1. The Morphology and Life-History of *Eimeria* (*Coccidium*) *avium*: a Sporozoön causing a fatal disease among young Grouse. By H. B. FANTHAM, D.Sc.Lond., B.A.Cantab., A.R.C.S., F.Z.S., Christ's College, Cambridge, Assistant to the Quick Professor of Biology, Cambridge, and Protozoologist to the Grouse Disease Inquiry.

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(Plates LV.-LVIII.* and Text-figure 65.)

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I. INTRODUCTION.

The subject of this memoir is a microscopic, protozoal parasite, which infests the lining epithelium of the alimentary canal of grouse. It belongs to the Coccidia, a group of parasitic Protozoa many of which are known to occur in the digestive tracts of both Vertebrates and Invertebrates. These minute organisms reproduce by means of resistant spores, and belong to that class of the Protozoa known as the Sporozoa. The Coccidia are of economic importance, inasmuch as they destroy the mucous membrane of the intestine of the host, thereby setting up enteritis which is accompanied by diarrhœa, and very often has a fatal effect upon the unfortunate animal harbouring the parasites, especially if the host be young.

Such a disease—termed coccidiosis—has long been known in rabbits, and is often fatal. Occasionally coccidiosis occurs in man. The life-history of a Coccidian parasite is complicated. There are two phases in the life-cycle:—(i) a multiplicative phase within the cells of the gut-epithelium of the host, and (ii) a reproductive phase leading, after a sexual act, to the formation of

* For explanation of the Plates see p. 690.

H.B. Fantham del.

E. Wilson, Cambridge.

EIMERIA (COCCIDIUM) AVIUM.
(Schizogony.)

H.B. Fantham del.

E. Wilson, Cambridge.

EIMERIA (COCCIDIUM) AVIUM.
(Macrogamete formation.)

H.B. Pantam del.

E. Wilson, Cambridge.

EIMERIA (COCCIDIUM) AVIUM.
(Microgametes, Sporogony.)

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EIMERIA (COCCIDIUM) AVIUM.
(Sporogony.)

resistant spores adapted for life outside the body of the host. The spores so formed are the means of spreading the parasite and lead to the infection of fresh hosts. The two phases were formerly considered to belong to separate parasites; but the occurrence of alternation of generations in the life-cycle of *Coccidia* was first suggested by R. Pfeiffer in 1892, conjugation was discovered in *Coccidia* by Schaudinn and Siedlecki (1897), and the complete life-cycle was demonstrated with a wealth of morphological and cytological detail in the celebrated memoir of the late Dr. Schaudinn (1900) on *Eimeria* (*Coccidium*) *schubergi*, parasitic in the gut of the centipede *Lithobius forficatus*.

Coccidia have been recorded from most of the great groups of the Metazoa, but very few *Coccidian* life-histories have been investigated completely.

Dr. Leiper, while working on helminthiasis in grouse in May 1909, noticed the occurrence of *Coccidian* cysts in large numbers in the gut of many grouse-chicks, with concomitant enteritis, very often proving fatal. The *Coccidian* cysts are oval and, at first sight, might easily be mistaken for eggs of worms. Leiper suggested that coccidiosis was a factor in "grouse disease," especially in young birds. Through the courtesy of Lord Lovat I was enabled to spend a few days early in June 1909 on a Scotch moor to investigate coccidiosis in grouse chicks*. Previously we had noticed, at various times, the occurrence of *Coccidian* cysts in adult grouse, but not in large numbers.

I have much pleasure in expressing my thanks to Lord Lovat, Dr. A. E. Shipley, Dr. E. A. Wilson, Dr. Hammond Smith, and Dr. Leiper for aiding my researches by procuring material for me, and to Professor Nuttall, in whose laboratory much of my work was done. I would also thank Mr. A. S. Leslie for placing me in communication with correspondents, and to members of the Grouse Disease Inquiry and many correspondents I am also indebted.

In this paper I wish to record my researches on the morphology and life-history of *Eimeria avium*, more especially as it occurs in the grouse. I would point out that the length of time at my disposal for these researches has been limited, only *one* season being available to me for procuring material, and I have had several other investigations to consider during the period, so that I was not able to give undivided attention to the elucidation of the Protozoa of grouse. However, the complete life-cycle of *Eimeria avium*, responsible for the dwindling of grouse broods in spring, is here set forth for the first time, so far as I am aware.

II. THE GENERIC NAMES *EIMERIA* AND *COCCIDIUM*.

Unfortunately, owing to the rule of priority, the generic name *Coccidium* (Leuckart 1879) no longer holds, but is replaced by that of *Eimeria* (A. Schneider 1875). I am in sympathy with

* The number of grouse chicks dying of coccidiosis on the moors is not easily estimated, for the chicks die in the heather, and their tiny corpses are rarely found.

Professor Minchin when he writes in a recent review:—"We regret to see the familiar generic name *Coccidium* replaced by *Eimeria*; this is one of those many cases where, in our opinion, rebellion against the law of priority in nomenclature is not only lawful but imperative" ('Nature,' March 3, 1910). It would save much confusion if the question of zoological nomenclature were settled by an international committee, as has been suggested by many able workers.

The *Coccidia* of birds were first recorded in fowls by Silvestrini and Rivolta (1873), under the name *Psorospermium avium*. Subsequently Railliet and Lucet (1891) recorded *Coccidia* from fowls, naming the parasite *C. tenellum*. I have followed Dofflein (1909) in naming the *Coccidia* of birds *Eimeria avium*. The *Coccidian* parasites were obtained from grouse chicks, and I have succeeded in transmitting the *Coccidia* of grouse to fowl chicks and to young pigeons.

III. METHODS.

In this investigation of coccidiosis both fresh and preserved materials were used. Samples of gut contents, taken from different regions, were examined fresh, and often these have been fixed wet with osmic or formalin vapour and stained by Delafield's hæmatoxylin or by Giemsa's stain. Such smears were sometimes useful for examining merozoites.

Oöcysts, because of the chitinous and almost impenetrable character of their walls, had to be examined fresh.

