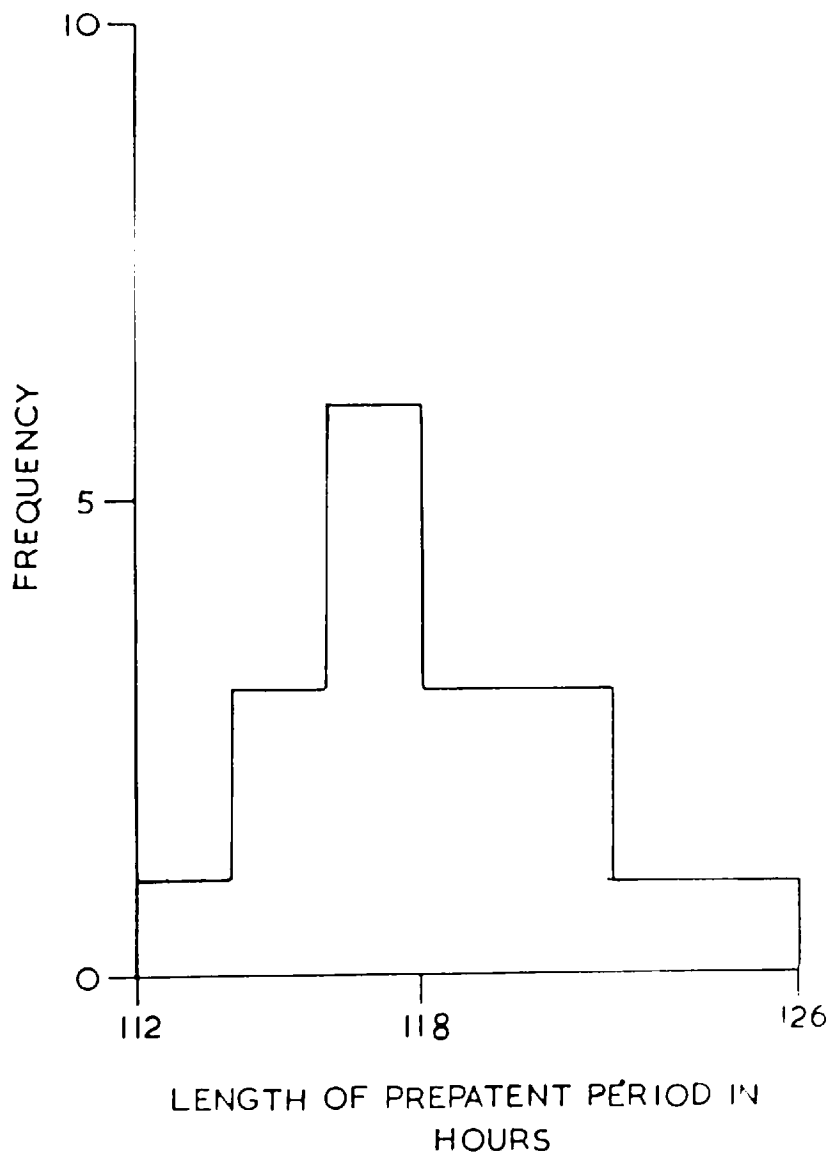


FIG. 14.

Infections of E. phasiani in the pheasant were self-limiting, oocyst production ceasing after a few days, if reinfection was prevented. The patent period, i.e. the number of days on which oocysts were passed, varied between ten and sixteen days, although very few oocysts were produced after the thirteenth day.

Fig. 15 shows the total daily oocyst output from these birds. The oocyst output increased at the start of the patent period to reach a peak around the second to fifth day, after which it gradually fell until cessation around day ten to sixteen.

Table 16. Oocyst Production in Graded Infections.

<u>Group</u>	<u>No. of Oocysts Administered</u>	<u>Patent Period</u>	<u>Av. No. of Oocysts produced per Bird</u>	<u>Approx. Oocyst Production per Oocyst administered</u>
1	1	3 days	151,000	151,000
2	50	10 "	4,442,000	88,400
3	5,000	11 "	7,908,400	1,600
4	10,000	13 "	12,698,850	1,270

Table 16 shows the oocyst production per oocyst administered in the graded infections. This shows E. phasiani to be a relatively poor producer of oocysts. Although within the range tested, with increased dose, the total number of oocysts increased ^{whilst} ~~but~~ the number produced per oocyst administered decreased considerably.

Table 16.

Group 1 ~~is~~ consists of 6 birds
whereas groups 2 3 & 4
consist of only 2 birds

This point should be made.

Some reference should be
made to Group I in the
paragraph following Table 16.

In this paragraph the word
whilst should be left out!

TOTAL DAILY OOCYST PRODUCTION IN BIRDS INFECTED WITH VARYING DOSES OF
E. PHASIANI

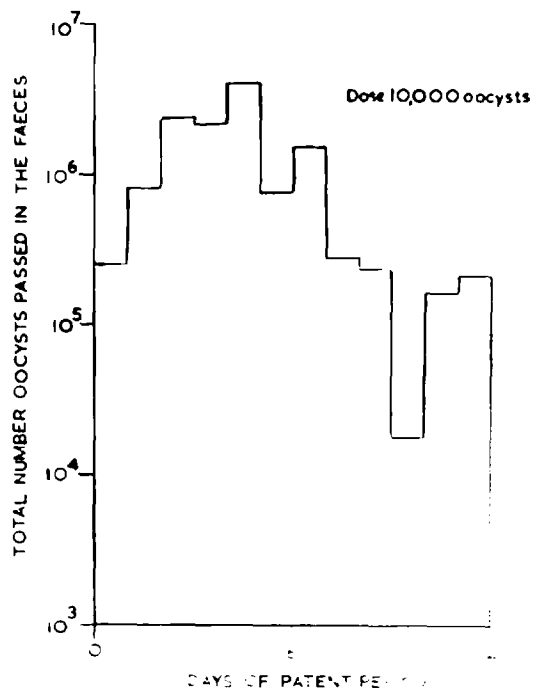
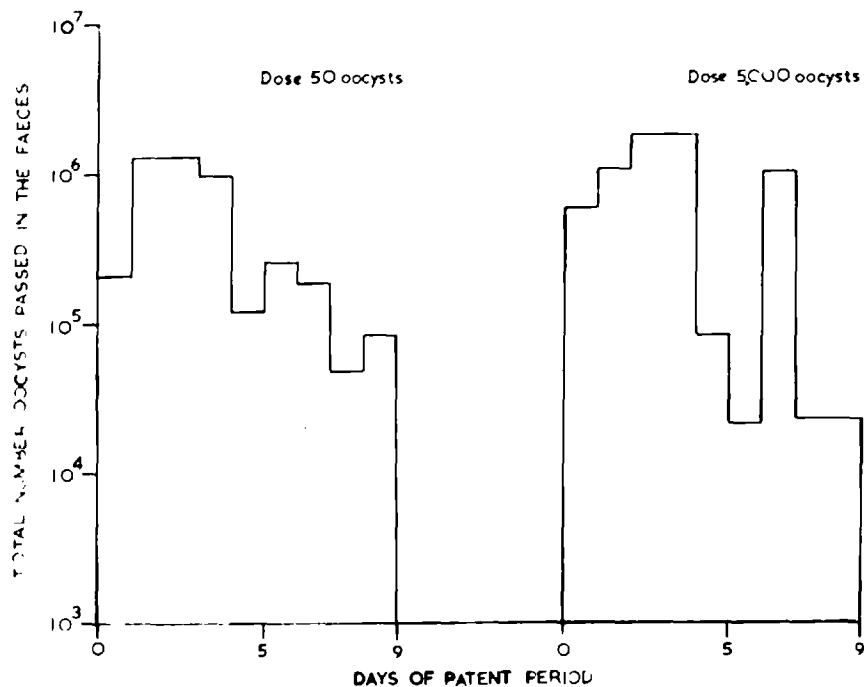


FIG. 15.

(ii) The Diurnal Output of Oocysts in *E. phasiani*.

The faeces of three pheasants, all infected with 20,000 oocysts of *E. phasiani* were examined to determine the pattern of oocyst output over a 24 hour period during the month of June. Faeces were collected each hour and the number of oocysts present estimated.

In all three cases there was a trend for the majority of oocysts to be produced in the period 14.00 - 22.00 hours (Fig. 16). The percentage of the total number produced in this time was 77.03% in Chick 36, 57.86% in Chick 37 and 82.40% in Chick 38. The minor peaks in the histograms, during the period 22.00 - 14.00 hours, may be due to the discharge of oocysts with the caecal contents.

Figs. 17, 18 and 19 compare the oocyst production per gramme of faeces passed (solid line) with the weight of faeces (dotted line) in the three birds. From this, there appears to be no correlation between the amount of faeces passed and the number of oocysts produced.

In order to test whether the diurnal periodicity of oocyst discharge was due to the host's influence rather than to an inherent periodicity of the parasite, the following experiment was performed. Oocysts were administered at different times during a 24 hour period. Thus, three groups each of four birds were infected with 10,000 oocysts per bird at 18.00 hours on 26.5.65, at 2.00 hours and 10.00 hours on 27.5.65, respectively and the number of oocysts passed during each two-hour interval for the first three days of the patent period was counted.

The majority of the oocysts on each day were produced in all

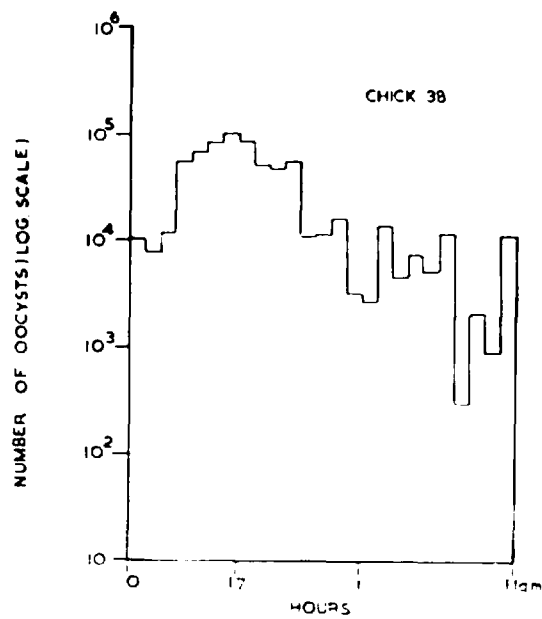
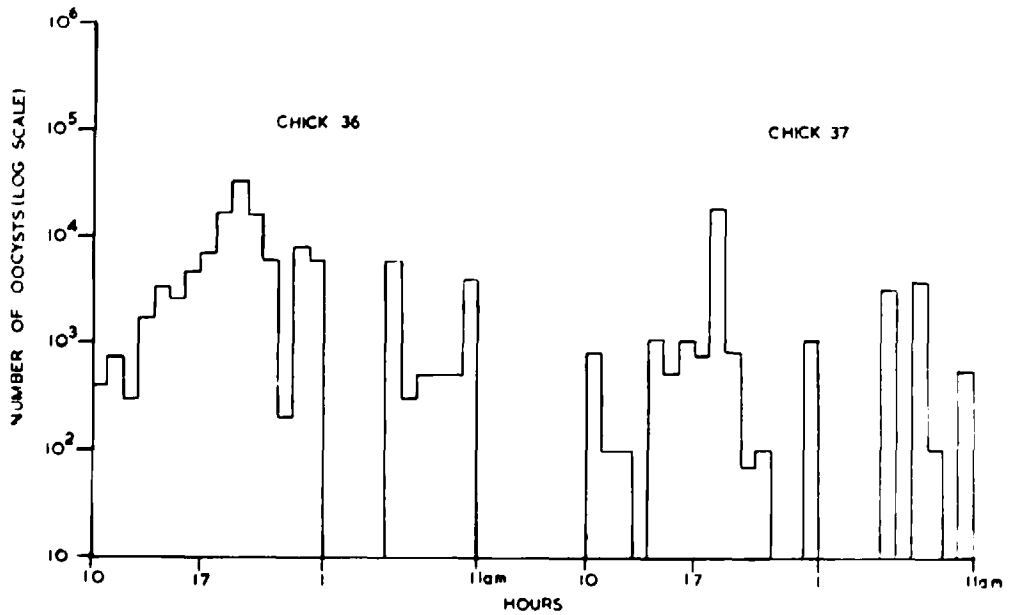
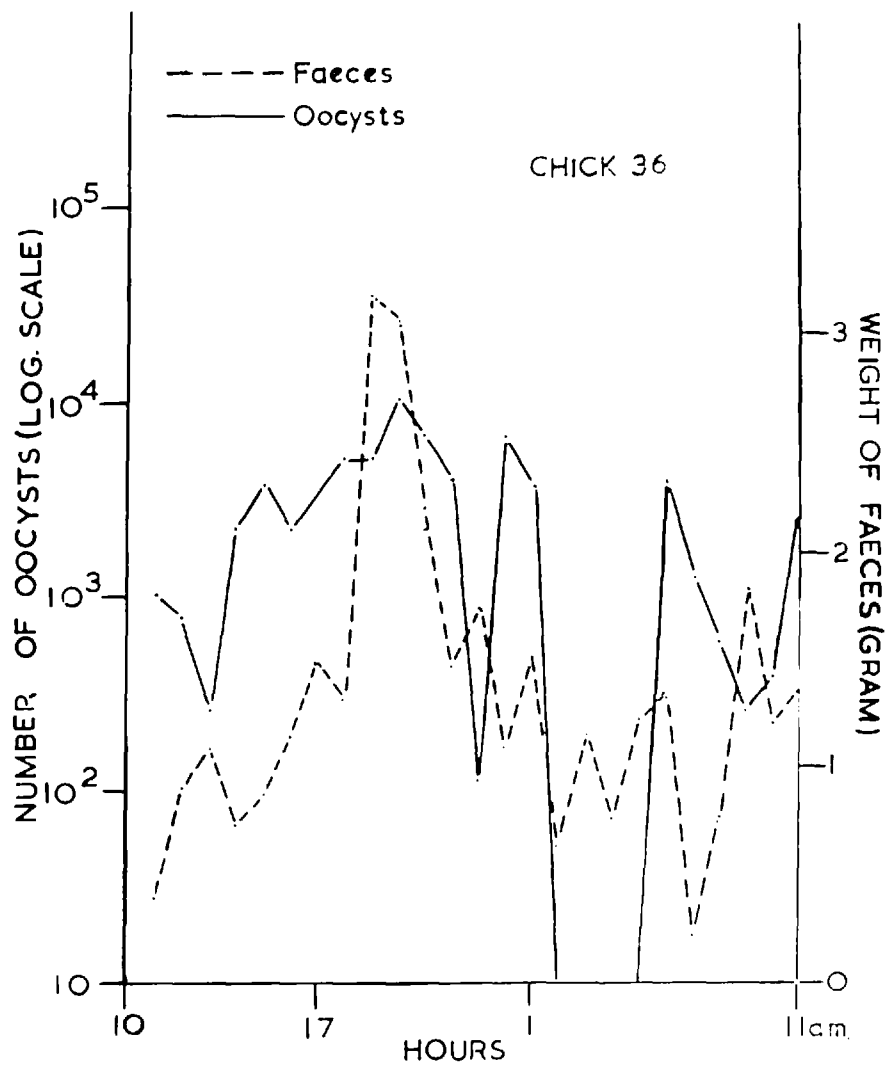
DIURNAL OUTPUT OF OOCYSTS OF EIMERIA PHASIANI

FIG 14

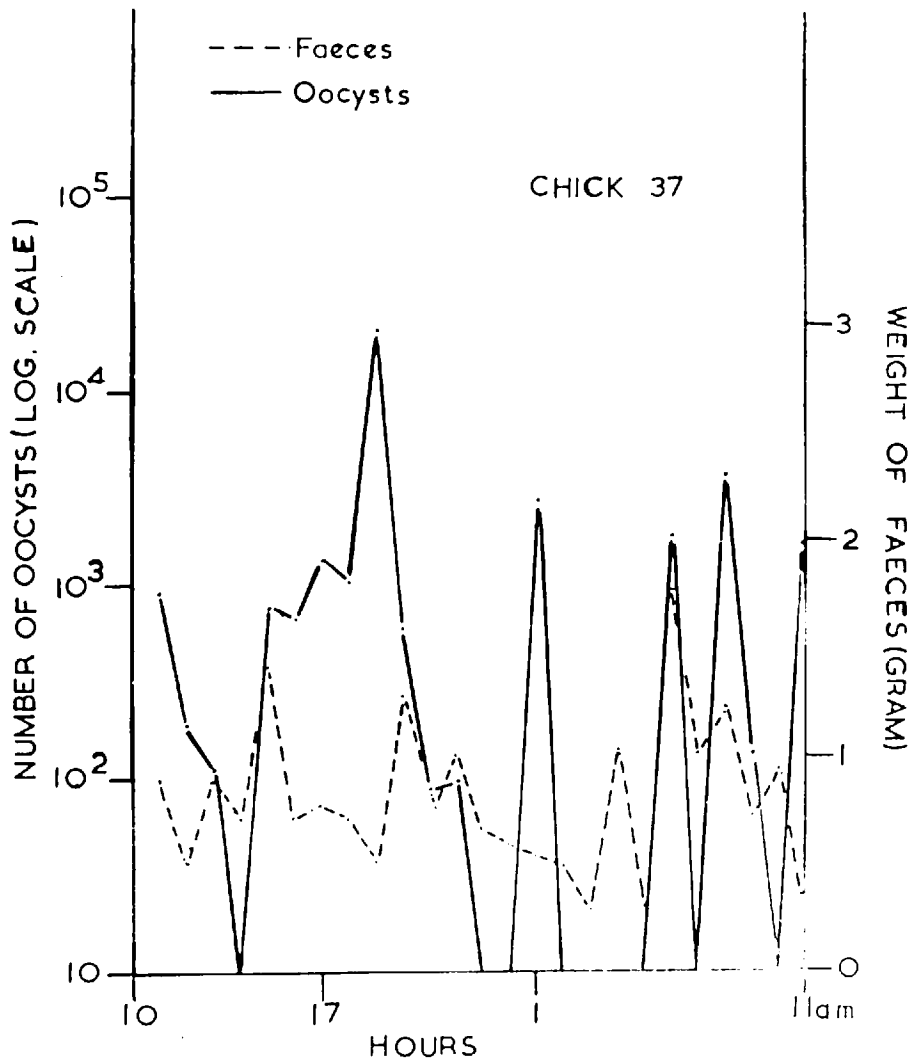
Diurnal output of oocysts of *Eimeria phasianis*



Number of oocysts per gram of faeces passed
per hour compared with total weight of faeces
passed per hour

FIG. 17

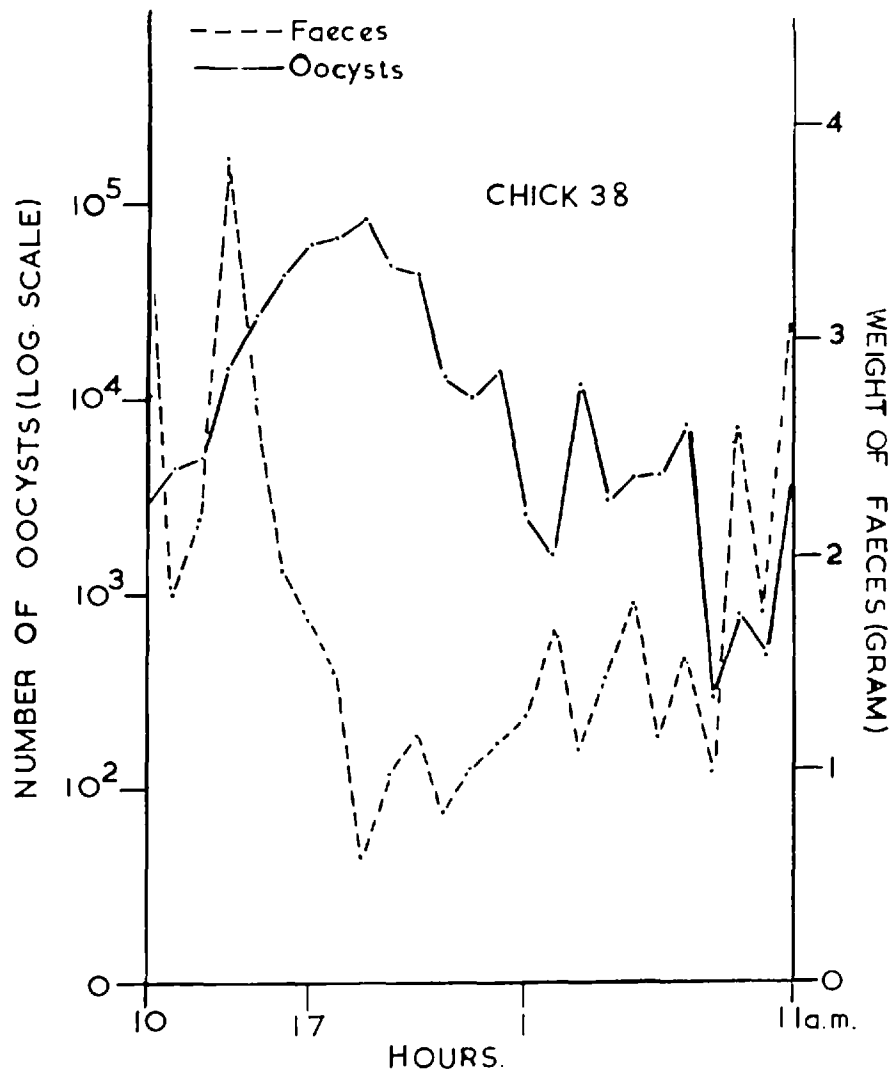
Diurnal output of oocysts of *Eimeria phasiani*



Number of oocysts per gram of faeces passed
per hour compared with total weight of faeces
passed per hour

FIG. 18

Diurnal output of oocysts of *Eimeria phasiani*



Number of oocysts per gram of faeces passed
per hours compared with total weight of faeces
passed per hour

FIG. 19

three groups during the period from 14.00 to 22.00 hours (Table 17) and the actual hourly peak of production was also similar (Fig. 20).

