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Eimeria avium: A morphological study.

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(With Plate 1 and 2.)

Contents.

I. Introduction and historical résumé.

The researches of COLE and HADLEY (1908, 1910) have shown the relation of a species of coccidium to a wide-spread and highly infectious disease of turkeys and other poultry, and those of MORSE (1908) and of HADLEY (1909) have shown the relation of the same coccidium [1]) to a fatal disease of young fowl, commonly known among poultrymen as "white diarrhea". The coccidium in question is probably identical with that described for poultry by EIMER (1870), RIVOLTA (1878), PODWYSSOZKI (1890), ECKHART (1903) and numerous other writers, and mentioned in several works on pathogenic protozoa under the head of *Coccidium avium* or *Coccidium tenellum.* Althougth next to the rabbit coccidium, this coccidium of poultry is probably the most commonly known parasite, there appears to have been published but few data bearing upon the morphology of organism. In connection with the practical handling of coccidiosis in poultry, a disease which each succeeding year is causing an increasing loss to the poultry industry of the United States, it was believed that no definite and permanently effective steps could be taken in preventing, or in intelligently combatting, this malady until some knowledge was at hand regarding the morphology and biology of the causative organism, including its distribution, life in the soil of the poultry yard, natural tenacity of life, resistance to chemical and thermal stimuli, relation to other micro-organisms, and also its life-cycle, both within and outside of its natural hosts. It is the aim of this study, of which the present paper constitutes the first part, to introduce certain facts gained from a study of the coccidium in question, as found in cases of coccidiosis studied in the poultry department of the Rhode Island Agricultural Experiment Station, Kingston, R. I., U. S. A. Here, the wide-spread occurrence of coccidiosis in various species of birds which frequent the poultry yards, and in many other wild birds as well (HADLEY 1910), has afforded an excellent opportunity for this work, and for gaining from supplementary laboratory experiments, a concise knowledge of the causative organism.

Historical résumé. — Coccidia were first observed by HAKE (1839) in the liver of the rabbit, although he did not recognize their true nature. The relation between these forms and the Psorosperms

[1]) Called by MORSE (op. cit.) and others *Coccidium tenellum.*

of JOHANNES MÜLLER and RETZIUS (1842) was recognized by REMAK in 1845. Little more was learned regarding their nature until 1879 when LEUKART, as a result of his study of the rabbit coccidium, separated them from the Myxosporida, and, giving them the name Coccidia, placed them with the Sporozoa.

Most of the early work upon the coccidia was confined to observations of the oöcysts, and it was many years before other stages of development were discovered. In fact, although the formation of sporoblasts was observed by KAUFMANN as early as 1847, the sporozoites were not discovered until 1884 when BALBIANI first found them in material from the rabbit. The first comprehensive revision of the previous work on coccidia was published by A. I. SCHNEIDER (1881). In this work SCHNEIDER distinguished between the Monosporidia, the Oligosporidia and the Polysporidia. The first group was characterized by having a single spore-containing body, the second by having two spore-containing bodies; and the third by having more than two. Into this system SCHNEIDER brought all the forms of coccidia which were known at that time.

This method of classification soon met with opposition. In 1890, R. PFEIFFER (1890) observed that in the coccidium of the rabbit there occurred a form which gave rise directly to small spores (the merozoites). These "merozoite-cysts" were analogous to SCHNEIDER's group of Monosporidia, and seemed to indicate that there might be a kind of dimorphism in the rabbit coccidium. This afterward proved a discovery of the greatest importance, but was contested by SCHNEIDER who could not accept PFEIFFER's conception of the indentity of the Monosporidia with one of the dimorphic forms of the rabbit coccidium (the merozoite-cyst). The following year, however, R. PFEIFFER (1891) confirmed the discovery of R. PFEIFFER and consequently inferred that SCHNEIDER's Monosporidia were only one form of reproduction of the coccidium, the other form of reproduction being by means of sporoblasts. L. PFEIFFER was also the first to distinguish between auto-infection, and extra-infection, and thus further extended the theory of dimorphism which was already winning greater favor. LABBÉ (1896), however, while admitting the simultaneous occurrence of both forms in the same organism believed they were distinct, and explained the condition on the basis of a double infection. LABBÉ also modified SCHNEIDER's original classification as follows: He separated all coccidia into the Oligoplastids, and the Polyplastids. The former were analogous to the Oligosporidia of SCHNEIDER. The Polyplastids,

however, he divided into the genera *Eimeria*, or coccidia which formed the sporoblasts, and the *Pfeifferia*, which divided into sporozoites directly. Of these last two genera, the former was equivalent to SCHNEIDER'S Polysporidia, while the latter were analogous to SCHNEIDER'S Monosporidia.