For preserved material, the best fixatives were found to be Schaudinn's fluid (corrosive-acetic-alcohol), and Bouin's fluid (picro-formol-acetic) to which a few drops of absolute alcohol were added. Schaudinn's fluid tends to shrink the tissues, while Bouin's fluid requires much washing out. Sections, 5μ to 6μ thick, were made of the duodenum and cæcum of infected birds, these parts of the digestive tract being especially examined. The chief stains used were Delafield's hæmatoxylin (either alone or counterstained with Orange G or eosin), which was found to be most useful, safranin and Lichtgrün, iron-hæmatoxylin (with or without van Gieson's picro-fuchsin) and paracarmine. On the whole the hæmatoxylin proved of most service.

IV. MORPHOLOGY.

On diluting some of the cæcal contents or faeces of a grouse chick suffering from coccidiosis, and examining the preparation microscopically, numerous oval cysts are seen (Pl. LVIII. figs. 61-66). Sometimes the cysts are also seen in the small intestine just beyond the duodenum. These cysts may have homogeneous contents, or, when older, may show four more or less well differentiated sporocysts within them (figs. 67-70). Each sporocyst, if ingested by another grouse, can develop two active, motile germs or sporozoites (figs. 71-76), which can penetrate

the intestinal epithelium—especially of the duodenum—and so begin a new infection. Though the oöcysts and spores are the most obvious external manifestation of coccidiosis, it is usual and certainly more convenient to begin the life-cycle of the *Coccidium* with the minute sporozoite (Pl. LV. fig. 2), the agent whereby primary infection is brought about.

(a) *The young, growing Parasite.*

The sporozoites are minute, falciform or vermicular bodies (Pl. LV. fig. 2) capable of fairly rapid movement and possessing great penetrative powers. They measure from 7μ to 10μ in length. The ends of the sporozoite are rather pointed, the extremity that moves foremost being slightly more acuminate than the posterior end. The general body cytoplasm is more or less homogeneous, exhibiting but very fine granulations. The nucleus has a definite rounded or oval contour. The chromatin is evenly distributed throughout the nucleus.

When the sporozoites are liberated from the investing sporocyst (Pl. LVIII. figs. 74–76), by the action of the pancreatic juice of the grouse, they are capable of active movement. The usual method of progression resembles that of the sporozoite or motile trophozoite of a Gregarine. The organism moves forwards with a slow gliding movement, the forward progression being facilitated by the secretion of a viscid proteid substance that rapidly hardens. On the smooth surface thus provided the coccidian sporozoite glides forward. The track of the sporozoite, as shown by its trail, can be stained, and the organism then shows the gelatinous or proteid material issuing from near the posterior region of its body (Pl. LV. fig. 2). During the gliding movement waves travel down the body of the sporozoite, recalling what is seen on a larger scale in the billowy undulations of the foot of a snail. On other occasions a more rapid movement of the sporozoite occurs. The two ends of the organism become approximated and then rapidly straightened, the effect being to propel the organism forwards much more quickly than when the gliding movement alone is used.

The sporozoite thus makes its way to an epithelial cell of the duodenum and proceeds to penetrate the cell. As it forces its way inwards (Pl. LV. figs. 3, 4), so the sporozoite curves on itself (fig. 7) and becomes round and immobile (figs. 5, 6, 8). The young, rounded parasite (figs. 8–10) is now in the trophic phase of existence and continues to grow for some time, feeding passively on the food-materials of the host-cell. During this period the parasite is called a trophozoite (figs. 8–12 a).

The nucleus of the trophozoite is approximately central in position, or sometimes to one side, and at first contains scattered granules of chromatin. It then becomes somewhat vesicular (figs. 8–11), and gradually the chromatin collects into a central karyosome, lying within the nuclear sap (fig. 12 a). The position

of the karyosome, however, is not centrally fixed; it may lie to one side of the nucleus (figs. 8, 10).

The growth of the trophozoite naturally affects that of the host-cell. The protoplasm of the latter becomes more and more tenuous, great hypertrophy of the host-cell occurring. This condition is maintained for some time and finally a limit is reached and atrophy sets in, the nucleus of the host-cell then appearing as a small, often crescentic mass (figs. 10, 14) to one side of the film (fig. 12) that represents the host-cell. A clear space often intervenes between the parasite and the enveloping epithelial film (figs. 9, 11, 12).

The trophozoite, having attained its full size (some 10μ to 12μ in diameter) within the host-cell, proceeds to divide, and the result of its division is to increase the number of parasites within the host. This stage in the existence of *Eimeria avium* is known as the schizont (agamont), and the method of multiplication is termed schizogony.

(b) *Schizogony.*

The schizont (fig. 12) is a more or less spherical parasite. At first it is uninucleate (fig. 12 *a*), but soon its nucleus begins to fragment (fig. 12 *b*). The division of the nucleus of the schizont is of the nature of multiple fragmentation rather than of a series of binary fissions of the nucleus and karyosome (figs. 13–18). The parasite is very small and it is not easy to follow the cytological details, even under the best and highest powers of magnification. Some of the portions of chromatin in multiple fragmentation may sometimes appear connected by thin strands (fig. 13) for a short period, but the fragments soon travel to the periphery of the schizont. The small daughter masses of chromatin, at first homogeneous, gradually show differentiation, becoming minutely vesicular with a dot of chromatin usually to one side (figs. 14–16), but occasionally central. Thus the nuclei of the future merozoites are forming their karyosomes early.

The daughter nuclei having migrated to the periphery of the mother cell (fig. 16), the cytoplasm of the schizont concentrates around them forming small ovoid masses (figs. 18, 19). The daughter forms so produced are the merozoites (agametes) which measure 6μ to 10μ in length. They gradually acquire a vermiform shape and arrange themselves around the remains of the protoplasm of the mother cell like the segments of an orange or the staves of a barrel (Pl. LV. figs. 20–22, Pl. LVI. figs. 23, 24). Owing to this method of grouping, the merozoites are said to be arranged “en barillet.” The groups, when ripe, soon break up and the individual merozoites are liberated. The movements of the merozoites, when free, resemble those of the sporozoites.