Table 17. Percentage Output of Oocysts during the Daily Period
14.00 - 22.00 hours.

<u>Day of</u> <u>Patent</u> <u>Period</u>	<u>Group 1</u> <u>Infected 18.00 hrs</u> <u>26.5.65</u>	<u>Group 2</u> <u>Infected 2.00 hrs</u> <u>27.5.65</u>	<u>Group 3</u> <u>Infected 10.00 hrs</u> <u>27.5.65</u>
1	95.33%	81.33%	
2	84.00%	85.19%	83.19%
3	75.57%	75.74%	70.89%

DIURNAL OOCYST OUTPUT OF GROUPS OF BIRDS INFECTED AT 8-HOUR INTERVALS WITH 10,000 OOCYSTS
OF E. PHASIANI

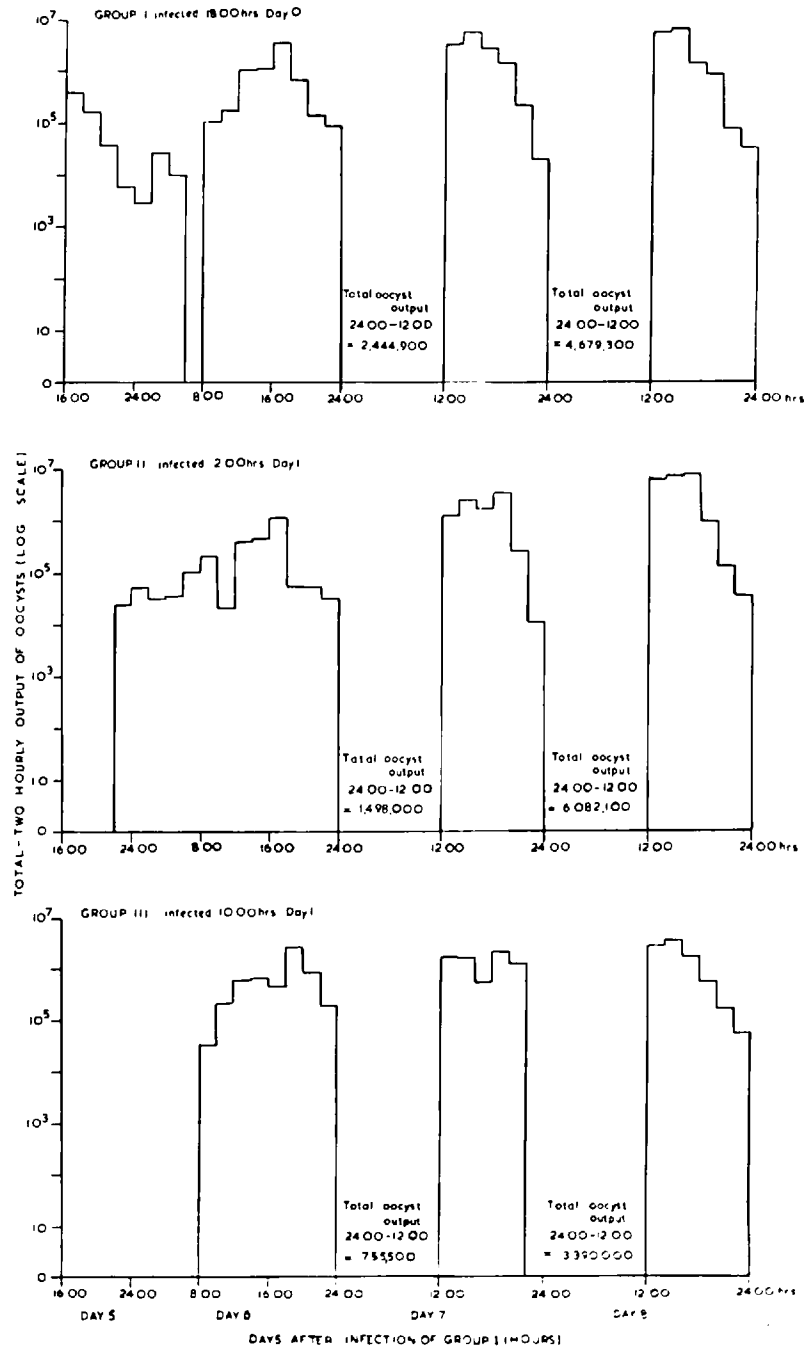


FIG. 20.

2. Studies on the Endogenous Stages.

(a) Localisation of the parasite.

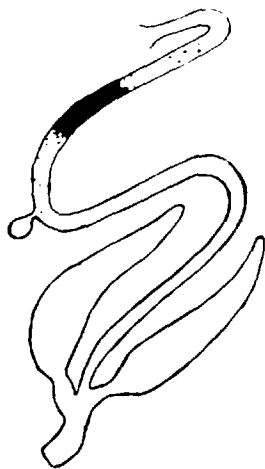
The whole life cycle was passed within the epithelial cells of the small intestine and caeca. The first asexual cycle was localised in a much smaller area than the second asexual cycle and there was a greater spread as the parasite underwent multiplication.

Thus, the first asexual cycle occurred in the first third of the small intestine, excluding the first half of the duodenum (Fig. 21a), the parasite being found mainly in the glands but also in the surface epithelium at the base of the villi. The second asexual cycle occurred in the epithelial cells covering the villi, extending for half to two thirds of the small intestine (Fig. 21b). The third asexual and the sexual cycles were found similarly distributed throughout the small intestine and additionally in the first half of the caeca; they were very rarely found in the rectum (Fig. 21c).

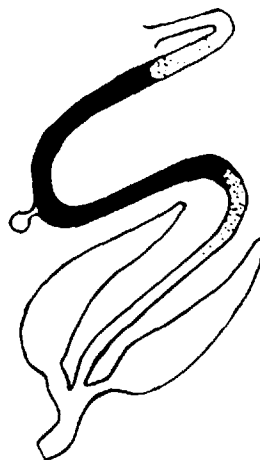
The stages of the second and third asexual cycles and the sexual cycle were found concentrated at the tips of the villi, the numbers present decreasing towards the base. Only on rare occasions were schizonts or gametocytes found in the glands.

(b) Sporozoites.

Free sporozoites were obtained after excystation, from the intestine of coccidia-free pheasants killed one hour after infection with 3×10^6 sporulated oocysts. Smears were examined fresh in normal saline

LOCALIZATION OF THE INTERNAL STAGES OF E. PHASIANI IN THE PHEASANT INTESTINE

a FIRST GENERATION SCHIZOGONY



b SECOND GENERATION SCHIZOGONY

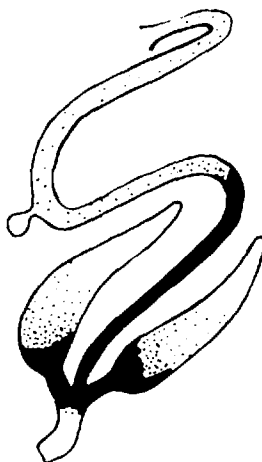
c THIRD GENERATION SCHIZOGONY &
SEXUAL CYCLE

FIG. 21

on a warm stage and also stained after fixation.

The sporozoites were slightly crescent-shaped, blunt at the posterior and pointed at the anterior ends, measuring $10.4 - 11.4 \mu \times 1.9 - 2.1 \mu$ (mean $10.8 \times 2.0 \mu$) (Fig. 22a). Each sporozoite had a large refractile globule at the posterior end which stained intense blue with Bromophenol blue, indicating the presence of protein (Fig. 22b). In Giemsa stained smears, the nucleus stained pink and the cytoplasm light blue with a few red granules at the anterior end (Fig. 22c).

Two types of movement were observed. Firstly, an elongation and contraction while remaining in one position and, secondly, a rapid gliding movement. When moving, a bulge appeared in the mid-length which was the position of the nucleus. This was confirmed in stained smears.

(c) First generation schizogony.

The sporozoites entered the host cells and developed inferior to the host cell nuclei. By 12 hours after infection, they had rounded off and usually contained a single nucleus (Fig. 22d). However, a few contained two nuclei. These trophozoites grew rapidly and commenced nuclear division, forming the first generation schizonts. By 24 hours after infection, the schizonts generally contained 4 to 8 nuclei (Fig. 22e) but an occasional one possessed as many as twenty. As the parasite grew it distended the host cell, pushing the host cell nucleus to one side and against the outer border of the cell. Thirty six hours after infection, nuclear division was almost complete, the schizonts possessing between

Explanation of Figure 22.

Camera lucida drawings showing the endogenous phases of E. phasiani Tyzzer 1929.

(All drawn from sections of intestine stained in Heidenhain's Iron Haematoxylin, except where stated).

- a. Sporozoite from fresh preparation.
- b. Sporozoite stained in Bromophenol blue.
- c. Sporozoite stained in Giemsa.
- d-f. Developmental stages of first generation schizont,
e.g. eosinophilic globule.
- g. Mature first generation schizont.
- h. First generation merozoite stained in Giemsa.
- i-j. Developmental stages of second generation schizont.
- k. Mature second generation schizont.
- l. Second generation merozoite stained in Giemsa.
- m-n. Developmental stages of third generation schizont.
- o, q. Mature third generation schizont.
- p. Third generation merozoite stained in Giemsa.
- r-t. Developmental stages of microgametocyte.
- u-w. Developmental stages of macrogamete.
- x. Oocyst within the host cell.

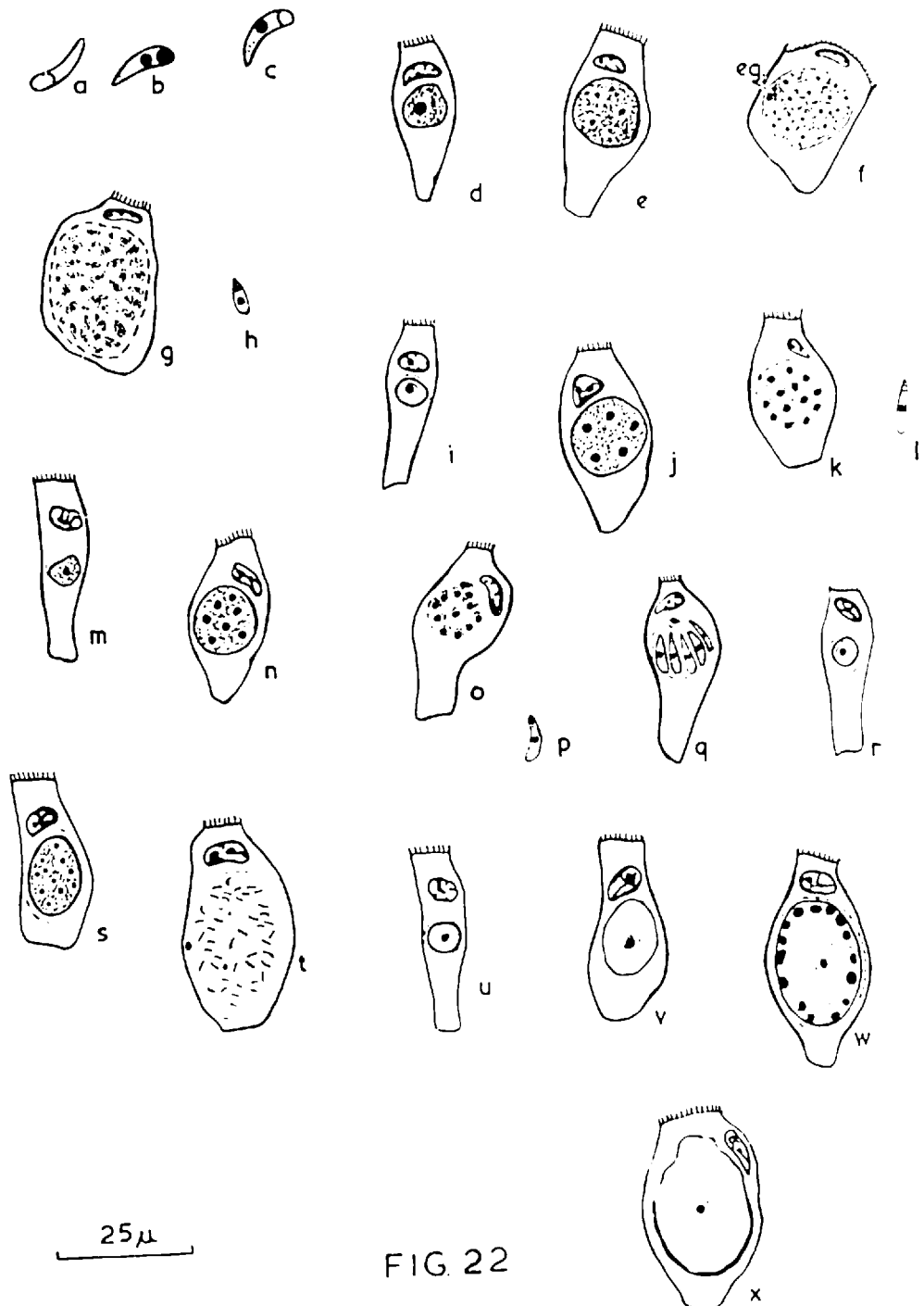


FIG. 22

50 and 100 nuclei (Fig. 22f). The nuclei of the schizonts consisted of a deeply staining karyosome, eccentrically placed in a light staining area which was surrounded by an unstained halo of nucleoplasm. This nuclear structure was similar in all stages of schizogony. An eosinophilic globule (e.g.) was present in all the developing stages of the first generation schizogony.

The cytoplasm of the schizonts became aggregated around the nuclei and 50-100 developing merozoites could be seen in a single schizont. The mature first generation schizonts, which measured $17.10 - 22.8 \mu \times 16.10 - 20.9 \mu$ (mean $20.9 \times 18.9 \mu$) and contained around 100 fully formed merozoites, were first observed at 48 hours after infection (Fig. 22g and Fig. 23). They could also be found as late as 60 hours after infection.

The first generation merozoites, measuring $4.5 \times 1.5 \mu$ (Fig. 22h), on examination on a warm stage were seen to move with a caterpillar-like motion. They progressed by elongating the anterior end, followed by a contraction of the posterior end. This motion was also observed for the second and third generation merozoites.

(d) Second generation schizogony.

The second generation schizogony was initiated by invasion of the epithelial cells by the first generation merozoites. These rounded off (Fig. 22i) and immediately commenced nuclear division. The second generation schizonts invariably developed inferior to the host cell nuclei, but in the instance of multiple infection of a single host cell this relation-

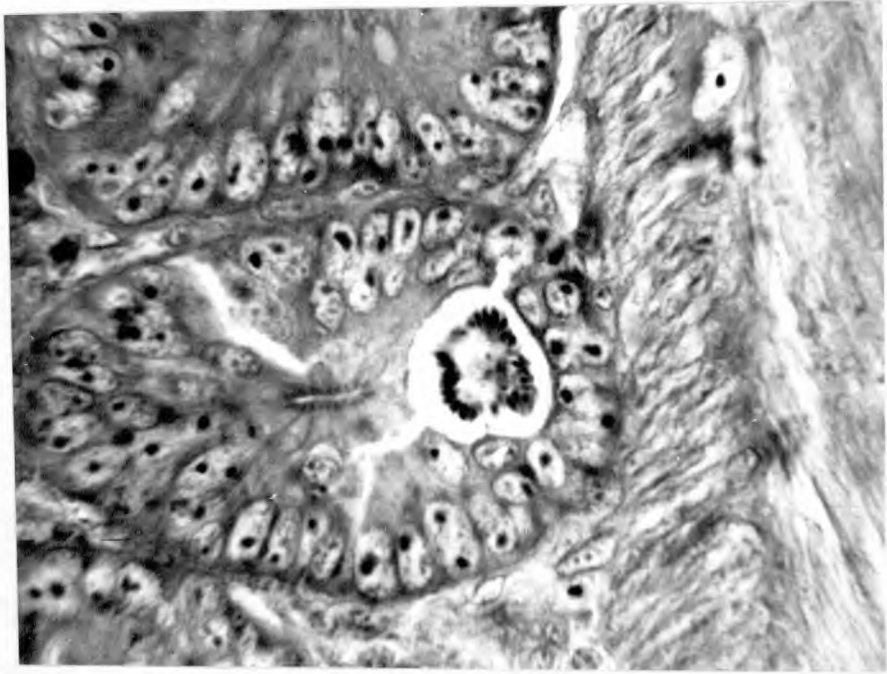


Fig. 23. First Generation Schizont stained in
Heidenhain's Iron Haematoxylin (X 1000)

ship was often disturbed. The schizonts grew and by 60 hours after infection contained 12 - 20 nuclei (Fig. 22j). No further nuclear division occurred and 12 - 20 merozoites were formed in a similar manner to those of the first generation schizonts. The mature schizont, measuring $8.6 - 11.4 \mu$ x $7.6 - 10.5 \mu$ (mean $9.8 \times 9.0 \mu$) (Fig. 22k and Fig. 24), was found by 66 hours after infection and the merozoites were released by 72 hours.

The second generation merozoites (Fig. 22l) which were larger than those of the first schizogony had a mean measurement of $7.4 \times 2.0 \mu$. In Giemsa stained smears the nucleus was near to the centre and numerous red granules were present at the pointed end.

(e) Third generation schizogony.

In most species of Eimeria the second generation of merozoites give rise to the sexual cycle but in this species a third asexual cycle occurred before any merozoites developed into gametocytes. Even as early as 72 hours after infection, trophozoites (Fig. 22m) were found in epithelial cells along the villi, lying inferior to the host cell nuclei. Multiple infections of cells were common. Division of the schizont progressed as in the other two asexual cycles and the mature schizont contained 12 - 16 merozoites (Fig. 22o and Fig. 25). The mature schizonts measured $7.1 - 8.5 \mu$ x $6.6 - 8.1 \mu$ (mean $7.7 \times 7.5 \mu$) and were observed as early as 96 hours after infection. They were also found alongside developing gametocytes at 108 hours and occasionally at 120 hours.

The third stage merozoites were smaller than those of the second

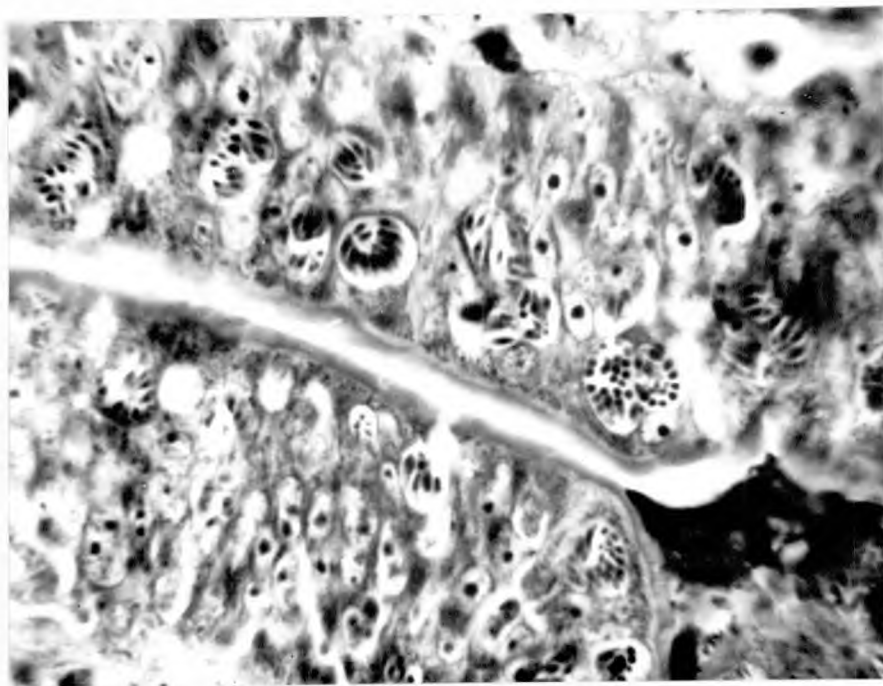


Fig. 24. Second Generation Schizont stained in
Heidenhain's Iron Haematoxylin (X1000)

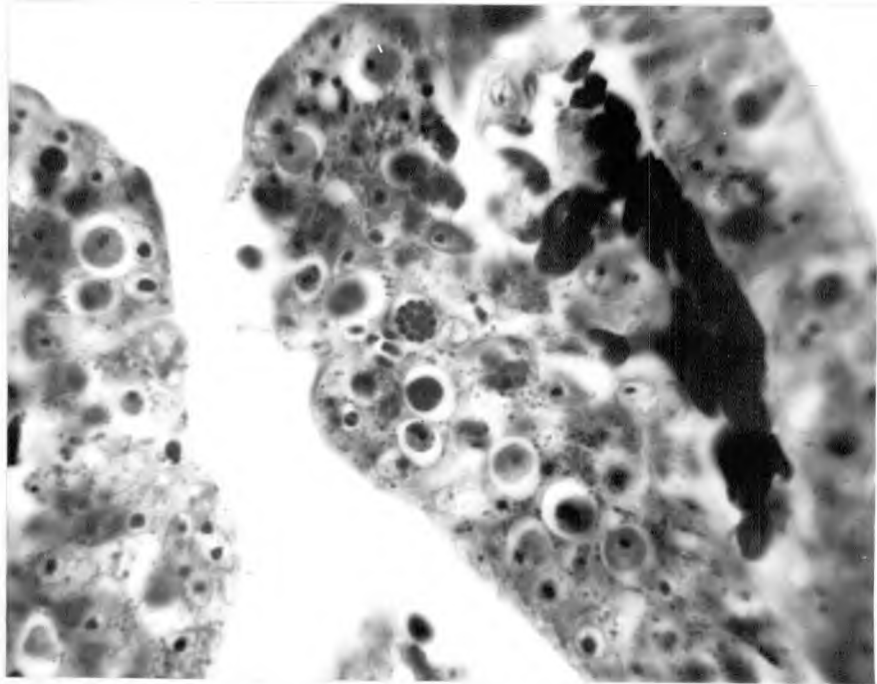


Fig. 25. Third Stage Schizont stained in
Heidenhain's Iron Haematoxylin (X 1000)

generation and measured $5.9 \times 2.0 \mu$ (Fig. 22p and Fig. 26).