The two views remained in opposition until 1897 when SIMOND (1897) performed an experiment which threw new light upon the controversy. SIMOND secured six young rabbits which he fed from birth, upon sterile milk. To one of them he fed rabbit coccidia by mixing them with milk. This rabbit, so fed, sickened in eight days and was killed on the eleventh. In the intestines were found both the *Eimeria* and *Pfeifferia* forms of LABBÉ. These observations of SIMOND threw more discredit upon the view of SCHNEIDER, while they, at the same time, furnished new evidence for the theory of dimorphism first enunciated by PFEIFFER (op. cit.). From this time on, evidence for the existence of two methods of development was extended by SCHUBERG, PODWYSSOZKI, SCHAUDINN, SIEDLECKI, and others, to other coccidia, and soon the theory of dimorphism, as now understood in the development of the merozoites and the sporozoites, gained general acceptance.

Although in the last twenty years many new species of coccidia have been discovered in many different species of animals, the coccidium of the rabbit, which was the first known, has remained the favorite subject of investigation, and careful observations have been made by METZNER (1903), and by WASIELEWSKI (1904). The latter writer has given us the fullest account yet published of the life history of *Eimeria stiedae.*

Although the common coccidium of poultry has likewise been known for many years, there has not been, so far as the writer knows any publication dealing, in a complete way, with either the morphology or the biology of this organism. The descriptions which exist are for the most part fragmentary. The first mention of coccidia as a pathogenic agent in birds was made by EIMER (1870) who saw the so-called "Psorospermien" in the intestines of sparrows and fowls. EIMER, however, made no observation on the morphology or development. The next references to coccidia in fowls was made by RIVOLTA and SILVESTRINI (1873), and again subsequently by RIVOLTA in 1878. RIVOLTA had observed in the submucosa of the intestines of dead fowls, white points the size of a poppy seed. These small sacs, which had a diameter of 40 μ to 80 μ enclosed a multitude of "navicelles" appearing like encapsulated gregarines,

having a length of 11,4 μ to 14 μ. Already in 1869 RIVOLTA (1869) had also observed in animals certain "psorosperms", and although EIMER (op. cit.) had suggested that the "gregarines" might develop into the "psorosperms", RIVOTA in his 1878 publication preferred to conclude that the two forms were distinct. This was because, first, the psorosperms were in epithelial cells, while the "gregarines" were in the submucosa; second, psorospermosis might be present without gregarinosis; third, crows might be affected with gregarines without manifesting psorospermosis. RIVOLTA left no record of the morphology of the elements described, aside from a brief verbal description.

Practically no further studies were made upon the coccidia of poultry until the work of RAILLET and LUCET. These writers (1890) describe an epidemic among geese, in which the epithelium of the uriniferous tubules was attacked by coccidia somewhat resembling *Eimeria stiedae* (*Coccidium oviforme*) of the rabbit's liver. These coccidia were however smaller and more round. The size is given as 20 μ to 22 μ by 13 μ to 16 μ. The encysted coccidia are also described. In a subsequent communication RAILLIET and LUCET (1890 b) mention *Coccidium tenellum* in fowls. This form is described as having poles which are equally round. The average size is given as 21 μ to 25 μ by 17 μ to 19 μ. All of the work of RAILLIET and LUCET on coccidiosis centers especially about the pathological features of the disease produced by the organisms, and very little detail is presented on the morphology of the organisms. It can scarcely be doubted, however, that the organisms observed by these writers in geese and in fowls, are identical with the coccidium which forms the subject of the present study, and which will here be called *Eimeria avium*.

Since the publication mentioned above, the coccidia have been mentioned from time to time by writers [1]) who were interested primarily in the clinical features of coccidiosis, and practically nothing was added to our concise knowledge of the morphology of the organism until COLE and HADLEY (1910) published a brief outline of the morphological features which characterized the coccidium found in poultry and wild birds in the United States.

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[1]) See PODWYSSOZKI (1890), MAC FADYEAN (1894, 1894 a), ECKHART (1903), MORSE (1908).

II. **Material, Methods and Technique.**

Material. — The material used in the present investigation came from cases of coccidiosis occurring in the eastern part of the United States. The greater amount, however, was from birds in the poultry yards of the Rhode Island Agricultural Experiment Station at Kingston, Rhode Island, where, for many years, coccidiosis has been prevalent. Some cases from which valuable material was obtained were typical cases of blackhead in turkeys, both young and old. In other instances the material was obtained from cases of intestinal coccidiosis in fowls, or from cases of coccidial "white diarrhea" of chicks, while still other specimens came from coccidial lesions in geese, ducks, pheasants, guinea fowl, pigeons, quail, grouse, woodcock, thrushes, juncoes, sparrows, and other wild birds. In all these birds the lesions may sometimes be observed macroscopically, although in the majority of instances, coccidiosis may be present in chronic form with no apparent macroscopic lesion.