The number of merozoites formed from a single schizont seems to vary. Eight to fourteen seem to be common numbers, but as many as twenty have been found.

The merozoites finally are slightly curved vermicules (Pl. LVI. figs. 23, 24), possessing a nucleus which may be approximately

central (Pl. LV. figs. 20, 21) or somewhat towards one end (Pl. LV. fig. 22, Pl. LVI. fig. 23). The nucleus of the merozoite is small, and the presence of a karyosome is often not very evident, though there is a small granule of chromatin—representing the karyosome—usually to one side of the nucleus (fig. 24). The ends of the merozoites are rather less pointed than those of the sporozoites, a feature that *E. avium* has in common with *E. schubergi* as described by Schaudinn.

When the merozoites reach a new host-cell, they enter, become round, and proceed to grow as trophozoites in the same way as did their parent organism, and undergo later nuclear fragmentation in a similar manner. As the result of this, many more merozoites are produced, and as schizogony may be continued through several generations, the destruction of the gut epithelium is very extensive (Pl. LV. fig. 1).

Towards the end of schizogony—especially in the cæcum—relatively smaller schizonts with larger and fewer merozoites (fig. 25), about five in number on the average, are produced. These larger merozoites appear to be formed near the end of infection, in company with large numbers of gametocytes, so far as evidence is available.

These differences in the schizonts might be taken by some investigators to be indicative of difference of species—in other words, that more than one species of *Coccidium* may occur in the gut of grouse. I do not state that this is not so, but personally prefer the view that the differences in the schizonts and merozoites noted are reflexes of the condition of nutriment of the parasite. Wenyon (1907) has some interesting observations on the variations in the schizogony of *E. falciformis* in the mouse, and states that the variations are due to the nourishment available for the parasite. Again, the species found in the liver and gut of the rabbit (*Coccidium oviforme* and *C. perforans*) are now usually united into one species, *Eimeria stiedæ*.

The merozoites originally produced in the duodenum pass lower down the gut and reach the cæca. At the ileo-cæcal junction, the epithelium is attacked again, and the merozoites rapidly grow to schizonts which produce new generations of merozoites, so that the cæca soon contain very large numbers of the parasites.

Probably coccidiosis set up in the duodenal wall is sufficient to kill very young chicks, *e. g.* chicks 8 to 10 days old, while older chicks dying at the age of about 4 to 6 weeks may have partially recovered from duodenal coccidiosis, but succumb to coccidiosis in the cæcum (typhlitic coccidiosis). In cases of intense duodenal coccidiosis, merozoites are found free in the intestinal contents and even in freshly shed fæces.

Sooner or later a limit is reached, both to the power of the grouse chick to provide nourishment for the parasite, and to the multiplicative capacity of the parasite itself. In other words, *the host begins to react on the parasite. Consequent on the now unfavourable environment, the parasite proceeds to form gametes, in order that its species may be perpetuated.* The

gametocytes or mother cells of the gametes (Pl. LVI. figs. 26-28, Pl. LVII. fig. 37) are modified schizonts which are of slow growth, and therefore can accumulate more reserve food-material in the form of granules within their cytoplasm. The processes leading to the formation of the gametes may be termed gametogony, which we may now consider.

(c) *Gametogony.*

Sexual differentiation is characteristic of the gametes of Coccidia, and in the case of *Eimeria avium* the differentiation is apparent in the gametocyte phase. Two forms of gametocytes can be distinguished. The first group are intra-cellular parasites containing large granules of food reserve within their cytoplasm. These are the macrogametocytes (Pl. LVI. fig. 26) which give rise each to one female gamete. On the other hand, the microgametocytes (Pl. LVII. fig. 37) or male progenitors contain a little reserve food-material in the form of very minute granules, distributed evenly throughout the body substance. Each microgametocyte gives rise to many microgametes.

The Macrogametocyte and Macrogamete.

The structure of the macrogametocyte and of the single macrogamete that arises from it is very difficult of interpretation in *Eimeria avium*, on account of the large amount of reserve food-material contained within the cytoplasm. Further, it is very difficult to draw the exact dividing line between the macrogametocyte and the female gamete, as the one gradually merges into the other. These forms vary from 11.8μ - 17.5μ in length, by 6μ - 11μ in breadth, as seen in sections. Many relatively large granules occur in the cytoplasm of the macrogametocyte. As the macrogametocyte grows from round to ovoid, these granules gradually concentrate to form larger, roundish, hæmatoxylin-staining granules, which are albuminoid (Pl. LVI. fig. 29), the chromatoid granules of many authors. There are also other, non-basic staining, granules known as plastinoid granules ("granules plastiques" of Thélohan and Labbé) composed of coccidin (Labbé). These ultimately are large and round (figs. 30-32) and are refractile in fresh preparations, where they appear yellow or greyish-green. They tend to shrink in preparations fixed with sublimate or sublimate acetic (fig. 29). The plastinoid granules occur between and among the chromatoid granules (figs. 30-32). In stained preparations* the granules are best seen by staining with iron-hæmatoxylin followed by van Gieson's picro-fuchsin, when the chromatoid granules appear blackish, while the plastinoid granules take on a uniform, yellowish hue. The plastinoid

* The reactions of the granules are discussed by Labbé (1896) and by Minchin (1903).

granules stain with Lichtgrün in marked contrast with the red of safranin taken up by the chromatoid granules. With Delafield's hæmatoxylin, the chromatoid granules stain intensely (figs. 29, 31, 32), and somewhat misleading appearances result, suggesting multiplication of the cell.

As the parasite grows, the chromatoid and plastinoid granules travel towards the periphery (figs. 29-32). The macrogametocyte at this time encysts within the epithelium, and the chitinous material of the cyst seems to be formed from the chromatoid granules. At any rate, the inner layer of the cyst-wall seems to take its origin therefrom (figs. 34-36). The formation of the cyst-wall from the chromatoid granules has been noted by Simond (1897) and Wasielewski (1904) in the case of *Coccidium oviforme* (*Eimeria stiedæ* of Stiles).