The morphological differences between the second and third generation schizonts were only slight. To confirm the existence of the separate generations, merozoites were isolated from infected birds at 72 hours and 96 hours respectively and were administered per rectum to 14-day old coccidia-free pheasant chicks; two birds received merozoites isolated at 72 hours and another two received merozoites isolated at 96 hours. The birds infected with 72 hour-merozoites produced oocysts 48 hours later and those infected with 96 hour-merozoites produced oocysts 24 hours later.

The difference in time before passing of oocysts by the two groups of birds was due to the 72 hour-merozoites passing through another generation of schizogony before entering into gametogony.

(f) Gametogony.

The sexual cycle of the parasite followed invasion of the intestinal epithelium by the third generation merozoites and, again took place in a position inferior to the host cell nucleus. The merozoites rounded off and, at this point, were indistinguishable from young schizonts (Figs. 22r, u). They developed either into macrogametes with a single nucleus or microgametocytes with many nuclei, much smaller than those of schizonts.

(i) Microgametocytes.

The microgametocyte exhibited repeated nuclear division (Fig. 22s and Fig. 27) without cytoplasmic separation. The mature microgametocytes,

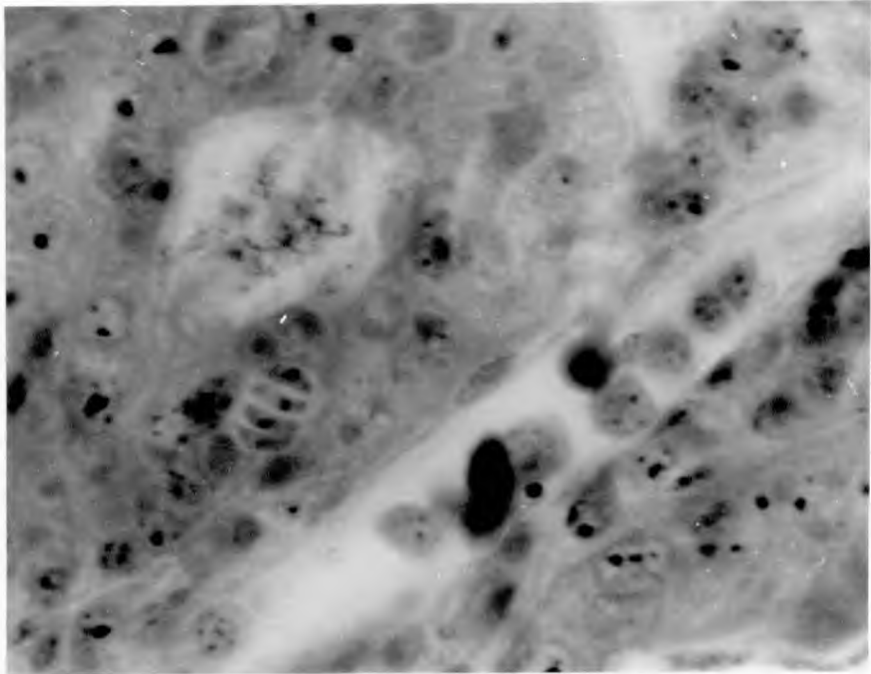


Fig. 26. Third Stage Schizont showing the Shape of the Merozoites, stained in Heidenhain's Iron Haematoxylin (X 1500)

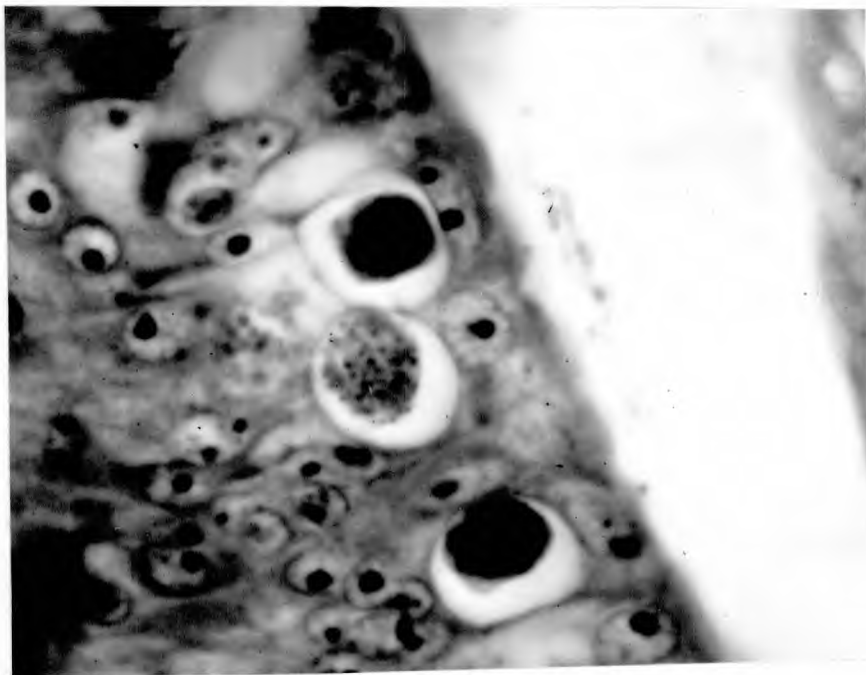


Fig. 27. Microgametocyte stained in Heidenhain's
Iron Haematoxylin (X 1600)

measuring $14.3 - 20.9 \mu \times 9.8 - 15.4 \mu$ (mean $15.6 \times 12.6 \mu$), were found by 120 hours after infection. The spindle-shaped microgametes, consisting mainly of nuclear material, were then separated off at the surface leaving a scattered residual body (Fig. 22t).

(ii) Macrogametes.

The macrogamete remained uninucleate during its development (Fig. 22 u-w) and grew rapidly in the host cell until it reached a maximum size of $16.1 - 22.3 \mu \times 12.3 - 15.5 \mu$ (mean $18.6 \times 13.9 \mu$) at 120 hours after infection (Fig. 22w and Fig. 28). The cytoplasm contained many large granules which stained densely black with Heidenhain's Iron Haematoxylin. These granules became localised at the periphery of the macrogamete and finally after fertilisation spread out to form the oocyst wall. The nucleus contained a large karyosome surrounded by an achromatic material. During the development, the karyosome decreased in size and was not always visible after fertilisation.

After the fertilisation of the macrogamete by a microgamete, the oocyst was formed in the host cell (Fig. 22x and Fig. 28) to be released later in the faeces.

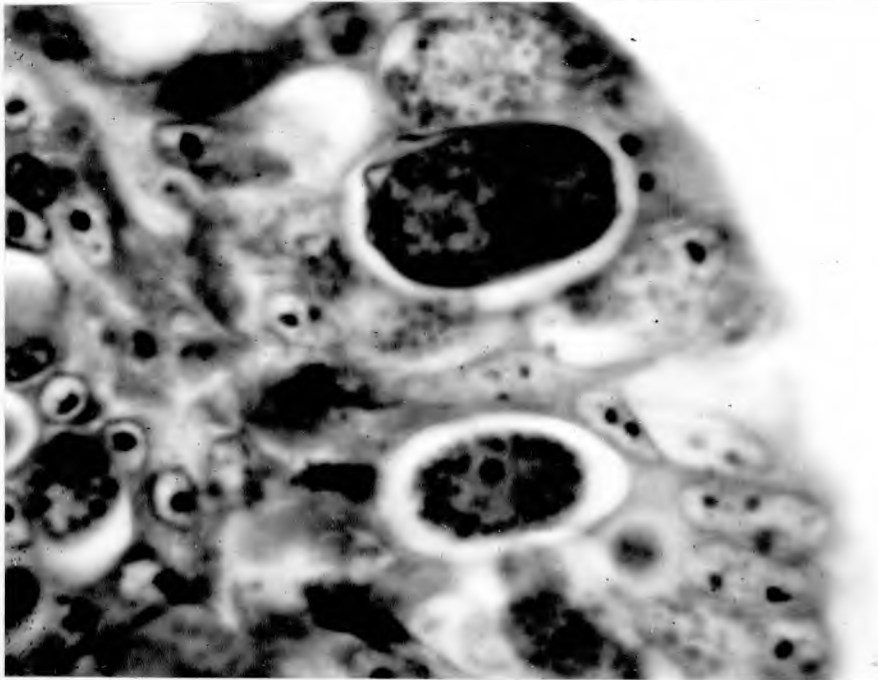


Fig. 28. Mature Macrogamete and Oocyst stained
in Heidenhain's Iron Haematoxylin (X 1600)

3. Pathogenicity.

(a) Symptoms and mortality.

E. phasiani may be fatal to chicks but the symptoms, including the mortality, vary greatly depending on the size of the infective dose. Table 18 shows the mortality rates of groups of chicks administered varying doses of sporulated oocysts.

Table 18. Effect of Size of Infective Dose on Mortality.

<u>Age of bird</u> <u>(weeks)</u>	<u>Dose</u>	<u>Mortality</u> *
2	10,000	0/8
3	10,000	0/5
3	25,000	0/8
2	100,000	3/6
3	100,000	2/5
2	500,000	5/5
3	1 x 10 ⁶	5/5

* numerator = number of birds dying: denominator = number of birds dosed.

In birds receiving 10,000 sporulated oocysts, few symptoms were shown although the faeces were very liquid. In birds administered 25,000 oocysts the symptoms were more marked. Little food was taken on the fourth and fifth days after infection leading to a reduction in the live weight gains as compared with uninfected controls (p. 88). Marked symptoms were observed in two and three week-old chicks given doses of 100,000 or more oocysts. They were normal for the first three days of infection but, as

before, on the fourth day they consumed less food than the uninfected birds, the feathers became ruffled and their gait unsteady. The faeces were very liquid, containing large amounts of mucus, and by the end of the fourth and during the fifth day they passed mucus tinged with small quantities of blood. The faeces also contained white mucus containing large numbers of third stage merozoites and cellular debris. Towards the end of the fifth day the liquid faeces contained many immature gametocytes, oocysts and much cellular debris. If the chicks survived this period the faeces gradually returned to normal during the patent period.

Approximately half the birds in groups infected with 100,000 oocysts died, the deaths occurring on the fifth day after infection. Birds of two and three weeks old, given doses of 500,000 or more oocysts all died, the deaths occurring late on the fourth day of infection, which coincided with the liberation of the third generation merozoites.

(b) The effect of the parasite on the live-weight gains of the host.

Apart from causing mortality in heavy infections, E. phasiani reduces the live-weight gains of chicks infected with smaller numbers of oocysts. Fifteen 3-week old poults of the same hatch, which had been kept under identical conditions were divided into three groups by random selection and were weighed daily for five days prior to and seventeen days after infection. Group I were uninfected controls, Group II were infected with 25,000 oocysts each and Group III with 100,000 oocysts each. Food and water were given ad lib. and the birds were kept in the same room, thus

ensuring constant conditions.

The control group remained uninfected from the day of hatching until the end of the experiment.

As the range in weights of the groups was wide, the mean weights of the groups were compared statistically, using the t-test, at five days and seventeen days after infection. The results are summarised in Table 19 and Fig. 29.

Table 19. The Effect of Infection on the Live-Weight Gains (grammes) of the Host.

Group	Day of Infection		5 Days after Infection		17 Days after Infection	
	Mean wt.	Range	Mean wt.	Range	Mean wt.	Range
I	131.0	119-146	174.8	145-207	283.2	203-324
II	133.9	124-148	143.6 t: 2.60	134-170 P < 0.05*	237.9 t: 1.57	183-304 P < 0.20*
III	131.6	121-150	136.1 t: 3.47	129-143 P < 0.01*	+	

* Significant difference from control group at the same age.

+ Figures not included due to the death of two birds at five days after infection.

On the day of infection the mean weights of the three groups were practically identical and continued to rise at a comparable rate for the first three days after infection. On the fourth day after infection the

EFFECT OF INFECTION ON LIVE-WEIGHT GAINS OF THE HOST

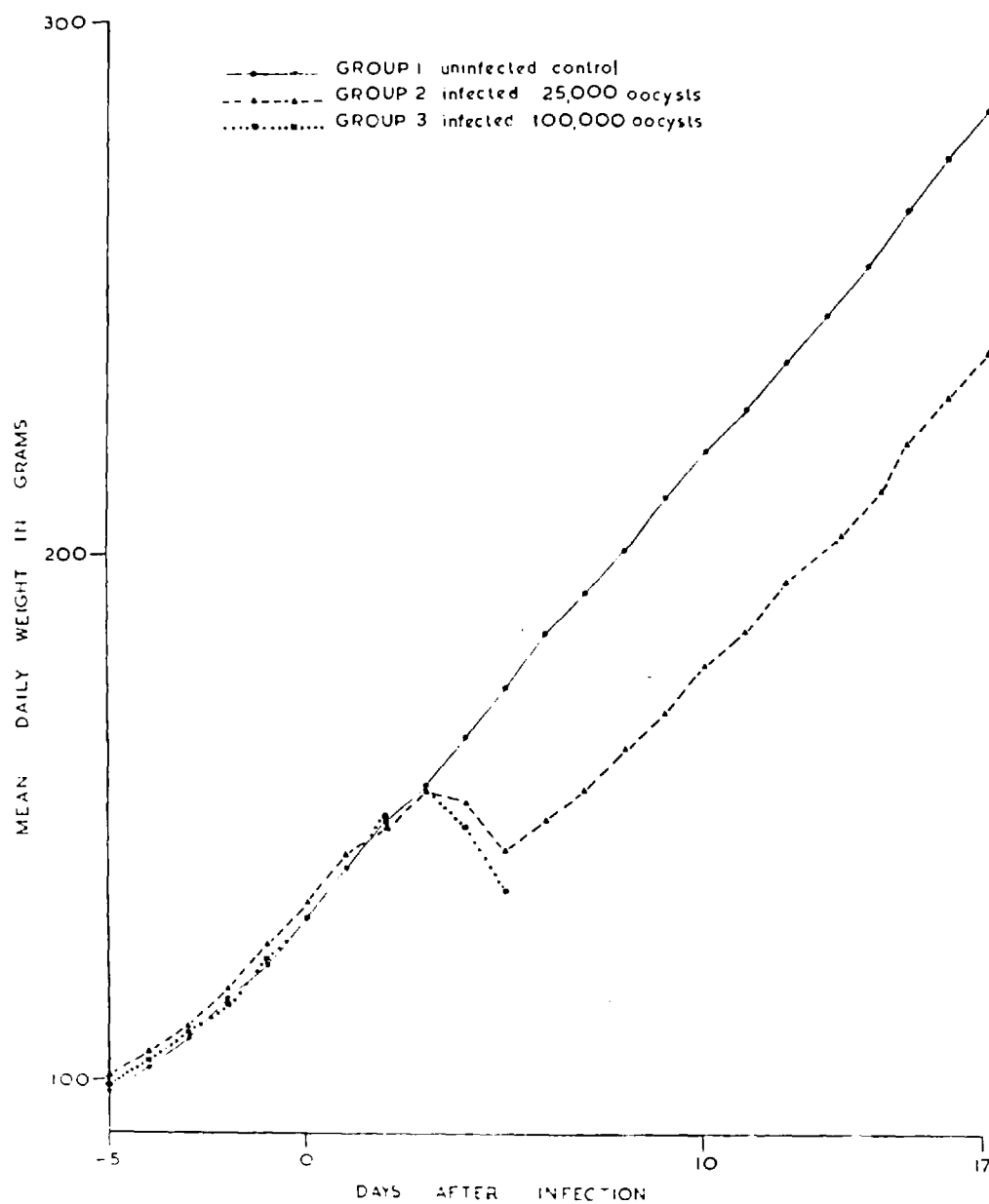


FIG 29

live-weight gains of the infected groups dropped slightly and to a greater extent on the fifth. The drops in weight were larger in the more heavily infected group. The differences in mean weights of the infected groups compared with the controls were significant at the $< 5\%$ level in Group II and $< 1\%$ in Group III. In Group III, given 100,000 oocysts, the two lighter birds died on the fifth day of infection and so this group could not be used in the statistical analysis after the fifth day.

After the fifth day of infection, the infected birds began to gain in weight and by the end of the infection the heaviest bird in Group II was nearly as heavy as the heaviest control bird. Although the mean weight of Group II was 45.3 gm. less than the control after seventeen days, it was only significant at the $< 20\%$ level due to the wide range of weights.

This shows the infected birds quickly regain their lost weight if re-infection is prevented and that an attack of coccidiosis produces groups of birds of uneven weights.

(c) Macroscopical pathology.

In chicks infected with 100,000 or more oocysts the appearance of the intestine was normal until the fourth day after infection when, owing to cessation of feeding, it became empty and flattened. By the end of the fourth day the intestine showed a haemorrhagic condition with petechial haemorrhages visible from both the mucosal and serosal surfaces. These haemorrhages were present in the upper half or two thirds of the small intestine. The mesenteric blood vessels of the intestine were

enlarged and the wall of the intestine was thickened and oedematous in the area of the haemorrhagy. The lumen was filled throughout its length with a mixture of mucus streaked with blood, third stage merozoites and much cellular debris. During the fifth day the intestine also contained immature gametocytes in the mucus and the terminal thirds of the caeca were slightly congested with orange clots, which contained immature gametocytes, third generation merozoites, blood cells and cellular debris. The small intestine was the site of all the three asexual cycles whilst the caeca contained only part of the third asexual cycle and part of the sexual cycle and so it was not surprising that the caeca were in a better condition than the small intestine.

When deaths occurred the birds were all in varying degrees of emaciation and the skin was firmly adherent to the underlying tissue. The heart, liver, kidneys, lungs and spleen were normal, the intestine being the only organ to show abnormalities.

Three-week old chicks given 25,000 oocysts showed the above characteristics in a milder form but in chicks administered only 10,000 oocysts and killed five days after infection there was negligible haemorrhage and the intestine contained white mucus with oocysts. Eight to nine days after infection with 10,000 oocysts the intestines were normal.

(d) Microscopical pathology.

(i) Histology of the normal intestine.

The normal histology of the intestine was studied in two 10-day

old coccidia-free pheasant chicks, sections being taken from the sites shown in Fig. 2.

The wall of the small intestine comprised the following layers:

- 1) an outer serosa;
- 2) a thin layer of longitudinal muscle and a much thicker inner layer of circular muscle between which were blood vessels, lymphatics and a nerve plexus;
- 3) a poorly developed submucosal layer; and
- 4) a well developed mucosa with projecting villi and intestinal glands occupying the whole of the lamina propria.

The villi of the small intestine were long, varying only slightly in length throughout the intestine, the duodenal villi being slightly longer. The villi were composed of connective tissue covered by an epithelium of simple columnar cells with prominent brush borders and many goblet cells. The crypts of Lieberkühn were shallow relative to the long villi and were composed of columnar cells with very few goblet cells. Mitoses in the intestine were confined to the short crypts where they occurred, mainly at the base.

The caeca were blind ending tubes arising at the junction of the small intestine and rectum. Both caeca were alike and consisted of three recognisably different regions:

- 1) a narrow isthmus arising from the ileo-rectal junction. This region had walls thickened by well developed muscle layers, and a narrow lumen. The mucosa was arranged in large folds, with smaller subsidiary ones

- on the main folds. A large amount of lymphatic tissue was present;
- 2) a mid-portion with a larger lumen and thinner walls. The mucosa was arranged in large folds, forming broader ones than in the isthmus, and without subsidiary folds; and
 - 3) a wide blind apex similar in structure to the mid-portion but the folds of the mucosa were smaller.