In the majority of cases the coccidia were isolated from the ceca, the intestines, or the duodenum. In several instances, however, they were obtained from the bile-duct, gizzard, proventriculus, mucous membranes of throat, cloaca, and from the excrement of birds affected with the disease in either acute or chronic form. During the course of the investigation the coccidia were studied in fresh preparations, in smears, and in sections. In all cases great value was attached to an examination of the same material at least by two, and if possible by all three methods. It may be said that this is the only procedure by which the relation of different histological elements to one another may be clearly ascertained, and misinterpretations avoided.

Fresh material was examined by placing it, together with a drop of serum, body fluid, or normal salt solution, upon a clean slide and imposing a cover glass which was gently pressed down. After the examination of such preparations, smears, or spreads were often made from them by pushing the cover glass across the slide. Before drying, these smears were fixed either in methyl alcohol, for staining by a rose-anilin-violet and methyl blue (VAN GIESON) method, or in absolute alcohol for staining by the ROMANOWSKY method.

Material to be sectioned was fixed in DOFLEIN'S corrosive-acetic-alcohol fixative, ZENKER'S fluid, or PETRUNKEVITCH'S fixing medium.

Generally speaking, DOFLEIN's medium gave the most satisfactory results. The sections were cut in paraffin, 3 μ to 12 μ in thickness.

The staining may be considered under the head of first, smears, then sections. The most satisfactory results in staining various stages of coccidia present in the smears, was found to be ROMANOWSKY's malarial stain.[1])

The VAN GIESON stain was also used with good effect, especially as a rough method for first detecting the presence of coccidia in the tissues. It gives, however, only slight differentiation of nuclear substance.[2])

The principle stains used for the sections were DELAFIELD's haematoxylin and eosin, iron haematoxylin and eosin, methylene blue and eosin. The first two gave good nuclear differentiation, but because of better differentiation of the tissue elements, the first proved rather more satisfactory. Long staining (24 hours) in a very weak haematoxylin (1 cc stain to 200 cc of water), with liberal use of acid alcohol in decolorizing, gave the best results. By this method the coccidia stain pink or lavender, with dark blue nuclei, while the nuclei of the tissue cells stain blue. Different staining methods are required to show to best advantage different stages of the coccidia. For instance, the merozoites can seldom be seen well in sections stained with haematoxylin and eosin, but are beautifully shown in smear preparations stained by the ROMANOWSKY method.

Since all of the stages of development of the coccidia are seldom to be seen in a single case, it is necessary to make the best use of such stages as each case offers, and to wait patiently for others which shall contain other desirable elements. The schizont stage

[1]) The method of using this stain for coccidia has been given elsewhere (HADLEY 1909a) but may be briefly stated as follows: the slides, after removal from the absolute alcohol are dried in air, then placed face downward in a shallow glass dish the bottom of which has been flooded with the stain. This has given the best results when prepared as follows: Mix sixteen to twenty drops of ROMANOWSKY's solution No. 1 with 10 cc of solution No. 2. The precipitate which forms is no hinderance to staining. Staining should be continued for 15 to 20 minutes, or until the nuclear elements stand out in clear reddish purple.

[2]) This stain was used as follows: To 10 cc of distilled water add 2 drops of a saturated alcoholic (95 %) solution of rose anilin violet, and 10 drops of a 50 per cent aqueous solution (saturated solution diluted one half) of methylene blue. When the smears are dry, after immersion in methyl alcohol, the slides are flooded with the stain and gently heated over a free flame until steam arises; where-upon the stain is poured off, and the slides are washed in water, dried by compression with filter paper, then in air, and examined directly in oil without use of a cover glass unless permanent mounts are desired.

of the organism is found commonly. Good representations of the mature macrogametocytes and microgametocytes, in large numbers, are less often met with, and usually not more than one in twenty-five cases show the merozoites, or the merozoite cysts to advantage. The birds usually live through the height of merozoite formation.

The cysts, in the early stages of development, are so common that they can be studied without difficulty in fresh preparations. Since, however, the later stages, involving the division into sporoblasts, are seldom, if ever, met with in the birds themselves, it is in all cases necessary to keep these in some condition in which their development may continue. Abnormal development frequently occurs in putrifying solutions. The best results were obtained by placing the cyst-containing material into Petri dishes or stender dishes, and covering it with a 5 per cent aqueous solution of potassium bichromate. In this medium the cysts will develop normally, and may be removed, from time to time, to watch the course of sporulation. When sporozoites are fully developed within the sporoblasts, the latter may be crushed, by pressure of the cover glass, and the sporozoites are left free for observation, or for staining by the iron-haematoxylin method.