The formation of the cyst of *Eimeria avium* takes place while the organism is still within the epithelium (figs. 34, 35), and therefore the parasite encysts precociously. The macrogamete at this stage is ovoid, and the number of chromatoid granules within it is reduced. The cyst formed is ultimately rather thick-walled, but a small aperture or micropyle, which may be in a slight depression, is left for the entry of the microgamete (Pl. LVII. fig. 47).

When Schaudinn investigated the life cycle of *E. schubergi*, he described a process of maturation of the macrogametocyte, whereby the karyosome of the nucleus was expelled in fragments. I regret that I am not at all sure as to the fate of the karyosome of the macrogametocyte of *E. avium*. Several causes combine to defeat the attempts made to elucidate this subject. In the first place, the entire parasite (*E. avium*) is much smaller than *E. schubergi* and its karyosome is not nearly so well differentiated a structure. Further, the presence of the chromatoid granules, which stain deeply with basic stains, much confuses the structure. Similar causes prevail in *E. oviforme* (*E. stiedæ*), as I can vouch from personal observation, and Wasielewski (1904, p. 54) states that he is unable to follow the maturation process in *E. oviforme*.

It may be that *E. avium* is like some other Coccidia (e.g. *C. lacazei*, *C. proprium*, *Adelea ovata*) where the karyosome is retained in the gamete and is left behind in the residual protoplasm of the oöcyst. On a few occasions, I have observed a small, chromatin-like granule in the oöcyst residuum (Pl. LVII. fig. 57), and this body may be the karyosome of the macrogametocyte. *E. avium* is distinguished by having a very small cystal residuum, which, together with the smallness of the karyosome, and indeed of the whole parasite, increases the difficulty of investigation. However, at the time of fertilisation, the distinctness of the karyosome of the macrogamete has disappeared (Pl. LVI. figs. 29-36), and the nucleus of the macrogamete appears to contain granules of chromatin which are rather indistinct.

The Microgametocyte and Microgametes.

The microgametocyte* (Pl. LVII. fig. 37) is an ovoid cell about $13\ \mu$ long and $8\ \mu$ broad in the specimens that I have seen. It possesses a central nucleus containing a karyosome. The process of the formation of the microgametes of *E. avium* is as follows:—The chromatin of the nucleus, largely concentrated in the karyosome, breaks up (figs. 38–40) into minute granules or chromidia which pass towards the surface of the cell, where they appear to form a *very* fine chromidial network (fig. 38). The chromidia then collect into groups or patches, arranged in the form of minute, irregular loops with central hollows (figs. 38, 39). These chromatic loops form a number of minute, flexible, rod-like bodies, composed almost entirely of chromatin (figs. 41–45). These are the young microgametes (figs. 43–46). The adult microgametes are small parasites, their chromatic bodies measuring $3\ \mu$ to $4\ \mu$ in length, possessing a rod-like, somewhat curved body composed of a core of chromatin which is surrounded by a tenuous film of cytoplasm, prolonged outwards to form two fine flagella (figs. 44, 46). The flagellum which I term the posterior flagellum trails behind the organism and is practically a continuation of its body; the other flagellum is at the opposite end of the body and so is termed the anterior flagellum. In life, the microgametes are capable of serpentine movements. Owing to the minute size of the microgamete (about $3\ \mu$ to $4\ \mu$ long) it is only with the greatest difficulty that the flagella can be discerned.

The whole of the microgametocyte is not used in the formation of the microgametes. When separation of the microgametes from their mother cell occurs, a large amount of the body-substance of the latter remains as a somewhat faint staining residuum, in which the pale staining remains of the karyosome (fig. 43, *cf.* fig. 38) can sometimes be distinguished. The residuum takes no further part in the vital activities of the parasite.

The microgametes are set free into the lumen of the gut and proceed to seek out the macrogametes.

(d) Fertilisation.

When the macrogamete has attained its maximum development, it often lies in the epithelium, near the outer edge of the tissue, or may even burst through the attenuate wall of the host-cell and so reach the margin of the lumen of the gut. The minute but active microgametes (Pl. LVII. fig. 46) meanwhile have broken away from the residual protoplasm of the host-cell, and swum out with rapid lashing movements of their flagella into the gut. Here they are attracted, possibly by some chemiotactic substance,

* Uninucleate microgametocytes seemed rare in preparations, for the cell rapidly proceeds to form many microgametes.

towards the macrogametes. The microgametes swarm round the micropyle of the macrogamete (Pl. LVII. fig. 47*) and several have been seen trying to enter it simultaneously. The nucleus of the macrogamete travels upwards nearer the micropyle, and before long, one microgamete effects an entry (fig. 47*), appears to bore its way into the female, reach the nucleus and finally be lost to view. The macrogamete secretes a plug of protoplasm across the micropyle, whereby other microgametes are excluded, and the individuals thus shut out degenerate (fig. 48). Such is the process of conjugation as seen in the living organism, and the evidence of stained preparations is fully confirmatory of what has just been described.

Owing to the presence of granules in the macrogamete it is extremely difficult to follow the subsequent stages of fertilisation. Occasionally there are indications of a fertilisation spindle (figs. 50, 51), but the nature of the material frequently precluded observation of the same.

The term "fertilisation spindle" is not exactly a happy one, for the object of that structure is the intimate intermingling of the chromatin of the uniting gametes.

(e) *Sporogony.*

After the microgamete has reached the nucleus of the macrogamete, fusion occurs and a zygote is produced. The contents of the zygote at first fill the oval oöcyst (Pl. LVII. figs. 49-52) but gradually they shrink away from the poles†. The oöcyst itself may increase slightly in size during the concentration of its contents, which ultimately form a globular mass, consisting of cytoplasm rich in fatty matters, within which is a nucleus, usually centrally placed.