There was no brush border on the epithelium of the caeca. Mucosal glands were present throughout the length of the caeca and contained more goblet cells than the epithelium of the mucosal folds.

No eosinophil leucocytes were seen in sections of any part of the intestine.

(ii) Infected tissue.

The host reacts immediately to the invasion of the intestine by E. phasiani by a rapid infiltration of large numbers of eosinophil leucocytes into the intestinal wall. Eosinophils which were not found in the intestine of coccidia-free pheasant chicks were detected in chicks which had received 3×10^6 oocysts as early as 2 hours after infection. They were present in the submucosa and even between the epithelial cells of the mucosa. They are found throughout the intestine, from the duodenum to the rectum, and not only in the area parasitised. This eosinophilia of the intestine was present throughout the prepatent period and as late as ten days after infection, which was the latest time examined for microscopical changes. Birds infected with both second and third generation

merozoites per rectum also exhibited this phenomenon.

No other changes were observed until four days after infection. The oedematous nature of the first half to two thirds of the small intestine was confirmed by the observation that the muscle fibres of the intestinal wall were separated to form spaces distended with fluid. This oedema was present for about seven days. The epithelial cells were cubical rather than columnar and were packed with schizonts. In some places the tips of the villi were broken off, releasing merozoites, cellular debris and blood cells into the lumen. By 108 hours after infection, the blood vessels were engorged, especially those of the serosa and there was ^{an} increase in the cellularity, particularly of eosinophils, of the mucosa.

After the release of the third generation merozoites the epithelial cells were rapidly replaced and the only pathological changes at five days were the increased vascularity, the increased cellularity of the sub-mucosa and the oedematous nature of the intestine. The lumen was packed with oocysts and cellular debris.

In birds given only small doses of oocysts, the recovery of the intestine was rapid so that by seven days there was little or no increased vascularity. At eight to ten days after infection the intestine was normal except for the presence of small numbers of gametocytes and an eosinophilia.

(c) Haematology.

The infected chicks reacted rapidly to E. phasiani by the

infiltration of the intestine by eosinophil leucocytes. Experiments were, therefore, carried out to see whether this was reflected in the circulatory blood constituents.

Blood was taken one day before and six days after infection from the wings of two birds infected with 25,000 oocysts and two infected with 100,000 oocysts. These counts were compared with those from two uninfected control chicks of the same age. Total erythrocyte, total leucocyte and differential leucocyte counts were made and the results are summarised in Tables 20 and 21, which also give the group means ($\bar{mT}$) and the total daily means ($\bar{mD}$).

Table 20. Total Erythrocyte and Total Leucocyte Counts of Infected Birds During Prepatent Period.

(a) Total erythrocytes per mm³ - all values x 10⁶.

Day	Uninfected Control	25,000 oocysts	100,000 oocysts	$\bar{mD}$
-1	2.98	3.01	2.89	2.96
0	2.92	3.01	2.90	2.94
1	2.69	2.77	2.81	2.76
2	2.81	2.82	2.80	2.81
3	2.88	2.92	2.89	2.90
4	2.90	2.91	2.87	2.89
5	2.92	2.86	2.83	2.87
6	3.00	2.83	2.84	2.89
$\bar{mT}$	2.89	2.89	2.85	2.88

(b) Total leucocytes per mm³ - all values x 10⁴.

Day	Uninfected Control	25,000 oocysts	100,000 oocysts	$\bar{mD}$
-1	2.00	2.67	3.00	2.56
0	1.67	2.67	2.00	2.11
1	2.00	2.00	1.67	1.89
2	3.00	2.00	2.34	2.45
3	2.67	2.00	2.34	2.34
4	2.34	2.00	2.34	2.23
5	2.67	2.00	2.34	2.34
6	2.34	2.34	2.00	2.23
$\bar{mT}$	2.34	2.21	2.25	2.27

Table 21. Differential Leucocyte Counts as Percentages of Total Count of Infected Birds during Prepatent Period.

(a) Lymphocytes.

Day	Uninfected Controls	25,000 oocysts	100,000 oocysts	$\bar{mD}$
-1	32	40	41	37.7
0	40	33	44	39.0
1	34	43	34	37.0
2	48	32	44	41.0
3	40	49	43	44.0
4	43	42	40	41.7
5	40	40	36	38.7
6	43	45	42	40.0
$\bar{mT}$	39.9	40.5	40.5	39.9

(b) Monocytes.

Day	Uninfected Controls	25,000 oocysts	100,000 oocysts	$\bar{mD}$
-1	9	6	8	7.7
0	6	8	6	6.7
1	5	8	8	7.0
2	4	9	6	6.3
3	8	6	7	7.0
4	9	8	10	9.0
5	5	5	4	4.7
6	10	7	8	8.3
$\bar{mT}$	7	7	7	7.1

(c) Neutrophils.

Day	Uninfected Controls	25,000 oocysts	100,000 oocysts	$\bar{mD}$
-1	48	46	49	47.7
0	49	47	45	47.0
1	50	40	48	46.0
2	44	48	45	45.7
3	50	40	40	43.3
4	40	43	45	42.7
5	46	50	48	48.0
6	41	46	40	42.3
$\bar{mT}$	46.0	45.0	45.0	45.3

(d) Eosinophils.

Day	Uninfected Controls	25,000 oocysts	100,000 oocysts	$\bar{mD}$
-1	2	1	1	1.3
0	1	1	1	1.0
1	2	1	2	1.7
2	1	1	1	1.0
3	0	1	1	0.7
4	2	2	1	1.7
5	1	1	2	1.3
6	2	2	2	2.0
$\bar{mT}$	1.4	1.3	1.4	1.4

(e) Basophils.

Day	Uninfected Controls	25,000 oocysts	100,000 oocysts	$\bar{mD}$
-1	9	7	1	5.7
0	4	11	4	6.3
1	9	8	9	8.7
2	4	10	4	6.0
3	2	4	9	5.0
4	6	5	4	5.0
5	7	4	10	7.0
6	4	10	8	7.3
$\bar{mT}$	5.8	7.3	6.3	6.4

At no time during the infection were significant differences noted in the blood picture although there was variation in all three groups under investigation.

4. Strains B2 and B3.

All the work on the life cycle of E. phasiani was performed with oocysts derived from the single oocyst strain B1. Oocysts of strains B2 and B3 were morphologically identical to those of B1 but, in order to be certain that they were of the same species, cross immunity tests were performed.

Nothing is known on the immunising power of E. phasiani and so the choice of the number of oocysts was based on a comparison with the work of Rose and Long (1962) on the coccidia of chickens.

Four groups of five 10-day old pheasant chicks were immunised against strain B1 by giving to each group three successively increasing doses of oocysts. Five hundred oocysts were given the first week and subsequent doses of 5,000 and 50,000 oocysts were given to each group at weekly intervals.

Five weeks after the initial infection, when only a few oocysts were being passed in the faeces, all the groups were challenged with 50,000 oocysts of strain B1. No increase in the oocyst output was noted and it was considered that the birds were immune to strain B1.

Two of the groups were then challenged with 50,000 oocysts of strain B2 and the other two with 50,000 oocysts of strain B3. Again no

oocyst production was noted after these challenges and so it was considered that strains B2 and B3 were identical to strain B1 and were, therefore, of the same species - E. phasiani.

A check was made to be sure that the oocysts of the second challenge, that is by B2 and B3, were still viable by administering 50,000 oocysts from the same culture to three 2-week old pheasants. All three showed symptoms of the disease and first passed oocysts on the fifth day after infection.

It is possible that the birds used in this immunising schedule had their intestines damaged by the successive infections so much that, by the time of the second challenge, they were too damaged to support a further infection. The only way of testing this was by infecting a group of "immunised" birds with another species but a second viable species from the pheasant was not available. The work of Rose and Long, using similar immunising schedules at similar time intervals with the pathogenic species of chicken coccidia showed no such tissue damage.

5. Cross Infection Experiments.

- (a) An attempt to infect the common partridge (Perdix perdix) and the domestic fowl (Gallus domesticus) with E. phasiani.

Five 8-day old partridge chicks were infected per os each with 150,000 sporulated oocysts of E. phasiani. The faeces were examined for a period of 26 days after infection but no oocysts were present. One bird

died on the tenth day after receiving the infective dose and showed no signs of infection in sections, smears or faeces.

A further five 8-day old partridge chicks were given 3×10^6 sporulated oocysts and three were killed at one hour and two killed at two hours after infection. The ~~infection~~^{intestine} contained a few intact oocysts, empty oocysts, free intact sporocysts and many liberated sporozoites. Free sporozoites were found throughout the intestine an hour after infection but they did not move when placed on a warm stage and appeared to be dead or dying. Sections and smears were made of the duodenum and small intestine but no developing stages of the parasite were observed.

A similar experiment was carried out with five 5-day old coccidia-free White Link domestic fowl chicks, receiving 150,000 oocysts of E. phasiani and with five others receiving 3×10^6 oocysts. No oocysts were passed by the first group during the examination of faeces over 21 days. Sporozoites were observed to excyst in the small intestine of the second group but no sign of development of the parasite in the intestine was observed.

A control group of five 10-day old coccidia-free pheasant chicks was infected with 150,000 oocysts from the same culture of E. phasiani. Three of these birds died of coccidiosis on the fifth day of infection and the other two passed oocysts in the faeces for twelve days. Therefore, the culture used in this experiment was viable.

It was, therefore, concluded that partridge chicks and fowl chicks were not susceptible to infection with E. phasiani.

(b) An attempt to infect the pheasant (*Phasianus colchicus*) with *E. tenella*.

E. tenella has been described from the pheasant by Haase (1939) and Muroma (1951). Further Clapham (1950) has suggested, without experimentation, that it might infect the pheasant. However, in the absence of cross infection experiments, these results are viewed with suspicion. It was, therefore, decided to attempt to infect the pheasant with *E. tenella*.

Six 3-week old coccidia-free pheasant poults were infected per os with 400,000 sporulated oocysts of the Houghton strain of *E. tenella*. The faeces were examined for 20 days after infection but no oocysts were passed.

Two 10-day old coccidia-free pheasant chicks were each infected with doses of 3×10^6 oocysts and were killed respectively one hour and two hours after infection. At one hour, excystation from the oocysts was seen to have taken place and many free sporocysts were found. Free sporozoites were found at both one and two hours after infection. At one hour a few had deteriorated in their activity and by two hours they had all ceased to show movement and were moribund.

Smears and sections were made from the small intestine and caeca but no sign of infection was observed.

A control group of four 5-day old coccidia-free White Link domestic fowl chicks were infected with 400,000 oocysts of the same culture of *E. tenella* as used above and all the birds died between the sixth and seventh days from acute caecal coccidiosis. Therefore, as the strain was still viable, it was concluded that pheasant chicks are not susceptible to

E. tenella.

- (c) An attempt to infect the pheasant and the domestic fowl with an Eimeria sp. from the red grouse (Lagopus scoticus).

An attempt was made to infect five 6-day old fowl chicks and five 10-day old pheasant chicks with an Eimeria species from the red grouse (Lagopus scoticus). This species is incompletely described (Appendix p.154-7) and so a specific name is not given.

Each bird was given 1,000 sporulated oocysts per os but none showed oocysts in the faeces during examination on the succeeding 21 days. Insufficient oocysts were available to determine if excystation could occur. It is unlikely that this species from the grouse can infect chickens or pheasants. No control of the viability of the oocysts used was possible because grouse chicks were not available and final proof of its inability to infect chickens and pheasants cannot be given.

6. Remarks on the In Vivo Excystation of Oocysts.

The process of excystation of E. phasiani and E. tenella was observed during the preceding experiments and the following facts emerged. The contents of the crop, gizzard and intestine were examined in normal saline.

The oocysts of E. phasiani and E. tenella remained unchanged whilst in the crop. The sporocysts were liberated from the oocysts whilst the latter were passing through the gizzard. The sporozoites were activated

and escaped from the liberated sporocysts only after they had reached the small intestine. Free sporozoites of E. phasiani were found in the duodenum as early as 20 minutes after ingestion of oocysts and by one hour after ingestion they were found as far down as the caecum. All empty sporocysts were undamaged except for the absence of the Steida body which indicates that the sporozoites must have escaped through the hole left at the apex of the sporocyst. In intact oocysts the sporozoites were not activated.

In attempts to infect pheasant poults with E. tenella, an hour after infection, free sporozoites were found mainly at the level of the yolk stalk and below, including the caecum. Most of the sporozoites in the duodenum were inactive and remained within the sporocyst, only a few free sporozoites were found at this level. This is in direct contrast to E. phasiani where the sporozoites are mainly liberated in the region of the duodenum. The majority of free sporozoites appeared moribund by two hours after infection.

7. Frequency of Occurrence of Coccidiosis in the Wild Adult Pheasant.

The frequency of occurrence of coccidiosis in the wild bird was determined by examination of seventy adult pheasants, collected in the field during the shooting season 1st October 1962 to 1st February 1963. Whenever possible smears and sections of the intestinal wall were examined for coccidial stages as well as faeces for oocysts. But out of seventy birds only nine were caught alive. The other birds, mostly shot on the various

estates listed in Tables 22, 23 and 24 had been dead for some time. In these cases the intestines were unsuitable for sectioning and the only guide to the incidence of coccidiosis was the presence of oocysts in the intestinal contents. A true picture of the incidence and degree of infection can only be obtained by examining live birds over a period of time, as they exhibit a diurnal periodicity in the output of oocysts.

Table 22a gives the combined frequency of distribution of two species found in the pheasant from six known localities. The true locality and sex of the birds listed under the name "MacFisheries" are unknown as these were intestines obtained from a shop owned by the MacFisheries Food Company. Table 22b gives the combined monthly incidence of the two species and Table 22c the frequency in the two sexes. Tables 23 and 24 give the frequency of distribution with respect to locality, month and sex, of E. phasiani and Eimeria sp., described in the Appendix (pp.149-153).

It can be seen that coccidiosis was very common in the pheasant, being found in 64.3% of the birds examined. The two species, E. phasiani and Eimeria sp. were equally prevalent, being found respectively in 60.0% and 55.7% of the birds. Mixed infections were found in all but nine infected birds, of these, six exhibited a pure infection of E. phasiani and three of Eimeria sp.

Coccidiosis of the pheasant was common in all the localities examined. E. phasiani was found in birds from Berkshire, Hampshire, Norfolk and Staffordshire and Eimeria sp. from Berkshire and Hampshire.

From the above figures there appears to be no monthly variation

Table 22. Survey of the Incidence of Coccidial Infection in the Wild Adult Pheasant.

(a) Rates of Infection in Different Localities.

Locality	No. Infected	% Infected	No. Uninfected	Total Examined
Sunninghill Park, Berkshire	3	100	0	3
Silwood Park, Berkshire	4	66.6	2	6
Windsor Great Pk, Berkshire	1	33.3	2	3
Burton upon Trent, Staffs.	1	50.0	1	2
Sutton Scotney, Hants.	18	64.3	10	28
MacFisheries	17	63.0	10	27
Norfolk	1	100	0	1
Total	45	64.3	25	70

(b) Monthly Incidence.

Month	No. Infected	% Infected	No. Uninfected	Total Examined
October 1962	3	100	0	3
November 1962	9	60	6	15
December 1962	20	66.6	10	30
January 1963	11	68.75	5	16
February 1963	2	33.3	4	6
Total	45		25	70

(c) Sex of Bird.

Sex	No. Infected	% Infected	No. Uninfected	Total Examined
Male	11	57.9	8	19
Female	18	75.0	6	24
Total	29		14	43

Table 23. Survey of the Incidence of *Eimeria phasiani* in the WildAdult Pheasant.(a) Localities.

Locality	No. Infected	% Infected	No. Uninfected	Total Examined
Sunninghill Park, Berkshire	3	100	0	3
Silwood Park, Berkshire	4	66.6	2	6
Windsor Great Pk, Berkshire	1	33.3	2	3
Burton upon Trent, Staffs.	1	50	1	2
Sutton Scotney, Hants.	17	60.7	11	28
MacFisheries	15	55.6	12	27
Norfolk	1	100	0	1
Total	42	60	28	70

(b) Monthly Incidence:

Month	No. Infected	% Infected	No. Uninfected	Total Examined
October 1962	3	100	0	3
November 1962	8	53.3	7	15
December 1962	19	63.3	21	30
January 1963	10	62.5	6	16
February 1963	2	33.3	4	6
Total	42		28	70

(c) Sex of Bird.

Sex	No. Infected	% Infected	No. Uninfected	Total Examined
Male	11	57.9	8	19
Female	16	75.0	6	24
Total	29		14	43

Table 24. Survey of the Incidence of Eimeria sp. in the Wild Adult Pheasant.

(a) Localities.

Locality	No. Infected	% Infected	No. Uninfected	Total Examined
Sunninghill Park, Berkshire	3	100	0	3
Silwood Park, Berkshire	4	66.6	2	6
Windsor Great Pk, Berkshire	1	33.3	2	3
Burton upon Trent, Staffs.	0	0	2	2
Sutton Scotney, Hants.	15	53.6	13	28
MacFisheries	16	59.3	11	27
Norfolk	0	0	1	1
Total	39	55.7	31	70

(b) Monthly Incidence.

Month	No. Infected	% Infected	No. Uninfected	Total Examined
October 1962	3	100	0	3
November 1962	8	53.3	7	15
December 1962	17	56.6	13	30
January 1963	9	56.25	7	16
February 1963	2	33.3	4	6
Total	39		31	70

(c) Sex of Bird.

Sex	No. Infected	% Infected	No. Uninfected	Total Examined
Male	11	57.9	8	19
Female	16	66.6	8	24
Total	27		16	43

in the frequency of any of the species during the shooting season 1962/3.

Little significance is attached to the differing frequencies of coccidiosis in the two sexes because of the small numbers examined.

8. Degree of Infection of *Eimeria phasiani* in the Wild Adult Pheasant.

In all wild adult birds examined alive, the infection was light and no deaths due to coccidiosis were recorded. The birds appeared to be of good weight and showed no outward symptoms of disease. At autopsy the intestine showed no macroscopic or microscopic pathological change, except that in sections an eosinophilia was observed.

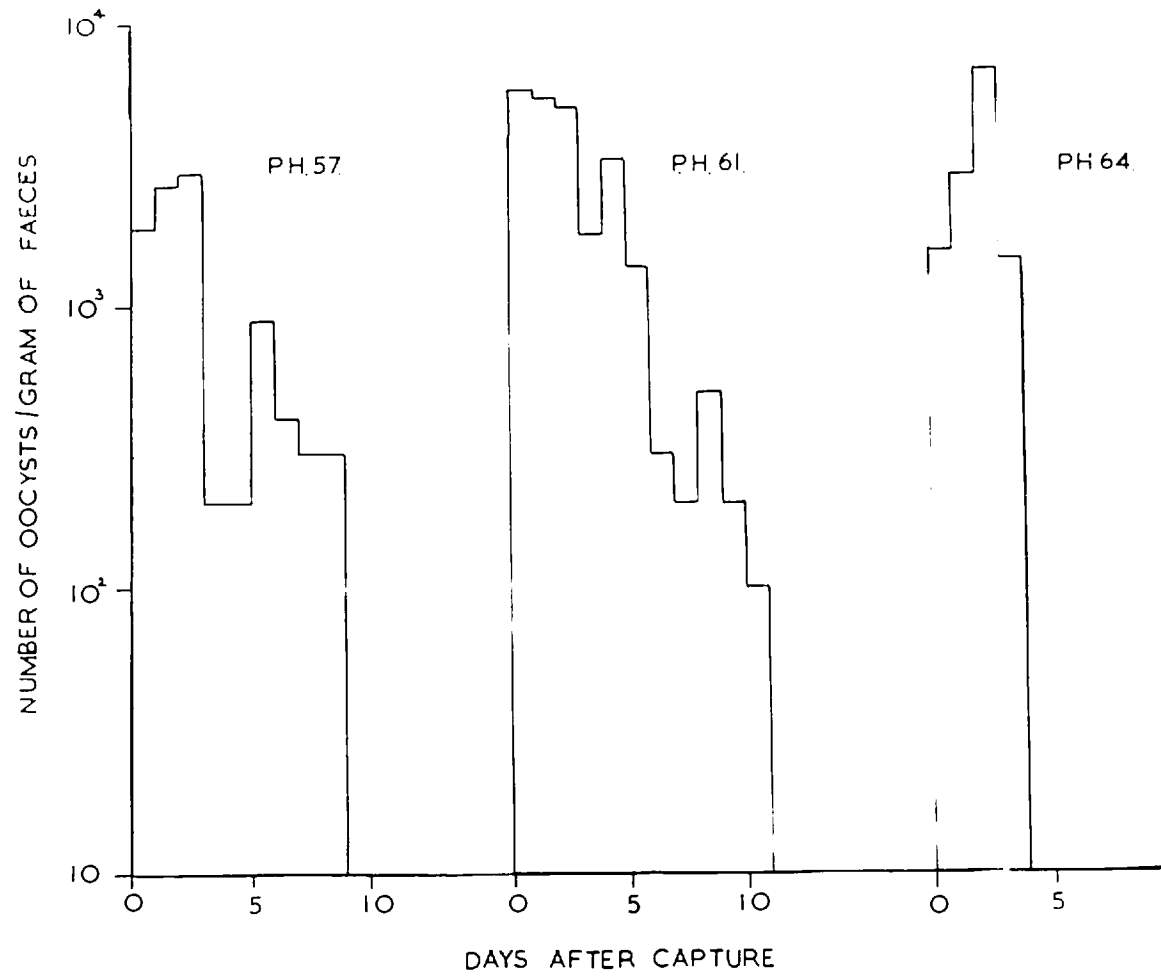
Three birds captured alive in the field exhibited a "pure" infection of *E. phasiani* and the oocyst output per day from the day of capture was recorded to determine the degree of infection. The oocyst production was light (Fig. 30) and in all cases ceased after a few days when reinfection was prevented. The maximum output per day was 7,300 oocysts/gramme of faeces in one bird. P.H. 64.