III. **The life cycle of the coccidium and the infective process.**

The life cycle of most species of the Tetrasporocystidae, is, in all probability very much the same, and has been described by other writers. In all cases of coccidial enteritis of poultry the disease is initiated by the ingestion, with the food or water, of a variable number of adult cysts (Pl. 1, Fig. 1), or cysts in which the sporulation has already commenced, as shown by the presence of the sporoblasts within the cysts (Figs. 4, 5, 6). Although the occasional infection of the mucous membrane of the mouth, throat, crop and proventriculus indicates that, under certain conditions, the cysts may sporulate before reaching the small intestines, in the majority of cases there is no general liberation of sporozoites before the cysts have reached the duodenum, and the thick cyst-wall has been acted upon and softened by the ferments or enzymes poured into the duodenal flexures from the pancreas, which lies between them. It is possible that in many cases, although a softening of the cyst wall occurs in the duodenum, the liberation of the sporo-

zoites does not take place until the cysts have reached the lower part of the small intestine, or more often, the ceca, in which the cysts may be retained for many days before they are passed on, and finally out of the intesinal tract; and it is probably due to the long retention of the cysts in the cecal pouches that the ceca are so frequently the original point of attack by the coccidia; and, in the case of the turkey, at least, usually show the first observable lesions. The duodenum and upper segment of the small intestine is more frequently involved in fowls, while, in the turkey, the ceca only are more often attached. The reason for this difference is not clear, although it may be due to the fact that sporulation is more rapid (though followed by a smaller number of actual infections) in the fowl than in the turkey. The reason for this, in turn, may lie in a difference in the character of the pancreatic fluids in the two species, and in their phisiological action upon the cyst wall, or upon the coccidia themselves.

However this may be, when the sporozoites have been liberated, they at once penetrate the nearest epithelial cells, and, within the cells, usually at the base, growing at the expence of the cell-contents, develop rapidly into schizonts (Pl. 1, Figs. 16—20). After a period of growth these manifest a division of the nucleus, which marks the beginning of the formation of the merozoites. These develop slowly, with their anterior ends imbedded in the "Restkörper" or residual body, until the merozoite cyst breaks down and liberates the merozoites, either in the tissues of the mucosa or in the cecal or intestinal content. If the former, there results a growth of the coccidial lesion at or near the original point of infection; if the latter, the infection is spread perhaps to adjacent, perhaps to remote regions of the epithelium or, perhaps, mucosa.

There is another point of interest in the present connection regarding the location of the coccidia when they have invaded the mucosa. Coccidia have usually been considered as parasites of the epithelial cells exclusively. The work of Cole and Hadley (op. cit.) demonstrated, however, that they were to found not only in endothelial cells, but also between these cells in the connective-tissue spaces. The large merozoite-cysts ordinarily develop in the mucosa, sometimes within the host cells, but more frequently not. How it happens that these stages of coccidium are found, as a rule, beneath the epithelium is a matter not well understood. Although it does not lie within the province of this paper to discuss matters appertaining to the pathology of coccidiosis (a subject which

will be dealt with in a subsequent communication), it may be stated here that in the coccidiosis of poultry there appears to be no safe ground for assuming that the presence of coccidia beneath the epithelium is evidence for a protective reaction on the part of the host animal, as assumed by THEOBALD SMITH (1910) for coccidiosis in the rabbit.

The schizogonous, endogenous, or auto-infective cycle, which has been discussed in the above paragraph, continues for a variable length of time before the merozoites, instead of all devoloping into more schizonts, begin to form sexual products, the macrogametes and the microgametocytes. As explained by SCHAUDINN (1902) for *Cyclospora carolitca*, the development of the sexual products and the subsequent emptying-out of the epithelial cells, may mark, in a certain number of cases, the crisis of the disease. It happens very frequently, however, that the endogenous and the exogenous cycles may be in effect at the same, time and in consequence the same area, or occasionally even the same cell, may contain three stages of the coccidia, schizont, macrogamete and microgametocyte. This condition can be explained only on the hypothesis that all the merozoites do not simultaneously form the sexual elements, but that some of them continue their course in the endogenous cycle. The cause of the schange from schizogony to sporogony has not yet been determined. That it is not, however, dependent upon differences in available food has been shown by WASIELIWSKI (1904) who reports finding three stages of *Eimeria stiedae* in a single epithelial cell in the rabbit; and this view is supported by the observation of the writer for the case of the coccidium in the turkey and in fowls.