The nucleus of the zygote proceeds to divide directly first into two (figs. 53, 56) and then into four (fig. 54), the divisions following one another very rapidly. The granular protoplasm segments around the nuclei, and four sporoblasts (fig. 57) are produced, each sporoblast separating from its neighbours as a small, rounded body (figs. 55-57). Occasionally oöcysts containing two ovoid masses of protoplasm (fig. 82) are seen, but as a rule the form containing four sporoblasts is the one found, the four sporoblasts being formed almost concurrently. The sporoblasts become ovoid (Pl. LVII. fig. 58; Pl. LVIII. fig. 68) and each gradually secretes a tough, chitinous sporocyst, usually differentiated as epispore and endospore, and so becomes a firm, resistant spore (Pl. LVIII. figs. 71-76). A minute amount of the cytoplasm of the zygote is not used in spore formation, but remains within the zygote as a small crystal residuum.

The sporocysts continue within the oöcysts for some time,

* See also Pl. LXII. fig. 3 of my paper on Experimental Coccidiosis (this Journal).

† In some cases the zygote contents of the oöcyst may be slightly nearer one pole than the other.

during which period each sporocyst undergoes developmental changes, leading to the production of actively motile sporozoites. The contents of the spore at first are homogeneous (Pl. LVIII. figs. 68-70), but gradually two refractile bodies or vacuoles appear at either end (fig. 73), and the protoplasm gradually concentrates into two masses, just internal to each vacuole. The nucleus is at first central, but divides into two, and the halves migrate to the opposite poles of the sporocyst (Pl. LVII. fig. 58). The protoplasmic masses gradually displace the polar vesicles, so that the two vacuoles move towards the centre and coalesce (Pl. LVIII. fig. 73), leaving nearly all the protoplasm of the sporocyst in two masses, one at each end (fig. 71). Each of the protoplasmic masses gradually becomes vermiform, extending along one edge of the spore (figs. 71, 72). Two vermiform sporozoites are thus formed (figs. 72, 74), sometimes with their more rounded ends placed at opposite ends of the sporocyst (tête-bêche) (figs. 72, 74, 75), sometimes with the slightly swollen ends side by side (Pl. LVII. fig. 59, Pl. LVIII. fig. 76), the sporozoites being capable of movement within the spore just previous to their escape. There is a slight sporal residuum.

The sporocysts when quite ripe tend to become more pointed at one end (Pl. LVII. fig. 60, Pl. LVIII. figs. 71, 75, 76), where a slight thickening or small Stieda's plate (fig. 71) may appear, which is a point of weakness, for here a rupture may occur under the action of the digestive juices of the fresh host, forming a sort of micropyle through which the sporozoites escape. Partially ruptured sporocysts are sometimes found (Pl. LVII. figs. 59, 60). In the case of grouse chicks dying from acute coccidiosis, ripe sporocysts have been found in the caecal walls themselves, as well as in the caecal contents, though usually mature sporocysts are found in caecal droppings that have been exposed.

The oöcysts of *Eimeria avium* show a fair amount of variation among themselves. Usually the oöcysts are oval (Pl. LVIII. figs. 65-68, 71, 72, 77, 78), actually measured specimens varying from 25μ to 35μ in length and from 14μ to 20μ in breadth. Sometimes the oöcysts are not oval but subspherical (fig. 70), and these are from 18μ to 20μ in diameter. Somewhat pyriform or egg-shaped oöcysts (fig. 69) are intermediate in size between the oval and subspherical forms. Morse (1908) noted the occurrence of both round and oval oöcysts when investigating white diarrhoea of fowls, in which coccidiosis played an important part.

Among the oöcysts of *E. avium* certain were found with somewhat squarish ends (fig. 78) while others had a slight depression at the apex (fig. 79), but their development was identical with that of the more common forms. Occasionally, oöcysts with two sporocysts only (figs. 81, 82) were found, but these were abnormal forms, as was also a parasite (fig. 83) in

which the cytoplasm extended in a cone or funnel-like fashion to the edge of the oöcyst.

The size and shape of the oöcysts are largely a factor of the space in which the macrogamete develops and the amount of food available for the parasite. When there are many *Eimeria* present in any particular region of the gut, the oöcysts produced are relatively small, while where abundance of space and nourishment are available, the oöcysts tend to be large.

From experiments made by feeding birds with Coccidian oöcysts, I conclude that schizogony takes from four to five days. Uninucleate oöcysts mature their sporocysts in two to three days. The period for the total life-history of the parasite would be from eight to ten days.

The larvæ and imagines of *Scatophaga stercoraria*, the dung-fly, ingest the oöcysts of *E. avium* along with the grouse fæces. The oöcysts pass through the bodies of the larvæ uninjured, and are scattered with the excrement, thus serving to disperse the spores to some extent.

V. SUMMARY OF THE LIFE-HISTORY OF *EIMERIA AVIUM*.

The life cycle of *Eimeria avium* is complicated, even though the organism completes its development within one host. The life-history may be represented diagrammatically as in text-fig. 65, A-T (p. 684). Beginning as a sporozoite (Pl. LV. fig. 2; text-fig. 65, A) liberated by the action of the pancreatic juice of the grouse, the parasite rapidly penetrates an epithelial cell of the duodenum (text-fig. 65, B), and, entering the cell, rounds up (text-fig. 65, C) and becomes a passive growing trophozoite (Pl. LV. figs. 3-7; text-fig. 65, D). After a period of rapid growth, during which time the trophozoite (figs. 8-11) practically destroys the cell harbouring it, the parasite enters upon an asexual, multiplicative phase termed schizogony.

The schizont is at first uninucleate (fig. 12; text-fig. 65, D) but the nucleus soon fragments (figs. 12, 13), the daughter nuclei migrate to the periphery (figs. 14-16; text-fig. 65, E), cytoplasm segregates around each (text-fig. 65, F), and the daughter forms thus produced become meridionally arranged, like the segments of an orange, the arrangement of the merozoites being "en barillet" (Pl. LV. figs. 17-22, Pl. LVI. figs. 23-25; text-fig. 65, G).