9. Strain Variance.

A search was made for strain variance with respect to drug action in *E. maxima* from the domestic fowl and *E. phasiani* from the pheasant. Three strains, Houghton, Weybridge and Celle, of *E. maxima* and two, strains Silwood B1 and Silwood B2, of *E. phasiani* were tested for a variation in response to 0.006% w/w sulphaquinoxaline, 0.006% w/w amprolium and 0.0125% w/w zoalene. The reaction of the parasite to the

OOCYST PRODUCTION IN INFECTIONS OF EIMERIA PHASIANI FROM THE FIELD

FIG. 30



drug was measured by the oocyst production and the live weight gains of the host as compared with those of uninfected, untreated controls, and infected untreated controls.

With the three strains of E. maxima no variance was observed, all three reacting to the drugs in a similar manner (Table 25 and Figs. 31 - 33) except for a slight difference in the reaction of the Celle strain to sulphaquinoxaline.

The Houghton and Weybridge strains were completely susceptible to sulphaquinoxaline, producing no oocysts and the hosts exhibiting similar live-weight gains to those of the uninfected control birds. The Celle strain produced a few oocysts on the seventh to ninth days after infection but the birds showed a similar weight gain to the uninfected controls.

All three were less susceptible to amprolium, producing almost as many oocysts as the infected controls but the birds exhibited a better live weight gain.

With zoalene treatment the infected birds showed a slightly better live-weight gain than those treated with amprolium and the oocyst production was cut to one third of that of the infected controls.

The two strains of E. phasiani both reacted to the three drugs in a similar manner (Table 26 and Figs. 34 and 35).

They were completely susceptible to sulphaquinoxaline, producing no oocysts and the hosts exhibiting a similar live-weight gain to the uninfected controls. They were less susceptible to zoalene and amprolium although zoalene gave slightly better results, with a better weight gain

Table 25. The Effect of Amprolium, Sulphaquinoxaline and Zoalene on the Oocyst Production of the Houghton, Weybridge and Celle Strains of E. maxima.

Strain	Group	Treatment	No. of Birds in Group	Oocyst Production in Millions						
				Days after Infection						Total per Group
				6	7	8	9	10	11	
Houghton	1	0.006% Amprolium	10	4.65	7.80	5.35	2.30	0.15	0	20.25
	2	0.006% Sulphaquinoxaline	10	0	0	0	0	0	0	0
	3	0.0125% Zoalene	10	0.20	5.30	3.80	0.10	0.10	0	9.80
	4	Untreated Infected Controls	10	3.95	8.65	3.65	5.10	0.30	0	21.65
Weybridge	1	0.006% Amprolium	10	1.00	18.10	7.80	0.35	0.10	0	27.35
	2	0.006% Sulphaquinoxaline	10	0	0	0	0	0	0	0
	3	0.0125% Zoalene	10	0.50	4.55	4.00	0.50	+	0	9.55
	4	Untreated Infected Controls	10	5.50	17.65	8.80	0.40	0.10	0	32.45
Celle	1	0.006% Amprolium	10	0.50	12.50	5.70	0.95	0.40	+	20.05
	2	0.006% Sulphaquinoxaline	10	0	0.75	1.05	0.10	+	0	1.90
	3	0.0125% Zoalene	10	0.20	5.85	2.30	0.70	0.10	0	9.15
	4	Untreated Infected Controls	10	1.85	15.20	6.50	1.35	0.20	+	25.10

THE AVERAGE WEIGHT GAINS OF GROUPS OF FOWL CHICKS INFECTED WITH
E MAXIMA AND TREATED WITH THREE COCCIDIOSTATS

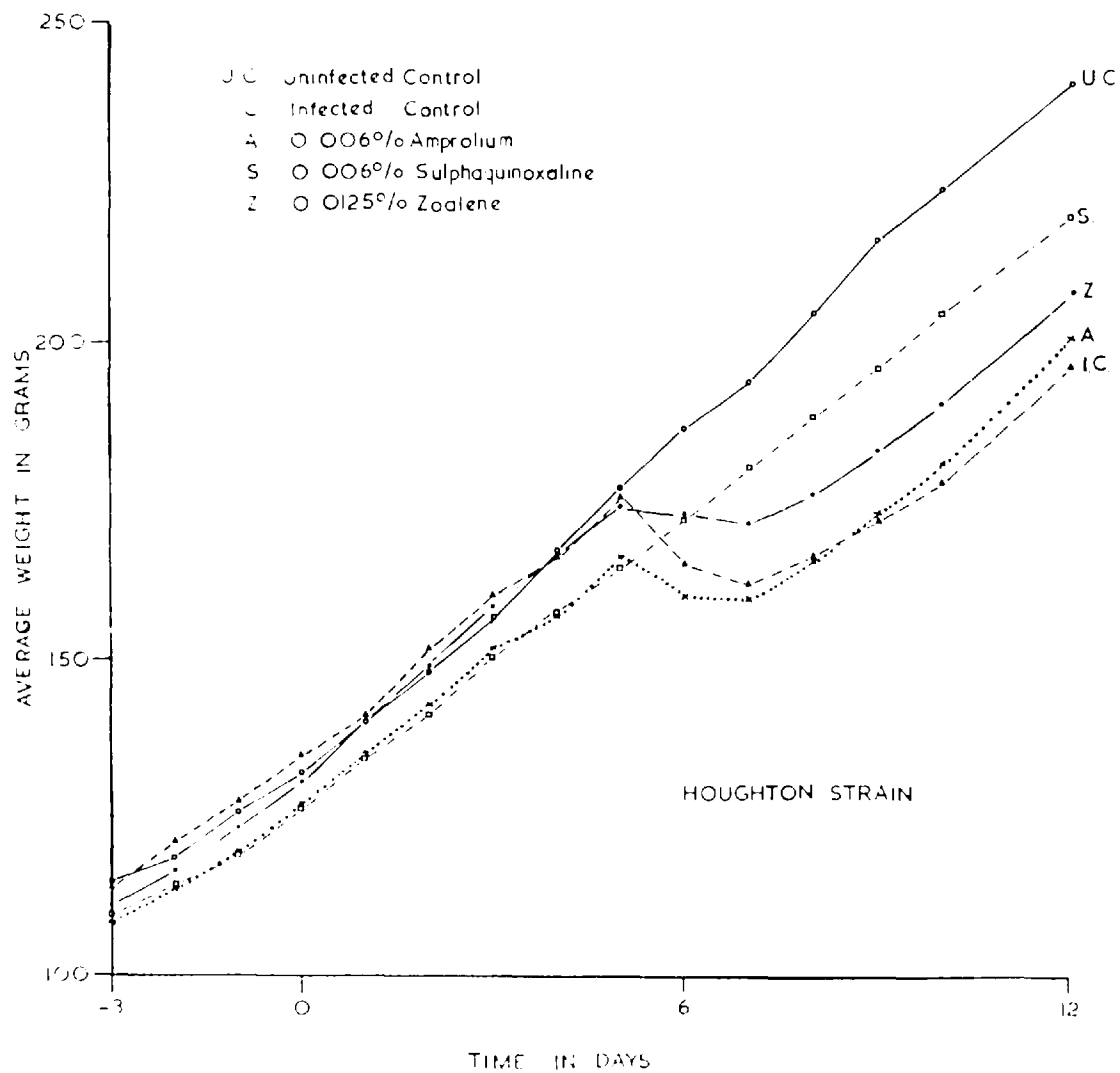


FIG 31

THE AVERAGE WEIGHT GAINS OF GROUPS OF FOWL CHICKS INFECTED WITH
E. MAXIMA AND TREATED WITH THREE COCCIDIOSTATS

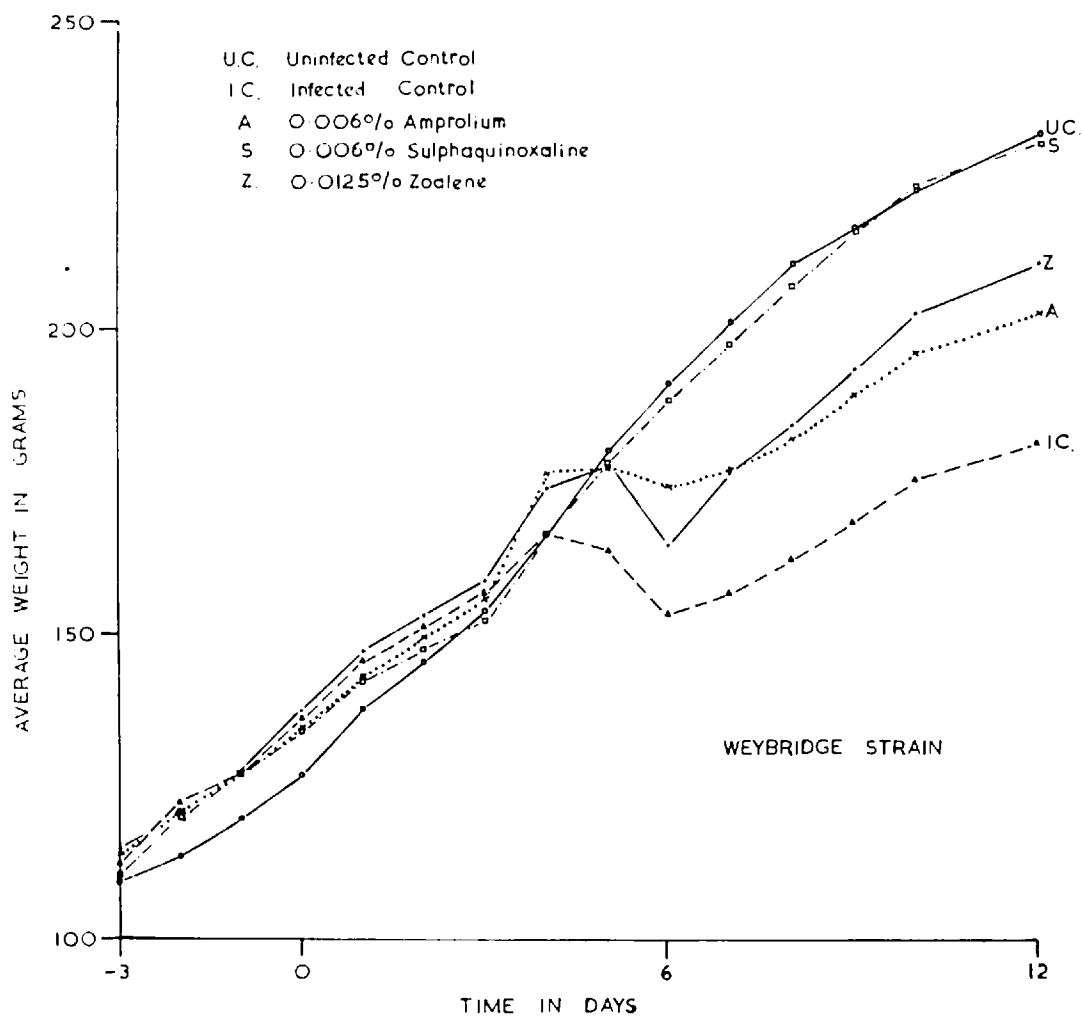


FIG. 32

THE AVERAGE WEIGHT GAINS OF GROUPS OF FOWL CHICKS INFECTED WITH
E. MAXIMA AND TREATED WITH THREE COCCIDIOSTATS

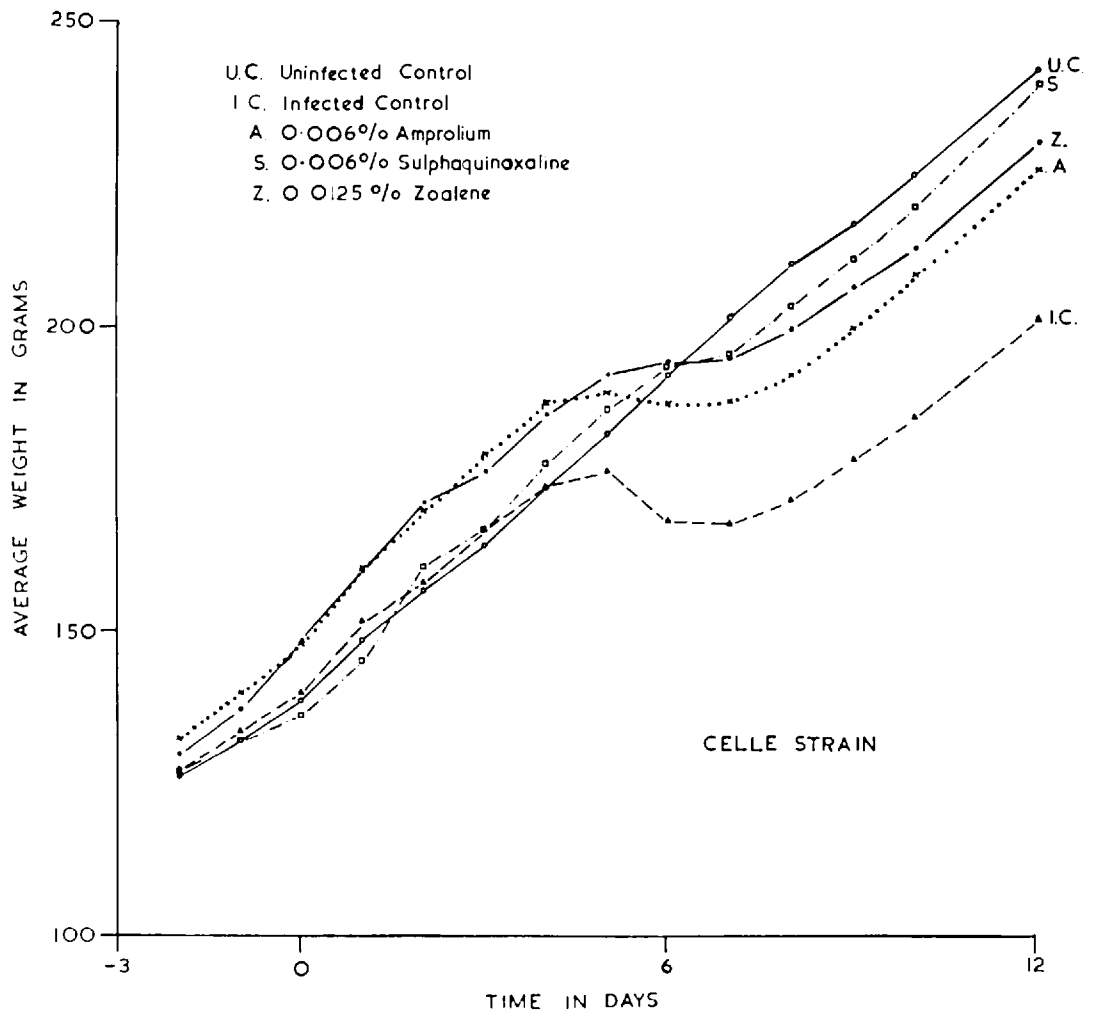


FIG. 33

Table 26. The Effect of Amprolium, Sulphaquinoxaline and Zoalene on the Oocyst Production of the Silwood Strains B1 and B2 of *E. phasianus*.

Strain	Group	Treatment	No. of Birds in Group	Oocyst Production in Millions											Total per Group
				Days after Infection											
				5	6	7	8	9	10	11	12	13	14		
B1	1	0.006% Amprolium	10	0	2.10	5.65	3.10	2.30	0.10	+	0	0	0	13.25	
	2	0.006% Sulpha- quinoxaline	10	0	0	0	0	0	0	0	0	0	0	0	
	3	0.0125% Zoalene	10	0	0.70	2.76	3.00	1.96	0.60	0	0	0	0	9.02	
	4	Untreated Infected Controls	10	5.30	10.30	14.70	8.60	9.76	4.30	3.40	0.60	+	+	56.96	
B2	1	0.006% Amprolium	10	0	3.50	6.31	2.36	1.00	0.60	0	0	0	0	13.77	
	2	0.006% Sulpha- quinoxaline	10	0	0	0	0	0	0	0	0	0	0	0	
	3	0.0125% Zoalene	10	0	0.35	3.36	3.01	+	0	0	0	0	0	6.72	
	4	Untreated Infected Controls	10	7.60	15.95	13.80	11.70	6.35	8.10	1.36	0.10	+	+	64.96	