When there exists an especially intense schizogonous development at any one point, this results, as has been shown, in an inflammation of the cecal or intestinal walls. Other intestinal parasites often take part in this process. In some cases, however, the cysts may have been formed for a long while, and the bird so affected is, therefore, a constant menace to others in the flock, because the mature cysts are constantly being liberated in its excrement.

The post mortem examination of large number of fowls dead of coccidiosis has shown that the duodenum is often the point of origin of the mature cysts which are found, later in the course of the disease, in the cecal tubes and lower intestine. In the epithelial cells of the duodenum of the majority of birds affected with coccidiosis may be found, at some stage in the disease, a variable number of micro- and macrogametocytes, oöcytes, and immature cysts. These

occur both in the cells that are still attached to the villi, and free in the lumen of the crypts; also in the main canal, into which they have fallen from the epithelial cells or from the adjoining connective-tissue spaces which originally contained them.

After the cysts have been formed within the epithelial cells and liberated from them, unless they are imprisoned by the consolidation of the area, they pass into the intestinal canal and are sooner or later eliminated. Just how long, after their formation the cysts are liberated from the body it is impossible to say. The experiments of the writer, however, in connection with artificial inoculation of young chicks by feeding cyst-containing material, show that cysts which are ingested may remain somewhere in the alimentary tract for at least ten days. Cysts which are formed in the ceca or duodenum of a bird probably may remain at least as long a time in the same bird but always without sporulation. In no instance has the writer observed a case which made it appear that the cysts developed and sporulated in the bird in which they had their origin, although Wasieliwski (op. cit) reports having observed this phenomenon in the rabbit, Morse (1908) suggest it for young chicks, and Tyzzer (1910) has reported the same phenomenon for *Cryptosporidium muris* of the mouse. They were never seen by the writer to advance in their development beyond the stage shown in Plate 1, Fig. 1, in which stage, if not earlier, they are liberated from the infected bird.

The preceeding details are sufficient to acquaint the reader briefly with the pathological conditions in the midst of which the coccidium in question was found and, for the most part, studied.

IV. **Description of the coccidium.**

The present section deals exclusively with the morphology and development of the coccidium. Data regarding the biological and physiological studies will appear in the forthcoming second part of this work. Since the mature encysted stage marks the beginning of sporogony, as well as the end of the exogenous cycle, the description of the various stages of the coccidium will begin with that of the mature cyst.

1. The mature cyst.

1. Shape. — The shape of the mature cysts (oöcysts), taken from the ceca or intestines of many different species of birds, and representing approximately the same stage of development, was usually elliptical or egg-shaped. In certain cases they appear round, although it is probable that this appearance is produced by the cysts presenting an end view. The relation of the two diameters [1]) of the cysts may vary within a wide latitude. Too high an index, as result of the pressure of the cover glass must, however, be guarded against. This may be done by mounting the cover glass upon wax feet. The exact limits of variation in the shape-index were 0,476 and 0,949. The average shape index for all cysts from all species of birds examined was 0,666. In certain cases the appearance of possessing great length may be brought about by the fact that the cysts, especially young ones, are still partly enclosed within epithelial cells the cytoplasm and nucleus of which is gathered at one pole of the cyst proper. It was not observed that any permanent difference in shape-index existed in different species of birds, except in the instance of the coccidia from sharp-tailled grouse. Here the average shape-index was 0,566, similar to that often found in the rabbit coccidium, *Eimeria stiedae.*

2. Size. — The variation in the size of the mature cysts from different birds, and occasionally from the same bird, may be very marked. The average size of all cysts from different species of birds was 14 μ by 21 μ. The largest cyst found came from the duodenum of a two-month-old chick; and measured 38,28 μ by 29,04 μ, thus having an index of 0,758. In one of the largest cysts the wall was 0.9 μ in tickness, and the nucleus measured 9,3 μ in diameter. The smallest cyst observed came from the ceca of a turkey suffering from blackhead, and measured 10,5 μ by 9 μ, thus having an index of 0,857.

In the rabbits kept at the poultry plant of the Experiment Station several were found to harbor coccidia. From these, the average size of the cysts was 28,65 μ by 18,1 μ. The largest cyst obtained from the intestines of a rabbit, which died of coccidiosis,

[1]) For convenience in making reference to the size of the cysts the term, shape-index has been introduced. The shape-index of a cyst is the result obtained by dividing the breadth by the length. For illustration: if a cyst measured 22 μ by 16 μ we would have index. Index $= \frac{16}{22} = 0{,}727$.

was 39.3 $\mu \times$ 24,2 μ giving a shape-index of 0,615 μ. As a rule the shape-index of the cysts from rabbits was smaller than the shape-index of the cysts from the grouse mentioned above.