Each merozoite is a small, vermicular organism, having a nucleus with a somewhat ill-defined karyosome usually to one side (fig. 24). The groups of merozoites break up (text-fig. 65, H), and the free germs seek out and enter an hitherto uninfected cell where the parasite again assumes the trophic phase and then undergoes division as before. Several successive generations of schizonts and merozoites are thus produced, resulting in a great destruction of the gut epithelium of the host. Finally a limit is

Text-fig. 65.

DIAGRAM of Life-cycle of *Eimeria (Coccidium) avium*.

D-H represent Schizogony. I-L, Gametogony. N-T, Sporogony.

Epithelial host-cells diagrammatically outlined.

A. Sporozoite which penetrates epithelial cell of the duodenum of the host. B. Sporozoite curving on itself before becoming rounded within the host cell. C. Young, uninucleate trophozoite. D. Fully grown trophozoite or uninucleate schizont.	E. Schizont with numerous daughter nuclei peripherally arranged, differentiating to form merozoites (seen in transverse section). F. Schizont showing further differentiation of merozoites (seen in oblique section).

reached to the ability of the host to provide nourishment and to the multiplicative powers of the parasite, and this results in the onset of sexual differentiation. Gametogony may occur both in the duodenum and cæcum.

Certain schizonts become considerably modified in one of two directions. In the first case, food-material accumulates, and a large, uninucleate food-laden form is produced (figs. 26-30). This is the macrogametocyte (text-fig. 65, I ♀), destined to give rise to a single macrogamete (text-fig. 65, J ♀). In the second instance (text-fig. 65, I ♂), nuclear multiplication of a finely granular parasite (Pl. LVII. fig. 37) occurs, and the many minute nuclei produced reach the periphery of the host-cell (figs. 38-42), and ultimately the now multinucleate microgametocyte (text-fig. 65, J ♂) gives rise to a large number of small, motile microgametes (figs. 43-46; text-fig. 65, K ♂). The macrogamete early on invests itself with a cyst-wall (figs. 35, 36) in which a thin part or micropyle is left for the entry of the microgamete (figs. 47, 48; text-fig. 65, K ♀). This oöcyst-wall is formed while the parasite is within the epithelium.

Fertilisation (figs. 47, 48; text-fig. 65, L) occurs—the process

Explanation of Text-fig. 65 continued (see opposite).

<p>G. Merozoites arranged "en barillet," about to issue from host cell. At one end of the cluster residual proto-plasm is seen.</p> <p>H. Free merozoites, each with a small karyosome.</p> <p>I. {
 ♀. Young macrogametocyte with chromatoid granules.
 ♂. Young microgametocyte with fine granulations.
 ♀. Macrogamete with round nucleus and chromatoid granules distributed through the cytoplasm.</p> <p>J. {
 ♂. Microgametocyte with nucleus divided to form a large number of bent rod-like portions, the future microgametes. Remains of karyosome seen in centre.
 ♀. Macrogamete showing plastinoid granules, the chromatoid granules being largely used up in forming the wall with which the macrogamete has invested itself, the remaining chromatoid granules being arranged between the plastinoid granules.</p> <p>K. {
♂. Microgametocyte with many biflagellate microgametes about to separate from it. Karyosome left at the centre.</p> <p>L. Fertilisation. One microgamete is penetrating the macrogamete, while other male cells are near the micropyle but will be excluded.</p>	M. Fertilisation. The male pronucleus which entered through a micropyle is lying above the female chromatin. Degenerating microgametes are shown outside the oöcyst. N. Oöcyst (encysted zygote) with protoplasmic contents filling it completely. Nucleus with signs of fertilisation spindle. O. Oöcyst with contents concentrated, forming a central, spherical mass which has a vacuole in the middle and the nucleus to one side. Many such cysts seen in infected caecal droppings. P. Oöcyst with four nuclei. Q. Oöcyst with contents segmented to form four rounded sporoblasts (as seen in fresh preparations). R. Oöcyst with four sporoblasts which have grown oval and are becoming sporocysts; the small cystal residuum seen to one side. S. Oöcyst with four sporocysts in each of which two sporozoites are differentiated. The oöcyst in this condition ultimately opens, liberating the sporocysts, the upper one of which is seen about to issue from the oöcyst. Slight cystal residuum. Sporol residuum in each sporocyst. T. Sporocyst which has issued from oöcyst. Two sporozoites are within it and have assumed the position most suitable for emergence.

has been watched in life—and the micropyle is then closed (figs. 49–51; text-fig. 65, M). The fertilised oöcyst (text-fig. 65, N) then passes into the lumen of the gut and is voided with the faeces of the grouse. The further development of the oöcyst largely depends on climatic conditions. Under the influence of warmth and moisture, the contents of the oval oöcyst (Pl. LVII. fig. 52, Pl. LVIII. figs. 61, 62, 64) shrink away from the poles and become a rounded, central mass (figs. 65, 77, 78; text-fig. 65, O). The nucleus rapidly divides into two (Pl. LVII. fig. 56), then four (fig. 54; text-fig. 65, P); each nucleus has protoplasm segregate around it (figs. 55, 67, 70; text-fig. 65, Q), a wall is secreted, and the net result is that four sporocysts (figs. 58, 71–76; text-fig. 65, R) are produced within the oöcyst. Within each sporocyst, two sporozoites gradually differentiate (figs. 58–60, 71–76; text-fig. 65, S), and when the sporocyst (text-fig. 65, T) is ingested by a new host, the sporozoites creep out of the sporocyst softened by the pancreatic juice of the new host and proceed to attack the epithelium of the gut, producing thereby the primary infection of the bird.