THE AVERAGE WEIGHT GAINS OF GROUPS OF PHEASANT CHICKS INFECTED
WITH E. PHASIANI AND TREATED WITH THREE COCCIDIOSTATS

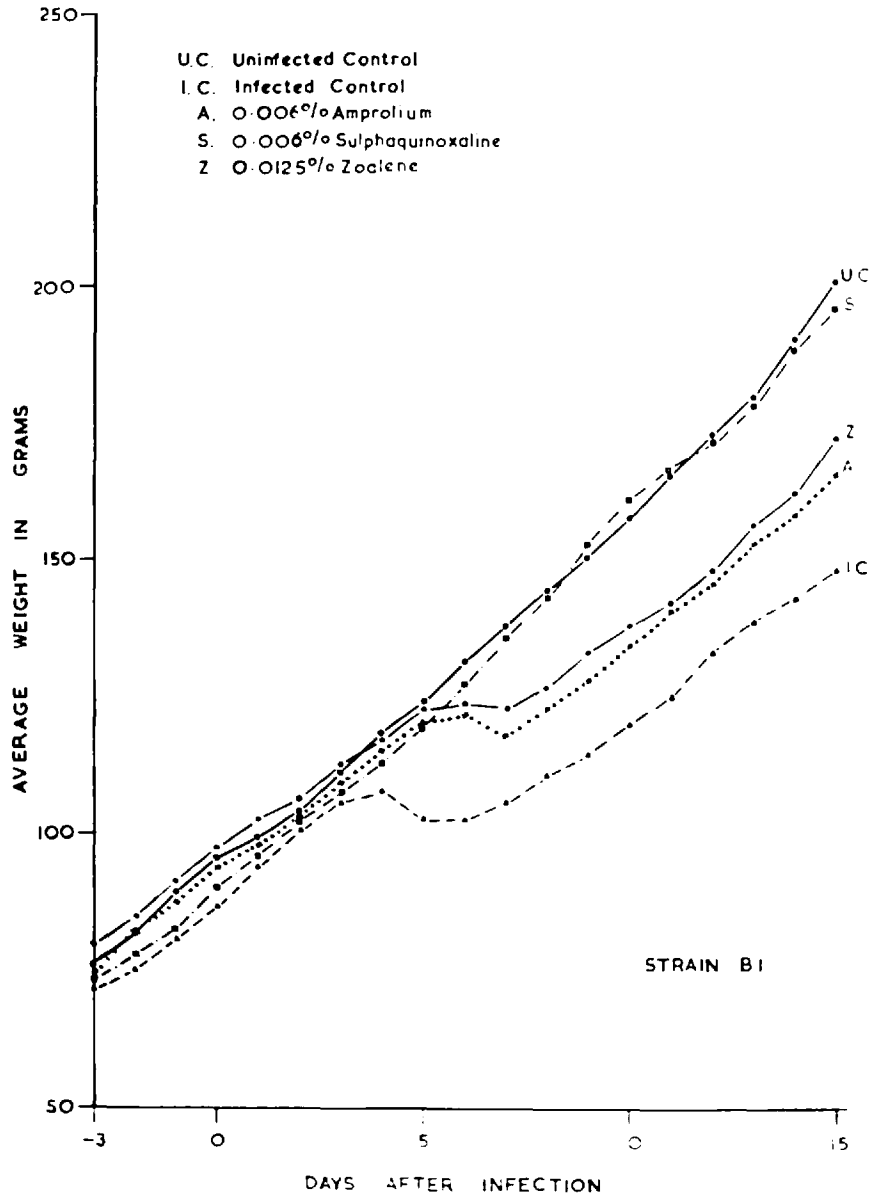


FIG 34

THE AVERAGE WEIGHT GAINS OF GROUPS OF PHEASANT CHICKS INFECTED
WITH E. PHASIANI AND TREATED WITH THREE COCCIDIOSTATS

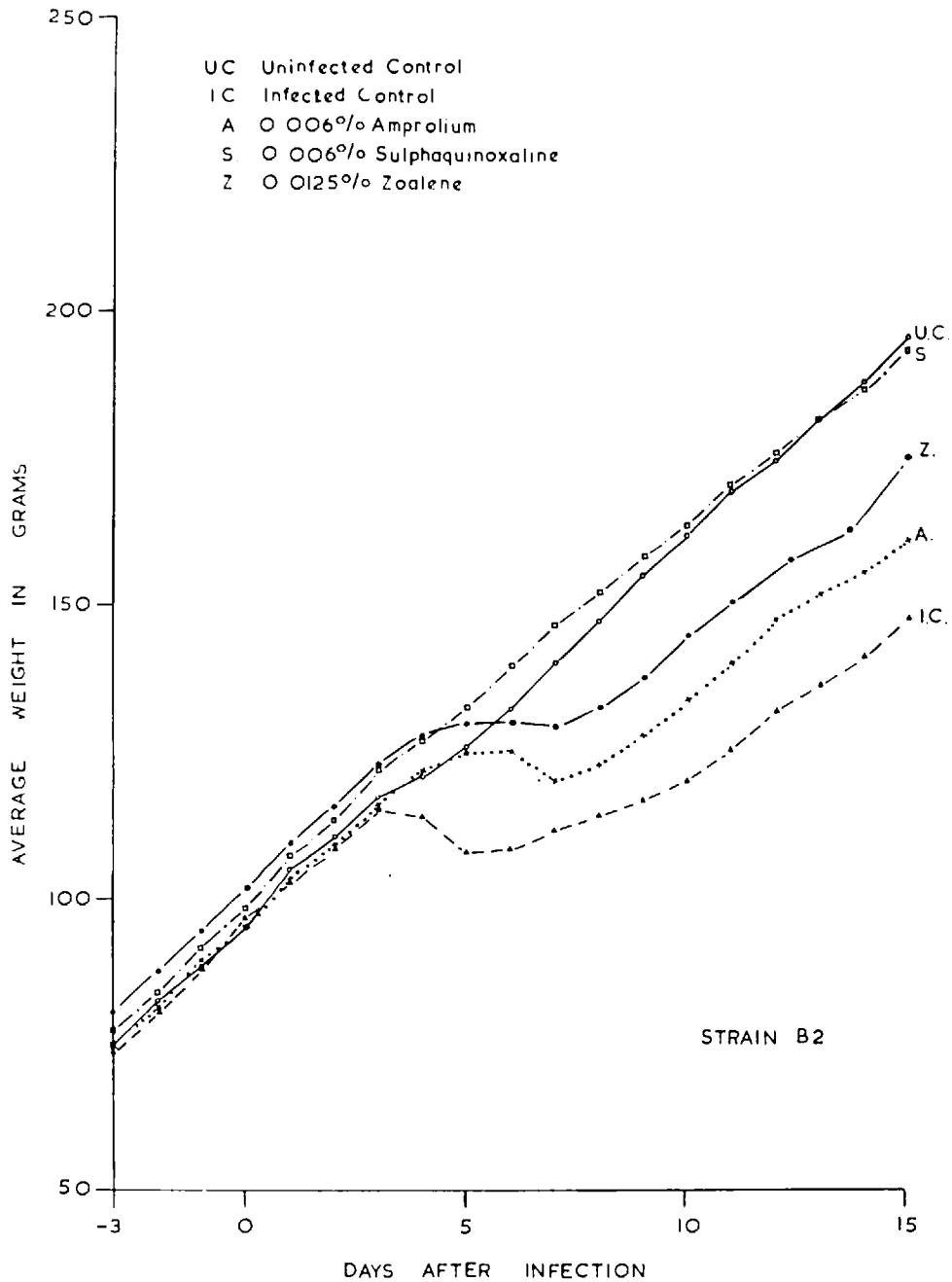


FIG. 35

and fewer oocysts produced than the amprolium treated group. Amprolium reduced the oocyst production by approximately one quarter of that of the infected untreated controls and the group also exhibited a better weight gain than those of the infected controls.

DISCUSSION

Tyzzer (1928) recommended the criteria for isolating and describing a species of Coccidia as the lengths of prepatent and patent periods, sporulation time, host specificity, characteristic distribution in the host, cross immunity, pathogenicity, morphology and the relationship of the parasite to the host cell. It was along these lines that the investigation of E. phasiani was conducted.

Previous work on the species has dealt only with oocyst morphology. Apart from simple records of the species (Becker, 1934 and Keymer, Rose, Beasley and Davies, 1962) only three descriptions of E. phasiani can be found. Tyzzer (1929) first named the species from the ring-necked pheasant (Phasianus colchicus torquatus) in the U.S.A. and his observations were later confirmed by Haase (1939) in Germany and Ormsbee (1939) also in the U.S.A. In none of these cases were the endogenous stages described, although Tyzzer did include a drawing of a schizont and the gametogony stages in his paper. Comparing the oocysts, there appears to be no doubt that the species in this study and that described by Tyzzer and the two other previous workers are one and the same, i.e. E. phasiani.

The size, shape and shape index (Length/Width) of the oocysts are very similar in all descriptions. Tyzzer gave the oocyst size as 23.0 x 15.9 μ , Haase as 23.2 x 16.2 or 15.7 μ and Ormsbee as 25 x 17 μ with which the figures of 24.74 x 17.14 μ obtained in this study closely agree. Haase found the measurements showed a simple normal distribution for both length

and width. The measurements of the oocysts in this work showed a marked heterogeneity for both length and width. This heterogeneity could not result from the presence of more than one species as the measurements were taken of oocysts of a 'pure line', established by infecting a pheasant chick with a single oocyst. However, the measurements were taken on different days of the patent period of oocysts from several different infections derived from the original 'pure line'. It was shown that measurements taken from separate infections vary significantly as do measurements taken on different days of the same infection and this variation explained the heterogeneity of the measurements when taken as a complete group.

Many authors have shown that measurements of oocysts of the same species vary significantly depending on the source of the samples taken. Fish (1931) with E. tenella, Cheissin (1947a and b) with E. magna, Becker, Zimmerman and Pattillo (1955) with E. necatrix and Becker et al. (1956) with E. necatrix, all showed that measurements of the same species but from different birds vary significantly. In E. phasiani, there was a tendency for the oocysts to increase in size as the infection progressed during the patent period although this was not observed in every bird infected with a similar dose. This is in agreement with the work of Fish (1931), Becker, Zimmerman and Pattillo (1955), Becker et al. (1956) and Duncan (1959), the last working with E. labbeana in the pigeon. It seems possible that a "crowding factor" may be operative, as in the one bird tested the smaller oocysts were produced when the oocyst output was high,

i.e. early in the patent period and larger ones when the output was low, i.e. late in the patent period. Massive infections involving numerous host cells probably do not allow the oocyst to reach its maximum size. This point of view is strengthened by the results of Jones (1932) with E. acervulina and Cheissin (1947a and b) with E. magna who both showed that the size of oocysts diminished as the dosage was increased and also that the smaller oocysts were produced early in the infection. It is highly improbable that crowding is the only factor involved, the diet and metabolism of the host and an inherent variability of the parasite may also play a part.

The results of experiments on the pattern of size variation during the patent period seem to vary greatly. Boughton (1930) with I. lacazei found the oocysts decreased in volume during the passage of the patent period whilst several other authors found no variation during the patent period. Kartchner and Becker (1930) with E. citelli, Becker, Hall and Hager (1933) with E. miyairii, Hall (1933) with I. lacazei and Doran and Jahn (1952) with E. mohavensis, all found no significant variation in the size of oocysts with the patent period. The size of the oocyst is clearly an unreliable criterion for species diagnosis especially if measurements have been taken from only one source.

No change in size of the oocysts of E. phasiani was observed during sporulation which agrees with the work of Becker, Zimmerman and Pattillo (1955) on E. brunetti.

The shape of oocysts is reasonably constant for a given species

(Davies, Joyner and Kendall, 1963) and in E. phasiani no variation in shape, as given by the shape index (L/W) was observed in separate infections, during the patent period or during sporulation. This is in direct agreement with Becker, Hall and Hager (1932), Becker, Zimmerman and Pattillo (1955) and Becker et al. (1956). The shape of the oocysts of E. phasiani was ellipsoidal with a mean shape index of 1.44. There is little difference between this value and the values of 1.45 (Tyzzer, 1929), 1.43 - 1.47 (Haase, 1939) and 1.41 (Ormsbee, 1939) given for the same species.

Sporulation is an important criterion in the identification of a species of Coccidia but a strict comparison with previous work may be made only when standard conditions are applied. The point at which an oocyst has completed sporulation is reasonably easy to ascertain as that time at which sporozoites have fully formed within the sporocyst. Most authors determine sporulation in this manner and such a method was applied to E. phasiani.

Sporulation is dependent on temperature (Pérard, 1925), humidity (Ellis, 1938) and oxygen tension (Wilson and Fairbairn, 1961) and for a strict comparison of sporulation times these factors must be identical. The sporulation time of E. phasiani was shown to increase with the temperature. A similar result was shown by Pérard (1925) with E. steida and E. perforans and Webster (1960) with E. neosciuri. At 6°C. the oocysts were dormant and above 25°C. abnormalities in sporulation were observed. A similar effect has been shown by Pérard, Chang (1937) with many coccidial species and Marquardt (1960) with E. zurnii.

The time of sporulation, which is used as one of the factors in species diagnosis, often varies greatly with the method of estimation. Some authors take the time of sporulation as that at which the first oocyst is seen to sporulate (Davies, 1964) and others as that at which the maximum number of oocysts are sporulated (Clarkson, 1960). However, the most suitable method for comparative purposes is that of Marquardt (1962) who takes the time as that required for 50% of the oocysts to complete sporulation.

Sporulation of 50% of the oocysts of E. phasiani was completed in 52 hours at 20°C. but it is difficult to compare this figure with the lower figures of the previous workers on the same species. Tyzzer (1929) gave a figure of 24 hours at room temperature but did not state at which temperature the room was kept. Haase (1939) stated sporulation was completed in 24 - 36 hours but did not give the temperature at which the figure was observed. Ormsbee (1939) also gave a figure of 24 - 36 hours and stated the temperature as 20°C. In no case was mention made of the percentage sporulation when the time was taken.

The oocyst of E. phasiani is easily distinguished from the other species found in the pheasant by its shape. It is the only species with ellipsoidal oocysts. E. dispersa Tyzzer 1929, E. langeroni Yakimoff and Hatschoulsky 1937, E. megalostomata Ormsbee 1939, E. pacifica Ormsbee 1939 and the two unnamed species described by Ormsbee (1939) and Davies, Joyner and Kendall (1963) are all ovoidal. E. phasiani may be further differentiated from E. megalostomata by the absence of a micropyle.

Table 27 compares the descriptions of the sporulated oocysts of E. phasiani given by the three previous authors with that of the present study.

Table 27. Characteristics of E. phasiani Tyzzer 1929.

	Tyzzer (1929)	Haase (1939)	Ormsbee (1939)	Present Study (Strain B)
Oocyst:				
Length	23 μ	23.2 μ (19.2-26.4)	25 μ (21-27)	27.14 μ (20.1-30.9)
Width	15.9 μ	16.2 or 15.7 (13.2-18.0)	17 μ (15-19)	17.14 μ (14.1-20.5)
Shape Index	1.45	1.43-1.47	1.41	1.44
Shape	Ellipsoidal	Elongate elliptical	Ellipsoidal	Ellipsoidal
Thickness of wall	-	0.7-1.0 μ	2 μ	1.59 μ
Membranes of wall	-	-	2	2
Polar Inclusion	1 or more refractile	"Allens Operculum"	2-3 refractile	1-3 refractile
Micropyle	-ve	?	-ve	-ve
Oocyst Residium	-ve	-ve	-ve	-ve
Sporocyst:				
Length	-	-	-	14.26 μ (12.9-15.9)
Width	-	-	-	6.69 μ (5.6-7.4)
Sporocyst Residium	-ve	1-4 refractile bodies	-ve	-ve
Steida Body	+ve	?	+ve	+ve
Sporulation Time	24 hrs at room temp.	24-36 hrs	24-36 hrs 20°C.	50% total in 52hrs at 20°C.
Position in Intestine	throughout small intestine & caeca	caecum	-	small intestine and caeca
Position in cell	inferior	-	-	inferior
Prepatent Period	5 days	-	-	117 hours
Patent Period	-	-	-	10-16 days

Only the description of Haase differs in any major respect and this appears to be more a difference of interpretation. In this study one to three refractile granules were present at the anterior pole of the oocyst, similar to the reports of Tyzzer (one or more granules) and Ormsbee (two to three granules). Haase reported a refractile thickening at the anterior pole of the oocyst but he considered it to be part of an operculum, calling it Allen's operculum and not a simple polar granule. As it does not appear in his photograph of the unsporulated oocyst, it is difficult to understand how an operculum, as he describes it, develops after the formation of the oocyst wall and during sporulation. Another point of disagreement with the present study is Haase's interpretation of the structure known as the Steida body. He stated that a thickening was present at the anterior end of the sporocyst similar in structure to a Steida body and it is difficult to understand why he preferred not to call it by this name. He also found a sporocystic residuum in the oocyst, consisting of one to four refractile granules. This was not observed in E. phasiani by the other two previous workers or the present author.

In this study the prepatent period of E. phasiani was found to be 117 hours which is in close agreement with the figure of five days given by Tyzzer.

No previous work has been performed on oocyst production and length of the patent period of E. phasiani. This species was found to be a relatively poor producer of oocysts, although the patent period of ten to sixteen days was fairly long. It was shown that, with an increase in

dosage, there was a marked reduction in the reproductive potential, as given by the approximate oocyst production per oocyst inoculated. It was as if the parasites had become too crowded to reproduce to their maximum extent. A similar phenomenon was shown by Brackett and Bliznick (1952) with five species of chicken coccidia. Two explanations may be given. Firstly, the destruction of the epithelium of the intestine by the successive schizogony stages may prevent some of the merozoites of the third stage schizogony entering host cells to develop into sexual stages. Secondly, it is possible that the early stages of the parasite stimulate antibody production by the host which interferes with oocyst production in later generations.

The present experiments showed that E. phasiani exhibited a diurnal periodicity in oocyst output, the majority of oocysts being produced between 14.00 and 20.00 hours. Similar periodicities have been shown by Boughton (1932 and 1933) and Anwar (1964) for I. lacazei and Schwalbach (1960) for an Isospora sp. from sparrows. All stated that under conditions of summer daylight the majority of oocysts were produced during the period 15.00 - 20.00 hours and that few oocysts were passed during the period 6.00 - 13.00 hours. A different periodicity was shown by Boughton (1937b) that 75% of the daily oocyst output of E. labbeana from the pigeon took place between the hours of 9.00 and 15.00 when the faecal output was at its lowest. No correlation between oocyst output and the amount of faeces passed was found in E. phasiani^{or} by Anwar (1964) in I. lacazei.

The results of Levine (1942), with five species of chicken

coccidia, suggested that the diurnal oocyst output was characteristic of the species. The pattern of oocyst discharge suggested that the formation of the oocysts was periodical but, Levine himself points out, that no definite conclusion can be drawn from the results he presented. However, in E. phasiani it is improbable that the diurnal periodicity is due to an inherent periodicity of the parasite, as three groups of birds infected with similar doses of E. phasiani but at eight hour intervals in a 24 hour period, all produced the majority of their daily oocyst output during the same 14.00 - 20.00 hour period. From this and the work of Boughton (1933), it seems more likely that the periodicity is of host influence. Boughton showed that with I. lacazei the periodicity could be altered by artificially changing the daylight hours of the host. He concluded that the periodicity was at least in part controlled by the general metabolism of the host which is in turn regulated by the diurnal habits set up in response to the alternation of light and darkness.

The present work with E. phasiani showed that there were three distinct asexual stages before the commencement of the sexual generation. In many Eimeria species there are only two schizogony cycles before the formation of gametocytes (Tyzzer, 1929 and Tyzzer, Theiler and Jones, 1932) although Schaudinn (1950) in his description of E. schubergi in the centipede considered that there were four or five asexual generations. Four asexual cycles have been described by Edgar and Seibold (1964) for E. mivati in the chicken and three for E. meleagridis in the turkey by Clarkson (1959). In some other species of Eimeria, however, only one schizogony cycle occurs

(Hammond, Bowman, Davis and Simms, 1946 for E. bovis and Lotze, 1953 for E. arloingi). One can only conclude that in the genus Eimeria there is no fixed number of schizogony cycles before the commencement of sporogony and that the number varies with the particular species concerned.

It is easy to distinguish the first stage schizonts of E. phasiani by their large size, greater number of merozoites than the other generations, the presence of an eosinophilic globule and by the fact that they are commonly found in the glands of the epithelium of the intestine. Neither of the further generations of schizogony are found in the glands. The distribution of the first generation schizogony was more limited in the small intestine than the other generations. One cannot distinguish the stages of the life cycle by their relationship with the host cell nuclei as all stages developed inferior to the nuclei. The only remarks made on the endogenous cycle were those of Tyzzer who stated that the gametocytes developed inferior to the host cell nuclei which in agreement with the present work.

The second and third generation schizonts are morphologically difficult to distinguish. They both develop inferior to the host cell nuclei but the second generation schizonts are slightly larger. They produce more merozoites, which themselves are longer and more slender than those of the third generation. A further point of contrast was that the second generation schizonts were found in the first half to two thirds of the small intestine whilst the third generation were found throughout the small intestine and the terminal half of the caeca. The distribution of

the sexual stages was similar to that of the third generation schizogony, as reported by Tyzzer for the species. Haase, however, stated that E. phasiani was found only in the caecum.

The separate existence of the last two schizogony cycles was confirmed by isolating merozoites from the hosts at 72 hours and at 96 hours and infecting these separately, per rectum, into two groups of coccidia-free pheasants. Both groups of merozoites produced infections, but the birds infected with 72-hour merozoites took 24 hours longer to produce oocysts than the birds infected with 96-hour merozoites. The 72-hour merozoites had to develop through a further cycle of schizogony before the commencement of gametogony. Confirmatory evidence of the presence of three asexual generations was obtained by counting the number of oocysts produced by a single oocyst infection. The mean oocyst production was 151,440. Two generations of schizogony would be insufficient to produce this number of oocysts even if the maximum number of merozoites in each generation were used to make the calculation (100,20).

Previous authors came to no definite conclusion as to the pathogenicity of E. phasiani. Becker (1934) suggested it only exerted a mild effect on the host whilst Tyzzer (1929) thought that continuous or heavy infections would seriously affect the health of the birds. In this study E. phasiani was fatal to young birds at doses of 100,000 or more oocysts and lighter doses produced a reduced live-weight gain. Natt and Herrick (1955) and Natt (1959) showed changes in the erythrocyte and leucocyte counts of the circulatory blood of the host, induced by E. tenella infections.

Clarkson (1958), however, could not observe this phenomenon with E. adenoeides infections. In the study of E. phasiani no such changes were observed, the blood picture of the infected birds being similar to that of the uninfected controls. The overall picture of the blood constituents was similar to that observed by Lucas and Jamroz (1961) for the ring-necked pheasant (Phasianus colchicus torquatus). However, an eosinophilia was observed in sections of the intestine of infected birds. This has also been observed by Clarkson (1958) with E. adenoeides and Hammond (1965) with E. bovis.

The species of Eimeria have a strict host specificity (Tyzzer, 1929; Patterson, 1933 and Becker, 1933) and so it was not surprising that in the present study it was found impossible to transmit E. phasiani to the partridge (Perdix perdix) or the domestic fowl. Patterson (1933) tried to infect the domestic fowl with E. dispersa and E. phasiani from the pheasant but was also unsuccessful. The unsuccessful attempt to infect the pheasant with E. tenella places a large element of doubt on the results of Haase (1939), Muroma (1951) and Clapham (1950) who all considered this species capable of infecting the pheasant. The mechanism of host specificity is unknown. Andrews (1927) stated that in birds other than the correct host oocysts are resistant to the digestive action and that the sporozoites are not released during the time taken to pass through the intestine. However, from the present study it appears that for closely allied birds, in this case gallinaceous birds, this does not apply. When E. phasiani was administered to the partridge and the domestic fowl and when E. tenella was

fed to the pheasant some excystation occurred although infections did not result. From the work of Doran and Farr (1962) and Farr and Doran (1962) on the excystation of chicken coccidia in vitro, it appears that excystation can be induced in a simple trypsin and bile salt solution and that the bile salts need not be those of the specific host. As the digestive mechanisms of the partridge, pheasant and domestic fowl are unlikely to be very different, it is quite understandable that the sporozoites of E. phasiani and E. tenella should excyst in these closely related birds. However, the cross infection experiments did not show the reason for non-infection after excystation, although they did suggest that the sporozoites failed to penetrate the epithelium of the intestine of the bird.

Thus, from the above results it can be concluded that the species described in this study as E. phasiani is the same as that described by Tyzzer (1929) and the previous authors under the same name and that the species is strictly host specific to the pheasant.

A second species of Eimeria was isolated from the small intestine of a wild adult pheasant but owing to the short breeding season of the host and the consequent short period of time for experimental work, a complete study of this species was not possible. No attempt is made to give a specific name to this species as it is considered unwise in the absence of knowledge of its endogenous cycle and pathogenicity and of cross-immunity tests. The incomplete description of this species is given on pp.149-53 of the Appendix.

The oocysts of this species were much smaller than those of

E. phasiani and were ovoidal in shape. They were similar morphologically to those described by Ormsbee (1939) as Type IV in size, shape, shape index, colour, and general oocyst structure (Table 28).

Table 28. Characteristics of the Eimeria sp. (Strain A) from the pheasant.