3. The cyst wall. — The mature encysted stage of the coccidium is characterized by a definite wall which surrounds the soft cytoplasmic contents. This wall appears, under the microscope, as double contoured; and, on the hypothesis that the distance of the two lines from each other, when in absolute focus, represents the thickness of the wall, it may be said that, in the mature stage, this wall varies in thickness between 0,4 μ and 0,9 μ. The thicker walls are usually found enclosing the larger cysts, as one would expect. The thickness varies with the development of the cyst, being much thinner, just before and after the period of maturity. The wall possesses also a marked rigidity, although it is somewhat more elastic in the earlier stages. This fact can be observed by examining the cysts while under pressure, and also from sections of material containing the mature cysts. The wall of the mature cyst is perfectly transparent and quite imperforate except for the micropyle which is present at or near one pole. This micropyle is usuallv plugged with a small cone-shaped remnant of the cytoplasm, which projects slightly into the cyst cavity, but not outside. The structure of the micropyle area can be seen most clearly in cysts which are either ready to release sporozoites (or sporoblasts) or in those which have failed to develop into sporoblasts, and are beginning to degenerate. Here the end of the cyst possessing the micropyle is the first to soften and appears microscopically as a single line (Pl. 1, Fig. 32), as contrasted with the double contoured wall present in all other regions. It usually gives a truncated appearance [1]) formed by the oval depression surrounding the micropyle.

Within the outer and heavier cyst wall, there sometimes appears an inner wall or membrane, which is more delicate. This was first described for *Eimeria stiedae* by Wasielewski (1904). This membrane, according to Wasielewski, folds around the sporoblasts during their formation. Other points regarding the origin, the changes in, and resistance of, the cyst wall at different times in the course of its development will be mentioned subsequently.

4. Cytoplasm and nucleus: The mature encysted coccidium, in contra-distinction to the preceeding, and also the subsequent,

[1]) Cysts presenting this appearance may be identical with the coccidium described by Raillet and Lucet (1891) as *C. truncatum.*

stages is further characterized by having the cytoplasm gathered into a spherical mass, which occupies the central part of the cyst and has a diameter nearly equal to the shorter diameter of the inner space. The cytoplasmic portion is, at this time, distinctly granular, and frequently possesses a greenish yellow appearance. The nucleus appears as a large, pale, slightly pinkish body located in the center of the cytoplasmic sphere. The average diameter of this is about 5 μ, although a few as great as 9 μ in diameter have been observed. In unstained preparations the nuclear material of the mature cyst appears homogeneous. In stained preparation, however, it is evident that by no means all of this nuclear area consists of chromatic material, for the only part of it which takes the chromatin stains strongly is a small central sphere, the karyosome, the average diameter of which as about 1 μ. Surrounding this central body lies a broad zone, corresponding to the pinkish nucleus as it is observed in fresh preparations. In the case of hematoxylin-eosin staining, it is observed that the achromatic nuclear material frequently takes the eosin more strongly than does the surrounding cytoplasm. In some cases, although the clear zone surrounding the karyosome may be considered as achromatic, there may be found, a very small smount of chromatin, usually in fine granules. The karyosome of the adult organism is invariably spherical, and appears to contain the greater amount of chromatic material, which is present in the nucleus, and which is dissipated again during those nuclear changes which precede the formation of sporoblasts.

Between the cytoplasmic ball and the wall of the mature cyst there is a homogeneous fluid in which the cytoplasm appears to float. It seems to be the residual substance left after the cytoplasmic granules have gathered at the center of the cyst. It shows no affinity for stains of any kind, and appears to have, as first suggested by WASIELEWSKI (1904), a slightly gelatinous consistency.

5. Comparison of the cysts with those of *Eimeria stiedae*: Several hundred cysts taken by the writer from rabbits suffering from coccidiosis gave the following measurements:

Average size of cysts	32,41 μ	$\times$	21,63 μ
Maximum „ „ „	40,08 μ	$\times$	25,05 μ
Minimum „ „ „	21,71 μ	$\times$	15,03 μ
Average shape-index	0,632		
Average thickness of wall	1,4 μ		
Maximum „ „ „	2,5 μ		
Minimum „ „ „	0,8 μ.		