The main differences between *Eimeria avium* (Silvestrini and Rivolta) and *E. schubergi* (Schaudinn) may be briefly summarised:—

1. *E. avium* is smaller than *E. schubergi*.
2. The merozoites of *E. avium* are arranged 'en barillet,' those of *E. schubergi* 'en rosace.'
3. Precocious encystment of *E. avium* occurs before fertilisation. This is not the case with *E. schubergi*.
4. Fertilisation in *E. avium* is micropylar; in *E. schubergi* a cone of reception is formed by the macrogamete.
5. The macrogamete of *E. avium* contains much more deeply staining reserve food-material than that of *E. schubergi*, thereby increasing the difficulty of minute examination of the parasite.
6. The cysts of *E. avium* are oval, those of *E. schubergi* are round.

VI. THE EFFECT OF *EIMERIA AVIUM* ON THE HOST.

The effect of coccidiosis on the grouse may now be considered briefly, fuller details regarding the symptoms of the disease and its effects being given in my paper relating to experimental coccidiosis (P. Z. S. 1910, pp. 708–722).

External effects.—The chief external evidence of coccidiosis is the pale colour and great fluidity of the caecal (soft) droppings of the grouse, the pale tint being due to myriads of oöcysts and the condition being that of diarrhœa. Sportsmen and keepers have noted diarrhœa as a symptom of "grouse disease." A similar disease in fowls is known among poultry-men as "white diarrhœa." As the Coccidian parasites cause great denudation

of the intestinal epithelium, digestive derangements are brought about, and consequent on this, malnutrition occurs and the bird becomes very emaciated and "anæmic." Feathering also is poor and ragged, leg weakness is fairly common, and a peculiar bluish tint is sometimes seen at the cere, ears and other parts.

Distribution and Effects on Internal Organs.

Eimeria avium appears to be purely a parasite of the gut of the grouse and does not affect such gut diverticula as the liver. The crop and gizzard of infected birds are rarely parasitised, though they may contain oöcysts in the condition in which they have been ingested with food. Examination of the duodenum shows that the sporocysts ingested with the food are attacked by the pancreatic juice (as I have proved by pancreatic digestion experiments, using both natural pancreatic juice and trypsin), and the sporozoites are set free. These invade the tissue of the duodenum, rapidly become schizonts and multiply, the result being that the duodenum is often riddled by the parasites, and consequently inflamed. Both the villi and the crypts of Lieberkühn are attacked, and the parasites have also been found, though much more rarely, in the submucosa. Great hypertrophy followed by atrophy of the epithelial host cell occurs, and the tissue attacked is often reduced to a finely granular, structureless mass. Desquamation of the gut is common, and epithelium containing various developmental stages of the parasite can be found floating free in the gut contents.

Some of the merozoites formed in the duodenum pass down the gut, reach the cæca and re-commence their life cycle there. Active schizogony and sporogony go on in the cæca*, chiefly in the epithelium, very rarely in the submucosa. Often the cæca are as heavily parasitised as the duodenum, whole areas being completely denuded of the epithelium, especially when the fertilised oöcysts pass outwards into the cæcal contents. The walls of the cæca are often rendered very thin and tender by the action, direct and indirect, of the parasite. Ripe oöcysts and sporocysts occur in the lumen of the cæca of dying chicks.

Podwyszożki (1890) stated that he found Coccidian oöcysts in the vitellus of eggs of fowls, especially in summer. He considered it possible that the cysts were derived from Coccidia in the oviduct of the mother, or perhaps from intestinal Coccidia which had ascended by way of the cloaca. I think that cloacal contamination was the more probable, for I have never seen Coccidia in the genitalia of adult grouse examined.

A reflex of coccidiosis is seen in the blood of infected birds, where polymorphonuclear leucocytosis is induced.

* Coccidiosis may sometimes occur along the entire length of the small intestine, and gametes may be formed far forward, in the duodenum.

Lesions caused by coccidia in the mucous membrane may admit bacteria to the circulation of the host. [Cobbett and Graham-Smith (1910), pp. 24, 25]. Rettger (1909) believes that "white diarrhoea" of fowls in America is due to a bacterium, while Morse (1908) considers that it is primarily due to coccidiosis. The discrepancy between the results of these American workers is thus capable of explanation.

VII. CONCLUDING REMARKS.

Eimeria avium of grouse is not restricted to this particular bird, for by administering faeces containing oöcysts from diseased grouse to young fowl chicks and pigeons, I have been able to reproduce the disease exactly as it occurs in grouse (p. 674). M'Fadyean (1894) found coccidiosis in pheasants, while "white diarrhoea" of fowls has been the subject of much investigation, particularly recently in America, where Morse (1908) and Hadley (1909) have worked on the subject. Morse's account of preventive measures is very good and he also notes coccidiosis in many other birds, but the figures of the complete life-cycle of the parasite are not yet published. Labbé (1896) also has described coccidiosis in certain marine birds.

Though coccidiosis is peculiarly fatal to grouse chicks during the first few weeks of their lives, adult grouse also can become infected, for I have examined an adult bird that probably died of coccidiosis. Old birds in the chronic condition serve as reservoirs of oöcysts and so may form sources of new infections on the moors. All infected corpses should be burned, *not* buried.

I may add that, while correcting the proofs of this memoir, I have been able to examine pheasant chicks dying from coccidiosis, the birds being obtained through the courtesy of Drs. Shipley and Hammond-Smith from various parts of England during June and July 1910.

The economic importance of coccidiosis in birds, especially in the young, is evident, and I trust that this work, which to the best of my belief is the first fully illustrated and detailed life-history of an avian Coccidium, may draw more attention to a subject both of great scientific interest and of practical importance.

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EXPLANATION OF PLATES LV.-LVIII.

All figures outlined with Abbé-Zeiss camera lucida, from stained preparations (sections of infected gut) except where otherwise stated. Zeiss 2 mm. apochromatic immersion objective and compensating ocular 8 usually employed. The magnification is about 1400 diameters, except where indicated otherwise. Many parasites are represented lying in portions of the degenerating epithelium surrounding them.

PLATE LV.

Fig. 1. Portion of gut (cæcum) of infected grouse to show mucous membrane riddled with parasites. Many stages in life-history of *Eimeria avium* shown therein.