Character	Ormsbee Type IV	Present Study Strain A
Oocyst:		
Length	18 μ	19.28 μ (16.8-23.5)
Width	15 μ	16.42 μ (13.4-20.1)
Shape Index	1.20	1.18
Shape	Ovoidal	Ovoidal
Thickness of Oocyst Wall	2.2 μ	2.0 μ
Colour of Oocyst Wall	yellowish brown	yellowish brown
No. of Membranes of Wall	-	2
Polar Inclusions	1 refractile	1 refractile
Oocyst Residuum	-	-ve
Sporocyst:		
Length	-	12.36 μ (10.6-13.9)
Width	-	6.47 μ (5.4 - 6.8)
Sporocyst Residuum	-	-ve
Steida Body	-	+ve
Sporulation Time	-	first sporulated in 36 hrs at 20°C.
Position in Intestine	-	Small Intestine
Prepatent Period	-	7 days

This species can be further distinguished from the other species from the pheasant with ovoidal oocysts by the presence of only a single refractile polar granule. It can be further distinguished from E. phasiani by the length of the prepatent period which was seven days against the figure of 117 hours for E. phasiani. Although no attempt can be made to name this species, there seems to be sufficient points to distinguish it from E. phasiani and to point to a similarity with the description of Type IV of Ormsbee.

From the results of the surveys on the incidence of the two species in wild adult pheasants, it can be seen that coccidiosis is common but that adults harbour only light infections. Sixty per cent. of the birds examined were infected with E. phasiani and 55.7% with Eimeria sp. These figures are somewhat low as compared with the figures of Madsen (1941) who stated that 82% of the adult pheasants in Denmark were infected with coccidia, although he did not name the species concerned. Ormsbee (1939) stated that there was a monthly variation in the incidence of pheasant coccidiosis during the months October to April but the number of birds examined was extremely low. This observation was not confirmed in this work. In most cases, including the present work, some birds have been examined dead and the presence of oocysts in the intestine taken as the criterion of infection. The only way of obtaining accurate figures is to examine the birds alive over a period of time as the birds exhibit a diurnal periodicity in oocyst output.

Ideally, the treatment of coccidiosis should be such that a

low level of infection is maintained and an immunity allowed to develop. However, continuous administration of a drug may lead to strain variance and the production of drug fastness as has been shown by several authors including Kantor, Kennet and Waletsky (1959) and Long (1963).

The search for strain variance in strains of E. maxima and E. phasiani to amprolium, sulphaquinoxaline and zoalene was unsuccessful. The results of medication by 0.006% w/w amprolium and 0.006% w/w sulphaquinoxaline were similar in the case of the Houghton strain of E. maxima to those of Long (1963) with the same strain. A similar effect was obtained with the Weybridge strain agreeing with the work of Davies and Joyner (1963) with this strain. No previous accounts of the reaction of either of these strains to 0.0125% w/w zoalene could be found but they reacted in a similar way to a strain E. maxima isolated by Clarke (1964). The Celle strain of E. maxima was isolated at Silwood from a field infection of the species from Celle, W. Germany and was susceptible to sulphaquinoxaline but less so to amprolium and zoalene. This was practically identical to the reaction of both the British strains.

Both of the Silwood strains of E. phasiani behaved identically to the three coccidiostats. However, as no previous work has been performed on the drug response of E. phasiani, the results are useful in giving a guide to the reaction of the species to the three drugs employed. E. phasiani was completely susceptible to 0.006% w/w sulphaquinoxaline but less so to 0.0125% w/w zoalene and 0.006% w/w amprolium. The last two drugs gave a certain protection, with zoalene the more efficient of the

two, but neither completely prevented a loss in live-weight gain.

Thus, protection from E. phasiani would be best obtained by using sulphaquinoxaline but a slightly lower level than 0.006% w/w in order to allow some development of the parasite and consequent immunity.

A species of Eimeria was isolated from the caeca of two adult red grouse (Lagopus scoticus) but as grouse chicks were not available and the grouse intestines received were too decayed for sectioning, the endogenous cycle of the parasite could not be studied. Also, a complete description of the oocyst could not be given as the oocysts were received already sporulated. Therefore, this species, of which a description is given on pp.154-6 of the Appendix, could not be identified further than to the genus Eimeria. There has been only one previous description of a species of Eimeria from the red grouse and this was by Fantham (1912) who described the species as E. avium. As this species was obviously a mixture of several, it is impossible to compare this description with that of the present study. The oocysts of this species were typically cylindrical and were $26.56 \times 16.07 \mu$ in size. The size and shape distinguish them from oocysts of species described from other species of grouse (Allen, 1934 and Galli-Valerio, 1929 and 1931).

Although this species is the first to be described from the red grouse since Fantham, it is considered unwise to identify it further than to the genus Eimeria as the description is incomplete.

SUMMARY

1. Seven out of the twelve 6-day old coccidia-free pheasant chicks became infected when administered single oocysts of Eimeria phasiani isolated from a wild adult pheasant.

2. Studies on the oocyst measurements of pure line infections of E. phasiani showed that the distribution of the measurements was heterogeneous. This heterogeneity was due to two factors. Firstly, there was a significant difference between the measurements of oocysts on separate days of the patent period, which showed the oocysts tended to increase in size during the patent period. Secondly, there was a significant difference between measurements taken from infections of the same species in different birds.

3. The probit of the percentage of oocysts sporulated was directly proportional to log. time within the range of 15°C. - 25°C. The optimum temperature for normal sporulation was 25°C. At 35°C. and above abnormalities were observed in sporulation.

4. The oocysts of E. phasiani were ellipsoidal in shape, with smooth, two-layered oocyst walls. On sporulation they contained four pyriform sporocysts, each with a Steida body and one to three refractile polar granules.

5. The prepatent period of E. phasiani was 117 hours and oocyst production occurred over a period of ten to sixteen days. There was a diurnal periodicity in the oocyst output which was not due to an inherent

periodicity of the parasite; the majority of the oocysts being produced during the period 14.00 - 22.00 hours. There was no relationship between the number of oocysts passed per hour and the amount of faeces passed.

6. There were three distinct generations of schizogony prior to gametogony. The first two schizogony cycles were restricted to the small intestine but the third generation and gametogony stages were found throughout the small intestine and in the lower half of the caeca.

7. E. phasiani was shown to be pathogenic to young pheasant poults. Doses of 100,000 or more oocysts proved fatal to birds up to three weeks old. Lesser doses produced losses in live-weight gains.

8. In cross infection experiments, the common partridge and the domestic fowl were not susceptible to E. phasiani and, the pheasant was not susceptible to E. tenella. However, some excystation of sporozoites of the two species did occur in birds other than the correct host.

9. In a field survey, coccidiosis was shown to be common in wild adult pheasants. Sixty per cent. of the birds examined were infected with E. phasiani and 55.7% were infected with a second species of Eimeria, incompletely described in the Appendix. All infections of adult pheasants in the field were light.

10. E. phasiani was completely susceptible to 0.006% w/w sulphaquinoxaline but less so to 0.006% w/w amprolium and 0.0125% w/w zoalene.

11. No strain variance to the three drugs mentioned above was found in two strains of E. phasiani or in three strains of E. maxima.

12. The oocysts of a second species of Eimeria, isolated from the small intestine of a wild adult pheasant, are described in the Appendix. The oocysts were ovoidal in shape, smaller in size than those of E. phasiani and possessed only one polar granule. The prepatent period of single oocyst infections was seven days.

13. The oocysts of a species of Eimeria from the red grouse (Lagopus scoticus) are also described in the Appendix. The oocysts were cylindrical in shape with smooth, double-layered walls. Only the sporulated oocysts were observed and these contained four pyriform sporocysts, each with a Steida body and two or three refractile polar granules. The pheasant and domestic fowl were not susceptible to this species.

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APPENDIX1. Eimeria sp. from the pheasant (*Phasianus colchicus*).

A second species of Eimeria was isolated from the small intestine of a wild adult pheasant, captured at Silwood Park. Unfortunately, time and lack of experimental birds, due to the restricted breeding season of the pheasant made it impossible to carry out a complete study of the exogenous or of any of the endogenous stages.

Pure lines of this species were obtained by isolating single oocysts from the infection in the field and administering these to coccidia-free pheasant chicks. Three birds out of five, treated in this way, became infected and all three passed oocysts late on the seventh day after infection. Oocysts were passed for three days; the total number produced being 150,000, 163,000 and 201,700 oocysts for the three birds.

The oocysts measured $16.8 - 23.5 \mu \times 13.4 - 20.1 \mu$ (mean $19.28 \pm 0.08 \mu \times 16.42 \pm 0.06 \mu$). These measurements were taken of oocysts from the three infections and the resulting distribution (Fig. 36 and Table A.7) was slightly heterogeneous. The oocysts were ovoidal in shape, S.I. 1.18 ± 0.003 . The cyst wall, 2.0μ in thickness, was uniform and smooth in outline, and consisted of two membranes. The oocyst appeared yellowish brown in artificial light.

At 20°C ., 36 hours was the minimum time taken for any of the oocysts to complete sporulation. In the sporulated oocyst (Fig. 36) four, elongate pyriform sporocysts, measuring $10.6 - 13.9 \mu \times 5.4 - 6.8 \mu$

DIMENSIONS OF OOCYSTS OF *EIMERIA* SP FROM *PHASIANUS COLCHICUS* COMPARED
WITH NORMAL DISTRIBUTION

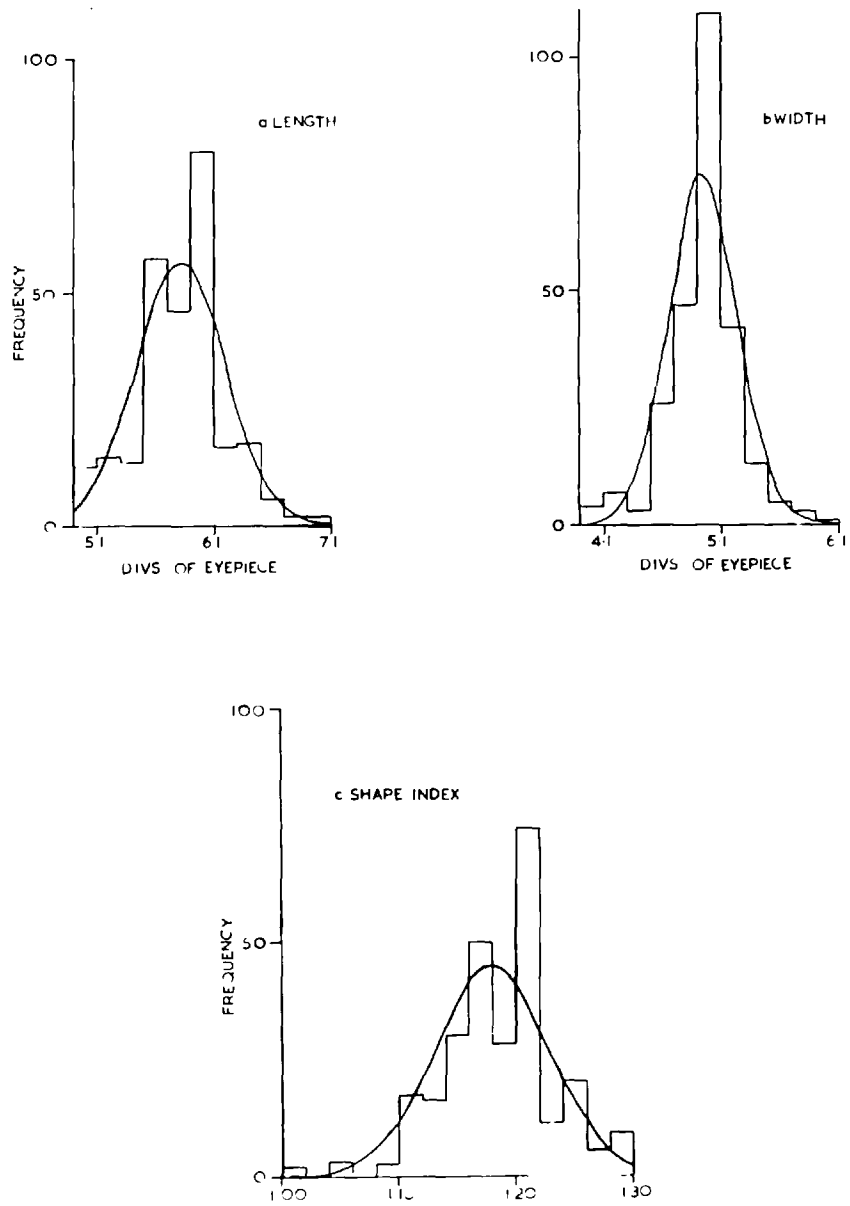


FIG 36

(mean $12.36 \pm 0.04 \mu \times 6.47 \pm 0.04 \mu$) were present. One refractile polar granule was present but a micropyle and oocystic and sporocystic residua were absent. There was a well developed Steida body at the anterior end of the sporocysts. Each sporocyst contained two elongate sporozoites, pointed at the anterior end and blunt at the posterior end with a central nucleus and a large refractile globule at the posterior end.

Table A.1. Variation in Size of Oocysts of Eimeria sp. in Pheasant.

Bird No.	Day of Patent Period	No. Measured	Length in μ				Width in μ				Range Shape Index	$\bar{m}$ S.I.
			Range	$\bar{m} \pm$ S.E.	S.	C.V.%	Range	$\bar{m} \pm$ S.E.	S.	C.V.%		
Chick 3	1 & 2	130	17.4-23.5	20.02 $\pm$ 0.10	1.20	5.99	14.4-20.1	16.87 $\pm$ 0.08	0.92	5.45	1.08-1.29	1.19
Chick 10	1 & 2	100	16.8-21.8	19.13 $\pm$ 0.11	1.15	6.01	13.4-17.8	16.25 $\pm$ 0.10	1.05	6.46	1.10-1.28	1.18
Chick 11	1	40	16.8-20.1	18.70 $\pm$ 0.16	1.05	5.61	13.7-16.8	16.13 $\pm$ 0.12	0.74	4.59	1.00-1.22	1.16

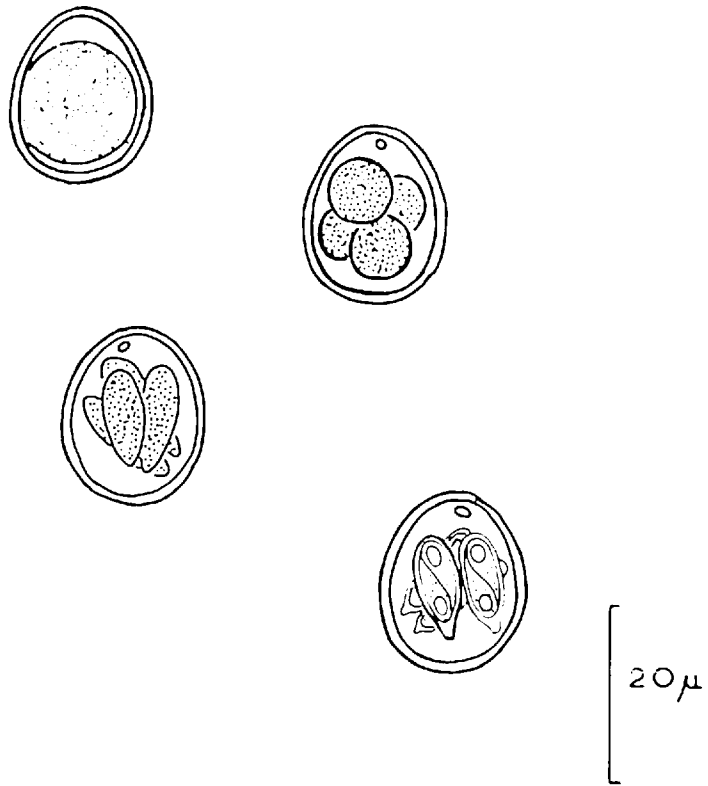


FIG. 37

2. Eimeria sp. from the red grouse (Lagopus scoticus).

Oocysts of a species of Eimeria were found in the caeca of three red grouse intestines, two of which were obtained from Staffordshire and one from Co. Tyrone, Northern Ireland. All the oocysts in the three infections were similar in appearance and were sporulated on receipt. Therefore, details of sporulation were not available. Each intestine was too decayed for histological examination and, as no grouse chicks were available, it was impossible to study the species further. An attempt to transmit it to the pheasant and domestic fowl was made but this was unsuccessful (p.103).

The oocysts measured $20.1 - 32.9 \mu \times 13.4 - 18.8 \mu$ (mean $26.56 \pm 0.19 \mu \times 16.07 \pm 0.08 \mu$). The distribution of the dimensions was slightly heterogeneous (Fig. 38 and Table A.8) but this was probably accounted for by the fact that measurements were taken of oocysts from three separate birds (Table A.2). The shape was typically cylindrical, S.I. 1.65, the opposite sides of the oocysts being nearly parallel throughout the middle third of the longitudinal axis (Fig. 39). The oocyst wall, 2.0μ in thickness, was smooth in outline consisting of two membranes. When viewed in artificial light the oocysts appeared light brownish.

Each oocyst contained four elongate pyriform sporocysts, measuring $12.0 - 14.0 \times 5.9 - 7.0 \mu$ (mean $13.07 \pm 0.04 \mu \times 6.54 \pm 0.03 \mu$). A Steida body was present at the pointed end of each sporocyst. Two or three refractile polar granules were present but a micropyle and oocystic and sporocystic residua were absent. Each sporocyst contained two elongate sporozoites pointed at the anterior and blunt at the posterior end where a large refractile globule was present.

DIMENSIONS OF OOCYSTS OF *EIMERIA* SP FROM THE RED GROUSE (*LAGOPUS SCOTICUS*)
 COMPARED WITH NORMAL DISTRIBUTIONS

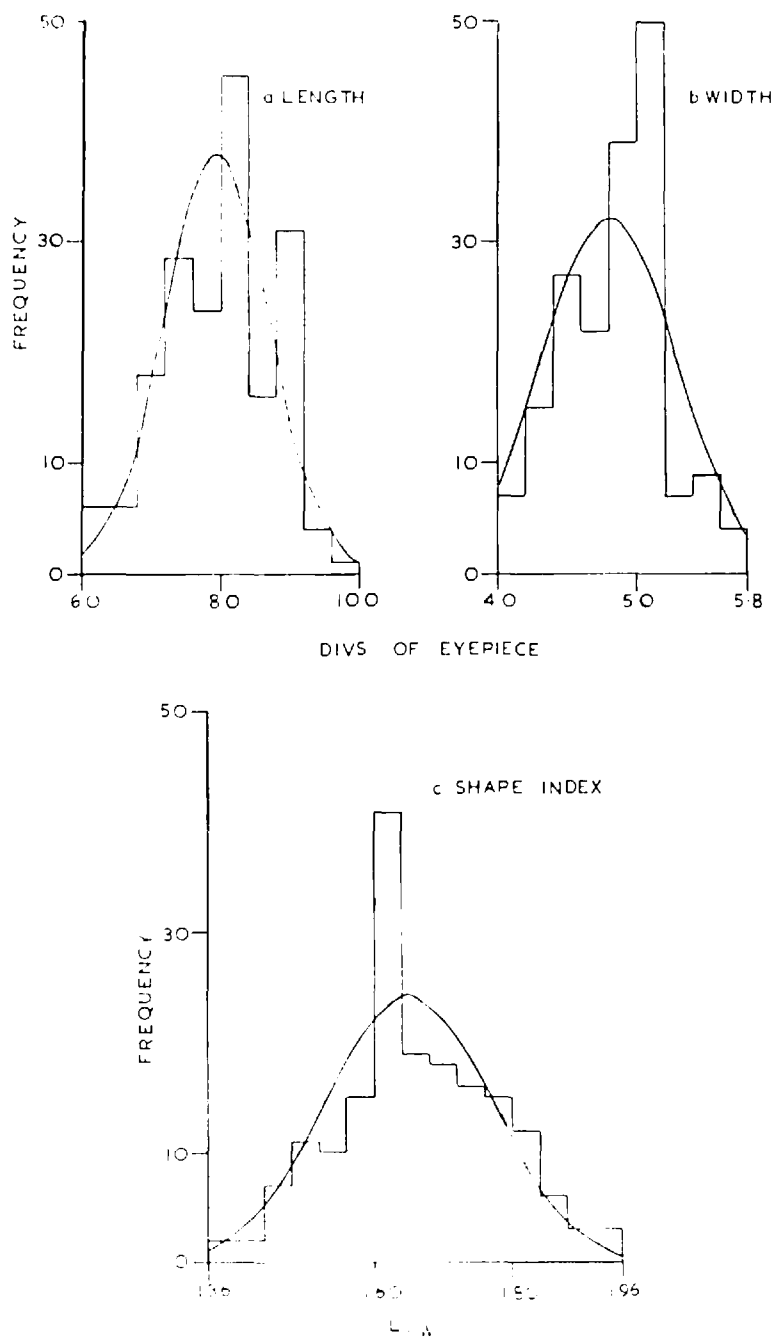
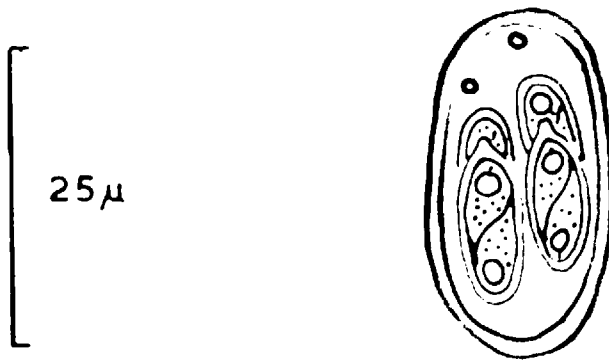


FIG 38

Table A.2. Variation in Dimensions of the Oocysts of Eimeria species from
the Red Grouse (Lagopus scoticus).