These measurements show that the cysts of *Eimeria stiedae* were much larger than those of the present coccidium, that the average shape-index is less than that of the present coccidium, and that the wall is considerably thicker (see p. 19). Moreover, the cysts of *Eimeria stiedae* frequently present a yellowish appearance not usually to be observed in the blackhead coccidium. In *Eimeria stiedae* this is probably due to the presence of a thin yellow, protective membrane, which is apparently lacking in *Eimeria avium.*[1])

2. The sporoblasts.

1. Origin: The first observable changes in the adult cyst, which culminate in the formation of the sporoblasts, involve modifications in the nuclear material only. The first nuclear division is preceded by the appearance of a spindle which stretches nearly across the cytoplasmic ball. A rapid division of the nucleus follows, the details of which were not observed. Hardly have the four daughter nuclei separated from each other when, in each, the second division begins, without any corresponding change in the cytoplasm. The four daughter nuclei then draw apart, and take positions near the periphery of the ball (Pl. 1, Fig. 4). Just what the nature of the finer nuclear changes (that is, the behavior of the karyosome) during this process may be, cannot now be stated, since the cyst wall is, at this time, quite impervious to both fixing and staining fluids. In fresh preparations, only the large, pale, whole nucleus can be seen.

In each of the spherical sporoblasts, the large nuclear ball can be observed (Pl. 1, Fig. 7). This at once divides into two daughter nuclei which take a position near to each other (Fig. 8), but on opposite sides of the sporoblast. An elongation of the whole body now occurs, forming, at first, an oval and later a spindle-shaped body (Figs. 9, 10), definitely pointed at one end, but rounded at the other, as shown in Fig. 11. Along with this change in shape, there develops over the outside of the spindle-shaped sporoblast, a thick membrane, which is similar to that formed over the cyst. The presence of these two membranes has served thus far to prevent following the further nuclear changes within the sporoblasts, although the continued changes in the cytoplasmic material may be observed for some little time. These changes involve, first, the appearance,

[1]) See in this connection p. 19.

near each pole of the sporoblast, of a greenish higly refractile sphere. These spheres increase in size and later one can observe, projecting from them, the borders of the two long sporozoites, which lie opposite to each another, and longitudinally in the sporoblast (Fig. 9). Somewhat later, there can be observed in about the center of each sporozoite a pinkish fleck, which is the first observable sign of the nucleus.

2. The fully developed sporoblasts: These in contrast to earlier stages, which were roughly spherical, are spindle-shaped, with one end definitely pointed as shown in Plate 1, Fig. 11. Their position within the cyst wall has all possible variations, but in the majority of cases the pointed end is toward the center (Fig. 6). The average size of the sporoblasts is 12 μ by 7 μ. They are characterized by the striking appearance of the two greenish refractive bodies which are present, one at either end of the sporoblast. These represent the curved ends of the two sporozoites which have developed, and between which lies a flattened, oval body, the "Restkörper". The nuclei of the sporozoites may be observed in fully mature specimens as elongated oval and slightly pinkish areas situated at about the middle part. The sporoblasts, as also the cysts, are surrounded by a thick resistant wall, which, in many cases, continues to protect them even after they have been liberated from the cyst. This wall, like that of the cyst, is transparent and very resistant to staining reagents.

At the more pointed end of the spindle may be seen frequently a small spherical body, the "STIEDA'sche Körperchen" (Pl. 1, Fig. 11). The size is usually less than 1,5 μ, but in sporoblasts which attain a length of 16 μ, the diameter of the STIEDA'sche body may attain 2,65 μ. While this has been explained by some writers (c. f. WASIELEWSKI 1904, p. 63) as the optical representation of a sort of sporoblast micropyle, this view is hardly tenable, and its true function is still little understood.

Whether or not, under natural conditions, the wall of the cyst or the wall of the sporoblast is ruptured first it is impossible to state definitely, but it is probable that both phenomena occur at about the same time; whereupon the sporozoites are thrown loose into the cecal or intestinal content. WASIELEWSKI has described in detail the liberation of the sporozoites of *Eimeria stiedae*. These, according to that writer, are first liberated from the sporoblast and then make their way, by many squirmings, out of the cysts through the micropyle. In the examination of many cysts by the present writer, none have been found containing free, mature sporozoites

while many free sporoblasts have been observed outside the cysts. In fresh preparations the writer has observed the liberation of the sporoblasts through the micropole. In these instances the sporoblast shot out suddenly and remained lying about 20 μ away from the micropyle. The thin wall of the cyst at this end was apparently split slightly. The other sporoblasts remained lying in the cyst cavity in a slightly changed position. Other points dealing with the liberation of the sporoblasts will be discussed in the second section of this work dealing with the biology of the coccidium.