2. Sporozoite from duodenum, showing its trail stained.
3. Sporozoite penetrating epithelium.
4. Young parasite just entered epithelium, by fine channel which is still open. The entering parasite is by the side of a slightly older (rounded) parasite or young trophozoite.
- 5, 6. Parasites beginning to become round.
7. Sporozoite, curved on itself, preparatory to becoming round. Duodenum.
8. Round, young parasite, with relatively vesicular nucleus containing karyosome.
9. Young trophozoite, as yet oval in shape, lying in relatively large space in the gut-wall.
- 10, 11. Round trophozoites, with large nuclei each containing a karyosome. Large space surrounding parasite shown in fig. 11.
12. (a) Uninucleate, full-grown trophozoite, becoming a schizont.
(b) Schizont in which nucleus is dividing. Duodenum.
13. Schizont in process of nuclear fragmentation.
- 14, 15, 16, 17. Schizonts containing many nuclei which go to the periphery of the cell. Very small karyosome differentiating to one side in each daughter nucleus.
- 18, 19. Developing merozoites.
- 20-22. Merozoites arranging themselves "en barillet," with residual protoplasm in middle or to one end.
Several smaller and younger parasites shown by the side of the "barillet" in fig. 22.

PLATE LVI.

Fig. 23, 24. Groups of merozoites, beginning to separate.

Fig. 23 magnification 1400. Fig. 24 magnification 1600.

25. Small schizont, containing five young merozoites—seen in cross-section. Cæcum.
26. Macrogametocyte, with granules of reserve food-material.
- 27, 28. Young macrogametocytes.
29. Longitudinal section of macrogamete, stained with Delafield's hæmatoxylin, showing deeply staining chromatoid granules, a few of which are surrounded by clearer areas of plastin.
30. Transverse section of older macrogamete, stained with iron hæmatoxylin and picro-fuchsin, with cyst just formed. Plastinoid granules well defined; chromatoid granules (largely used in cyst formation) small and in between the plastinoid granules.
- 31, 32. Macrogametes, in section, as stained by Delafield's hæmatoxylin. Chromatoid and plastinoid granules alternating. Plastinoid granules do not stain with hæmatoxylin. $\times 1600$.
Young macrogametocytes seen in the vicinity.
- 33, 34. Macrogametes with chromatoid and plastinoid granules, after careful staining with iron hæmatoxylin and picro-fuchsin. In fig. 34 chromatoid granules are seen passing to the periphery, to form the inner wall of the mature macrogamete.
- 35, 36. Mature macrogametes. Remains of chromatoid granules seen attached to inner wall of cyst.

PLATE LVII.

Fig. 37. Microgametocyte or male mother cell.

38-40. Nuclear fragmentation of microgametocyte. In fig. 38 remains of parent karyosome seen. In fig. 40 young microgametes arranged along meridians.

41, 42. Young microgametes at periphery of mother cell.

43-45. Separation of the clusters of microgametes.

46. Free microgametes.

47. Fertilisation. One microgamete has gained access to the female pronucleus. The micropyle has closed, and the microgametes left outside will degenerate.

48. Fertilised oöcyst. Zygote nucleus beginning to form a fertilisation spindle. Remains of microgametes on outside of cyst.

49. Zygote in oöcyst just after fertilisation.

50, 51. Show "fertilisation spindle"—not very clearly differentiated in *Eimeria avium*.

52, 53. Longitudinal and transverse sections of oöcysts, containing rather a large amount of reserve material—as judged by staining (hæmatoxylin). In fig. 53 the zygote nucleus shows signs of dividing.

54. Oöcyst with four nuclei, one ready for each sporoblast. Safranin, Lichtgrün.

55. Small oöcyst with four round sporoblasts. Paracarmine.

56. Oöcyst, showing segmentation into four sporoblasts. From lumen of cæcum of dying grouse chick.

57. Oöcyst, with four ovoid sporocysts, and crystal residuum, containing minute dot of chromatin (? remains of karyosome of macrogamete). Cut obliquely.

58. Oöcysts with four sporocysts showing commencement of differentiation (polar separation of nuclei) into sporozoites. Paracarmine.

59, 60. Sporocysts, each with two sporozoites. Dehiscence represented in fig. 60.

PLATE LVIII.

Mostly from fresh preparations. $\times 1000$.

Fig. 61. Zygote nearly filling oöcyst. Culture of cæcal contents of grouse containing such oöcysts made and examined for four days subsequently.

62, 63, 64, 66. Development at beginning of second day.

65. Cyst uninucleate, such as is frequently seen in fresh fæces.

67. Oöcyst with four sporoblasts, as seen at end of second day, and on third day.

68. Oöcyst with four ovoid sporoblasts, as seen in cultures on the third day.

69-70. Illustrate three common forms of oöcyst of *E. avium*—oval, egg-shaped, and rounded. There is variation among the oöcysts.

71. Oöcyst with four sporocysts, in each of which sporozoites are differentiating.

72. Oöcyst with four sporocysts, nearly ripe, each containing two sporozoites. From partially dried cæcal droppings, taken from grouse moor.

73. Ripe oöcyst dehiscing—one sporocyst outside, with sporozoite nuclei at poles, and central vacuole. Sporocyst inside oöcyst shows polar vacuoles.

74. Free sporocyst, from fæces. Sporocyst contains two sporozoites, arranged "tête bêche," and sporal residuum.

75, 76. Sporocysts, each with two sporozoites.

77. Oöcyst, commonly occurring in fresh droppings.

78. Oöcyst with somewhat square ends.

79. Oöcyst with depression (thin place in cyst-wall) at one end.

80. Zygote in oöcyst—zygote nucleus dividing into two. Stained preparation.

81. Oöcyst containing two precociously separated sporoblasts. There should be, normally, four sporoblasts. Probably the oöcyst represented will degenerate. Stained preparation.

82. Oöcyst with two sporoblasts. Very few of these seen. Possibly abnormal. The four sporoblasts should be formed almost concurrently in the normal cyst.

83. Oöcyst showing protoplasmic contents in form of cone-like projection attached at one point to the cyst. Development retarded, after keeping three weeks in water.