Bird No.	No. Measured	Length in μ				Width in μ				Range Shape Index	$\bar{m}$ S.I.
		Range	$\bar{m} \pm S.E.$	S.	C.V.%	Range	$\bar{m} \pm S.E.$	S.	C.V.%		
G.1.	80	21.1-30.5	25.83 $\pm$ 0.26	2.31	8.94	13.4-17.4	15.85 $\pm$ 0.10	0.86	5.43	1.36-1.88	1.63
G.2.	50	20.1-29.5	25.42 $\pm$ 0.24	1.71	6.73	13.4-16.8	15.36 $\pm$ 0.16	1.14	7.42	1.40-1.94	1.65
G.3.	50	24.1-32.9	28.84 $\pm$ 0.28	2.05	7.11	13.4-18.8	17.05 $\pm$ 0.16	1.16	6.80	1.38-1.94	1.69



SPORULATED OOCYST OF EIMERIA SP.
FROM LAGOPUS SCOTICUS

FIG. 39

Explanation of Table A.3.

S. of S.	=	Sum of Squares.
D.F.	=	Degrees of Freedom.
M.Sq.	=	Mean Square.
F.	=	Variance Ratio.
P.	=	Probability.
$x_1, x_2, x_3 \dots x_{10}$	=	equal intervals of time, i.e. days of patent period.
y	=	sum of volumes of 20 oocysts.
$\xi_1^1, \xi_2^1 \dots \xi_5^1$	=	orthogonal polynomials of degrees 1, 2, ...5 corresponding to x , $x^2 \dots x^5$.
$\eta_1^1, \eta_2^1 \dots \eta_5^1$	=	covariances with y .
$(\xi^1)^2$	=	sum of squares of ξ^1 .
λ	=	multiplier to avoid fractional values of ξ^1 .
$\hat{y}$	=	value of y on the fitted line.

Table A.3. Complete Analysis of Variation of Size of Oocysts during the Patent Period of *E. phasiani* in Chick 38 set out in successive Stages.

1. Analysis of Total Variance (Volumes of oocysts).

Source	S. of S.	D.F.	M.Sq.	F.	P.
a Between Times	22646	9	2516	a/b	< 0.1%
b Within Times	103202	190	543	=4.63	
c Total	125848	199			

2. Analysis of Between Times Variance by Method of Orthogonal Polynomials using Table XXIII, $n = 10$ in "Statistical Methods for Biological, Agricultural and Medical Research" by Fisher and Yates.

(a)

Totals	y	$y \sum_1^6$	$y \sum_2^6$	$y \sum_3^6$	$y \sum_4^6$	$y \sum_5^6$	b_1
x_1	3440	-30960	+20640	-144480	+ 61920	-20640	
x_2	3581	-25067	+ 7162	+ 50134	- 78782	+50134	
x_3	3699	-18495	- 3699	+129465	- 62883	- 3699	
x_4	3792	-11376	-11376	+117552	+ 11376	-41712	
x_5	3880	- 3880	-15520	+ 46560	+ 69840	-23280	
x_6	3870	+ 3870	-15480	- 46440	+ 69660	+23220	
x_7	3929	+11787	-11787	-121799	+ 11787	+43219	
x_8	4077	+20385	- 4077	-142695	- 69309	+ 4077	
x_9	4006	+28042	+ 8012	- 56084	- 88132	-56084	
x_{10}	4176	+37584	+25056	+175392	+ 75168	+25056	
Σ		+11890	- 1069	+ 7605	+ 645	+ 291	1.80
Σ^2		141372100	1142761	57836025	416025	84681	
$(\sum_1^6)^2_n$		6600	2640	171600	57200	15600	
$\Sigma^2 / (\sum_1^6)^2_n$		21420	433	337	7.27	5.43	

The increase in y for every unit increase in x.

$$b = \lambda b_1 = 3.60$$

- (b) The contributions to the sum of squares are quoted in Table 8 (ii) (page 44), which shows that a rectilinear relationship adequately fits the data.

3. To fit a straight line to the graph.

$$\begin{aligned}\hat{Y} &= \bar{y} + b_1 x \\ &= \bar{y} + b(x - \bar{x}) \dots \dots \dots (1)\end{aligned}$$

Being rectilinear, the line must pass through the point $\bar{x}$, $\bar{y}$ and using the above formula 1, when $x = 10$

$$\begin{aligned}\hat{Y} &= 192.26 + 3.60 (10 - 5.5) \\ &= 192.26 + 36.0 - 19.80 \\ &= 208.48\end{aligned}$$

From the above two points the straight line can be drawn. The value $b = 3.60$ indicates that there is an increase of 3.6 in y for every unit increase in x .

Table A.4. Test of Significance of Fitting b to the data for variation in size of oocysts during the Patent Period in the Pilot Sample - Chick 15.

(i) Length

Source	S. of S.	D.F.	M.Sq.	F.	P.
Effect of Fitting b	4.49	1	4.49	9.76	<1.0%
Quadratic and Higher	5.09	11	0.46		
Between Times	9.58	12			

(ii) Width.

Source	S. of S.	D.F.	M.Sq.	F.	P.
Effect of Fitting b	1.53	1	1.53	5.46	< 5.0%
Quadratic and Higher	3.09	11	0.28		
Between Times	4.62	12			

(iii) Volume (LW^2).

Source	S. of S.	D.F.	M.Sq.	F.	P.
Effect of Fitting b	21704	1	21704	7.13	<5.0%
Quadratic and Higher	33465	11	3042		
Between Times	54169	12			

Table A.5. List of Random Samples of Volumes of Oocysts used in the Analysis of Variance of the Volume (LW^2) of Oocysts from Different Infected Birds.

(All values in eyepiece units and taken on the same day of the patent period).

	Chick 7	Chick 9	Chick 15	Chick 38	Chick 41	Chick 42	Chick 104	Chick 106	$\bar{x}$	Σx
	242.00	200.00	172.80	175.00	168.07	194.69	131.63	139.66		
	175.00	175.00	180.07	161.28	175.00	175.00	165.67	139.66		
	216.32	144.00	139.66	185.00	165.00	180.00	168.07	133.65		
	227.45	143.89	161.28	175.00	126.96	205.50	146.00	168.07		
	250.88	242.00	175.00	180.00	156.67	200.01	141.77	156.67		
	218.70	175.00	180.00	227.45	175.00	175.00	158.98	139.66		
	175.00	257.45	165.00	152.06	150.21	219.10	168.07	154.37		
	216.32	242.00	143.89	156.67	139.66	165.89	168.07	170.47		
	180.00	219.10	158.98	200.01	156.67	180.00	131.63	156.67		
	176.87	180.00	105.84	180.00	143.89	161.28	158.98	168.07		
Σc	2078.54	1978.14	1582.52	1792.47	1557.13	1856.47	1538.87	1526.95		13911.09
$\bar{c}$	207.85	197.81	158.25	179.25	155.71	185.65	153.89	152.70	173.89	

Tables A.6, A.7 and A.8.

Tests for Goodness of Fit of data with Normal expectation: chi-squared.

KEY

x	=	Variate, in eyepiece units.
$(x - \bar{x})$	=	Deviate
$(x - \bar{x})/s$	=	Normal deviate
z	=	Ordinate
$100.z/\sum(z)$	=	Percentage frequency
E	=	$nz/\sum(z)$ = Expected frequency
O	=	Observed frequency
χ^2	=	$\sum (O - E)^2/E$

Table A.6. E. phasiani from the pheasant.

n = 1439
s = 0.49
 $\bar{x}$ = 7.38

Length

x	$x - \bar{x}$	$x - \bar{x} / s$	Z	E	O	χ^2
6.1	-1.28	-2.61	0.0132	7.76	6	0.3992
6.3	-1.08	-2.20	0.0355	20.88	0	20.88
6.5	-0.88	-1.79	0.0804	47.28	19	16.915
6.7	-0.68	-1.39	0.1518	89.26	92	0.119
6.9	-0.48	-0.98	0.2468	145.13	109	8.99
7.1	-0.28	-0.57	0.3391	199.40	291	42.82
7.3	-0.08	-0.16	0.3939	231.63	214	1.34
7.5	+0.12	+0.24	0.3876	227.92	182	9.25
7.7	+0.32	+0.65	0.3230	189.94	132	17.67
7.9	+0.52	+1.06	0.2275	133.78	163	7.08
8.1	+0.72	+1.47	0.1354	79.62	169	103.14
8.3	+0.92	+1.88	0.0681	40.05	32	1.62
8.5	+1.12	+2.28	0.0297	17.46	10	3.19
8.7	+1.32	+2.69	0.0107	6.29	10	2.19
8.9	+1.52	+3.10	0.0033	1.94	3	0.579
9.1	+1.72	+3.51	0.0009	0.53	6	56.45
9.3	+1.92	+3.92	0.0002	0.12	1	6.45
Total			2.4471	1438.99	1439	299.0822

Table A. 6 Continued. E. phasianus from the Pheasant.

n = 1439

s = 0.33

 $\bar{x}$ = 5.11Width

x	$x - \bar{x}$	$x - \bar{x} / s$	Z	E	O	χ^2
4.10	-1.01	-3.06	0.0006	0.53	2	4.075
4.30	-0.81	-2.45	0.0198	17.50	4	10.43
4.50	-0.61	-1.85	0.0721	63.71	13	40.36
4.70	-0.41	-1.24	0.1849	163.38	112	16.16
4.90	-0.21	-0.64	0.3251	287.27	262	2.222
5.10	-0.01	-0.30	0.3814	337.02	439	30.86
5.30	+0.19	+0.58	0.3372	297.96	282	0.8549
5.50	+0.39	+1.18	0.1989	175.75	189	0.9989
5.70	+0.59	+1.79	0.0804	71.04	79	0.8919
5.90	+0.79	+2.39	0.0229	20.24	26	1.639
6.10	+0.99	+3.00	0.0044	3.89	27	137.29
6.30	+1.19	+3.61	0.0006	0.53	3	11.51
6.50	+1.29	+3.91	0.0002	0.18	1	3.736
Total			1.6285	1439.00	1439	261.0277

Table A.6 Continued. E. phasianus from the pheasant.

n = 1439
s = 0.056
 $\bar{x}$ = 1.44

Shape Index (L/W)

x	$x - \bar{x}$	$(x - \bar{x})/s$	Z	E	O	χ^2
1.27	-0.17	-3.04	0.0044	2.26	1	0.7025
1.29	-0.15	-2.68	0.0110	5.66	0	5.66
1.31	-0.13	-2.32	0.0270	13.89	6	4.4818
1.33	-0.11	-1.96	0.0584	30.03	21	2.7153
1.35	-0.09	-1.61	0.1092	56.15	24	18.408
1.37	-0.07	-1.25	0.1826	93.90	36	36.702
1.39	-0.05	-0.89	0.2685	138.07	57	47.602
1.41	-0.03	-0.54	0.3448	177.30	197	2.189
1.43	-0.01	-0.18	0.3925	201.83	303	51.72
1.45	+0.01	+0.18	0.3925	201.83	240	7.602
1.47	+0.03	+0.54	0.3448	177.30	234	18.132
1.49	+0.05	+0.89	0.2685	138.07	119	2.634
1.51	+0.07	+1.25	0.1826	93.90	105	1.312
1.53	+0.09	+1.61	0.1092	56.15	35	7.9665
1.55	+0.11	+1.96	0.0584	30.03	30	0.00003
1.57	+0.13	+2.32	0.0270	13.89	13	0.057
1.59	+0.15	+2.68	0.0110	5.66	6	0.0204
1.61	+0.17	+3.04	0.0044	2.26	6	6.189
1.63	+0.19	+3.39	0.0012	0.62	5	30.94
1.65	+0.21	+3.75	0.0004	0.21	1	2.972
Total			2.7984	1439.01	1439	248.00553

Table A.7. Eimeria sp. from the Pheasant.

$n = 270$
 $s = 0.38$
 $\bar{x} = 5.81$

Length

x	$x - \bar{x}$	$x - \bar{x} / s$	Z	E	O	χ^2
5.0	-0.81	-2.13	0.0413	5.95	13	8.35
5.2	-0.61	-1.60	0.1109	16.00	15	0.0625
5.4	-0.41	-1.08	0.2227	32.11	14	20.91
5.6	-0.21	-0.55	0.3429	49.44	57	1.44
5.8	-0.01	-0.26	0.3857	55.61	46	1.66
6.0	+0.19	+0.50	0.3521	50.77	80	16.83
6.2	+0.39	+1.03	0.2347	33.84	17	8.38
6.4	+0.59	+1.55	0.1200	17.30	18	0.028
6.6	+0.79	+2.08	0.0459	6.62	6	0.058
6.8	+0.99	+2.61	0.0132	1.90	2	0.0053
7.0	+1.19	+3.13	0.0033	0.48	2	0.563
Total			1.8727	270.02	270.00	58.2868

Table A.7 Continued. Eimeria sp. from the Pheasant.

n = 270

s = 0.29

 $\bar{x}$ = 4.93Width

x	$x - \bar{x}$	$x - \bar{x} / s$	Z	E	O	χ^2
4.0	-0.93	-3.21	0.0024	0.45	4	28.00
4.2	-0.73	-2.52	0.0167	3.11	7	4.866
4.4	-0.53	-1.83	0.0748	13.94	3	8.586
4.6	-0.33	-1.14	0.2083	38.81	26	4.228
4.8	-0.13	-0.45	0.3605	67.17	47	6.057
5.0	+0.07	+0.24	0.3876	72.22	119	18.73
5.2	+0.27	+0.93	0.2589	48.24	42	0.8072
5.4	+0.47	+1.62	0.1074	20.01	13	2.456
5.6	+0.67	+2.31	0.0277	5.16	5	0.0496
5.8	+0.87	+3.00	0.0044	0.82	3	5.796
6.0	+1.07	+3.69	0.0004	0.07	1	12.36
Total			1.4491	270.00	270	80.8118

Table A.7 Continued. Eimeria sp. from the Pheasant.

$n = 270$
 $s = 0.048$
 $\bar{x} = 1.18$

Shape Index (L/W)

x	$x - \bar{x}$	$x - \bar{x} / s$	Z	E	O	χ^2
1.01	-0.17	-3.54	0.0009	0.10	2	36.1
1.03	-0.15	-3.13	0.0033	0.37	0	0.37
1.05	-0.13	-2.71	0.0101	1.15	3	7.063
1.07	-0.11	-2.29	0.0290	3.29	0	3.29
1.09	-0.09	-1.88	0.0681	7.72	5	0.9454
1.11	-0.07	-1.46	0.1374	15.58	17	0.1294
1.13	-0.05	-1.04	0.2323	26.35	16	4.065
1.15	-0.03	-0.63	0.3271	37.10	30	1.359
1.17	-0.01	-0.21	0.3902	44.25	50	0.7599
1.19	+0.01	+0.21	0.3902	44.25	28	5.968
1.21	+0.03	+0.63	0.3271	37.10	74	36.70
1.23	+0.05	+1.04	0.2323	26.35	11	8.942
1.25	+0.07	+1.46	0.1374	15.58	20	1.254
1.27	+0.09	+1.88	0.0681	7.72	5	0.9454
1.29	+0.11	+2.29	0.0290	3.29	9	9.910
Total			2.3825	270.20	270	117.8011

Table A.8. Eimeria sp. from Lagopus scoticus.

$$n = 180$$

$$s = 0.77$$

$$\bar{x} = 7.92$$

Length

x	$x - \bar{x}$	$x - \bar{x} / s$	Z	E	O	χ^2
6.2	-1.72	-2.23	0.0332	3.13	6	2.632
6.6	-1.32	-1.71	0.0925	8.72	6	0.8484
7.0	-0.92	-1.19	0.1965	18.53	18	0.0152
7.4	-0.52	-0.68	0.3166	29.86	29	0.0248
7.8	-0.12	-0.16	0.3939	37.14	24	4.649
8.2	+0.28	+0.36	0.3739	35.26	45	2.691
8.6	+0.68	+0.88	0.2709	25.55	16	3.57
9.0	+1.08	+1.40	0.1479	13.95	31	20.84
9.4	+1.48	+1.92	0.0632	5.96	4	0.6446
9.8	+1.88	+2.44	0.0203	1.91	1	0.4336
Total			1.9089	180.01	180	36.3486

Table A.8 continued. Eimeria sp. from Lagopus scoticus.

n = 180

s = 0.48

 $\bar{x}$ = 4.79Width

x	$x - \bar{x}$	$x - \bar{x} / s$	Z	E	O	χ^2
4.1	-0.69	-1.44	0.1415	11.36	7	1.673
4.3	-0.49	-1.02	0.2371	19.04	15	0.8572
4.5	-0.29	-0.60	0.3332	26.76	27	0.0215
4.7	-0.09	-0.19	0.3918	31.46	22	2.845
4.9	+0.11	+0.23	0.3885	31.20	39	2.482
5.1	+0.31	+0.65	0.3230	25.94	50	22.32
5.3	+0.51	+1.06	0.2275	18.27	7	6.952
5.5	+0.71	+1.48	0.1334	10.71	9	0.2730
5.7	+0.91	+1.90	0.0656	5.28	4	0.3103
Total			2.2416	180.02	180	37.7340

Table A.8 Continued. Eimeria sp. from Lagopus scoticus.

$$n = 180$$

$$s = 0.12$$

$$\bar{x} = 1.65$$

Shape Index (L/W)

x	$x - \bar{x}$	$x - \bar{x} / s$	Z	E	O	χ^2
1.38	-0.27	-2.25	0.0317	1.93	2	0.0254
1.42	-0.23	-1.92	0.0632	3.84	2	0.8817
1.46	-0.19	-1.58	0.1145	6.96	7	0.0023
1.50	-0.15	-1.25	0.1826	11.09	11	0.0073
1.54	-0.11	-0.92	0.2613	15.87	10	2.171
1.58	-0.07	-0.58	0.3372	20.48	15	1.466
1.62	-0.03	-0.25	0.3867	23.49	41	13.05
1.66	+0.01	+0.08	0.3977	24.16	19	1.102
1.70	+0.05	+0.42	0.3653	22.19	18	0.7911
1.74	+0.09	+0.75	0.3011	18.29	16	0.2867
1.78	+0.13	+1.08	0.2227	13.53	15	0.1597
1.82	+0.17	+1.42	0.1456	8.85	12	1.121
1.86	+0.21	+1.75	0.0863	5.24	6	0.1102
1.90	+0.25	+2.08	0.0459	2.79	3	0.1581
1.94	+0.29	+2.42	0.0213	1.29	3	2.267
Total			2.9631	180.00	180	23.5995

THE LIFE CYCLE AND PATHOGENICITY OF *EIMERIA PHASIANI* FROM THE PHEASANT.

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The development of *Eimeria phasiani* was studied from pure-line infections obtained by feeding single isolated oocysts to 6-day old coccidia-free pheasant chicks. Seven out of twelve chicks receiving single oocysts became infected, passing oocysts on the 6th day. It was found impossible to transmit *E. phasiani* either to partridge or domestic fowl chicks.

The endogenous phase of the life cycle consists of three generations of schizogony followed by gametogony. The first two generations of schizogony are restricted to the first $2/3$ of the small intestine whilst the third generation and gametogony stages are found throughout the small intestine and the lower $1/3$ of the caeca. All stages develop between the host cell nucleus and the basement of the cell.

The first generation schizonts are mature by 48 hours after infection and contain 50 - 100 merozoites measuring $4.5 \times 1.5 \mu$. By 66 hours the second generation schizonts ($9.83 \times 9.03 \mu$) are mature and give rise to 12 - 20 merozoites ($7.43 \times 2.0 \mu$). The third generation schizonts, maturing by 84 - 90 hours, are smaller than the second ($7.70 \times 7.50 \mu$) and produce 12 - 16 merozoites ($5.9 \times 2.0 \mu$). Gametogony follows the third generation of schizogony. The prepatent period averaged 117 hours in 22 experimental infections. The patent period ranged between 10 and 16 days.

The oocysts ($25.16 \times 17.45 \mu$: Shape Index 1.44) are ellipsoidal with a smooth, light brownish wall consisting of two membranes. Sporulation of 50% of the oocysts was completed in 52 hours at 20°C .

An autopsy of birds given doses of 10^6 + oocysts and killed at 84 hours, the intestine showed petechial haemorrhages from the mucous and serosal surfaces, the lumen being filled with mucus speckled with blood and containing merozoites. In most cases the faeces were loose and mucoid.

In sections a marked eosinophilia was present but this was not demonstrable in differential leucocyte counts. Birds given doses of 25,000 and 100,000 oocysts exhibited a reduction in weight gains.

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