3. Abnormal spore-formation. Under certain conditions there may occur very wide departures from the normal process of spore-formation. WASIELEWSKI (op. cit.) has already described several of these for *Eimeria stiedae*. It may be briefly said that the abnormalities in the present coccidium are, except for the presence of the "Restkörper", similar, and include the following variations from the normal: 1) the formation of only two sporoblasts; 2) the formation of eight sporoblasts and one residual body; 3) the formation of one sporoblast and of one bean-like body; 4) the formation of sporoblasts of different sizes and shapes; 5) the formation of parts of sporozoites directly out of the surface of malformed sporoblasts. Most of these forms undergo only a restricted development and then die.

4. Comparison with the sporoblasts of *Eimeria stiedae*. The average size of the sporoblasts of *Eimeria stiedae*, as ascertained by the writer, was 15,6 $\mu \times 9\ \mu$; the maximum size was 18,4 $\mu \times 11{,}7\ \mu$. It thus appears that these are rather larger than those of *Eimeria avium* (12 $\mu \times 7\ \mu$). It is to be observed also that the wall of the sporoblasts in the former instance is thicker. The "STIEDA'sche Körperchen" is observable in both forms; more seldom in *Eimeria avium*.

5. The "Restkörper". The presence or absence of a residual body, or "Restkörper", remaining after the sporoblasts have been formed, has been used by many writers as a criterion of the specific identy of coccidia. DOFLEIN (1901) in his classification of the Coccidia, separates *C. schubergi*, *C. falciforme*, and *C. salamandrae*, which possess no Restkörper, from *C. bigeminum*, *C. avium*, and *C. pfeifferi*, which do possess a Restkörper. *Eimeria stiedae* (*C. cuniculi*), however, LABBÉ places in both groups, thus indicating that sometimes this organism may possess a residual body, and again it may not. This characteristic has been mentioned for *Eimeria stiedae* by WASIELEWSKI (1904), who states, however, that a Restkörper

is present in the greater number of cases. Among other writers, some have described the "Restkörper" and some have not. From these circumstances it is apparent that the presence or absence of the residual body cannot always serve as a satisfactory criterion for the separation of species among the coccidia.

In the coccidium under consideration the residual body is seldom observed. When present it is seldom large, usually being composed of a few closely adhering granules, which almost never take the form of a ball showing granulations such as those observable in *Eimeria stiedae*. The fact that in all species of coccidia in which it is normally present, it can vary greatly in size suggests, however, that the presence or absence may be, in many cases, merely the expression of the results of differences in the nutritive conditions for the coccidia, existing in the host animal. The average size of the Restkörper of *Eimeria stiedae*, as ascertained by the writer was 7,7 μ. The maximum size was 11,7 μ, and the minimum 3,3 μ. Only about 8 percent of the cysts revealed no residual body. The shape was usually spherical and the texture coarsely granular. In *Eimeria stiedae* the occurrence of the residual body is much more frequent than in the coccidium under discussion. Its presence therefore has a certain value in differentiating these two forms.

3. The sporozoites.

The fact that the walls of the mature cysts are very resistant to all staining materials, yet tried, makes it exceedingly difficult to stain either the sporoblasts or the sporozoites while these are yet within the latter. For this reason, the evolution of the nuclear material is difficult to follow.

1. The mature sporozoites. After their liberation from the sporoblasts, the sporozoites appear as elongated, slightly curved bodies, pointed at the anterior end. The color of the cytoplasm is greenish, while the nucleus appears slightly pink. The cytoplasm has a granular texture and the nucleus lies in about the center of the sporozoite (Pl. 1, Figs. 12, 13). The average length of the newly liberated sporozoite is 10 μ to 14 μ while the breadth is 3 μ to 5 μ. In smears stained by the iron-hematoxylin method the average length is 12 μ. It thus appears that the sporozoites are longer than the sporoblasts and broader than the merozoites. They, moreover, seldom manifest the sickle shape, which is characteristic of the latter, and are more flattened dorso-ventrally, like a

Planarian. When mounted in normal saline solution or in serum, under a cover glass, they manifest a very slow gliding movement with no appreciable change of shape.

It is presumable that, under normal conditions, the sporozoites penetrate the epithelial cells soon after liberation from the sporoblasts. Here a rapidc hange of shape occurs, in which the sporozoite becomes much fore-shortened and rounded. This change of shape within the epithelial cells has not been observed by the writer, but similar changes in shape occur in "cultures"; and here the development of the sporozoites has been followed through the changes in form shown in Pl. 1, Figs. 13—19; after this they die. The pointed anterior end is lost, and the form becomes, first roughly oval, then spherical. During this time, the nucleus changes in shape from long oval to spherical. No division of the nucleus has been observed by the writer before the sporozoite has penetrated the epithelial cell; and it appears that a certain period of growth of the sporozoite is necessary before the nuclear changes begin. This is in contra-distinction to the behavior of the merozoite nucleus, in which the division may begin even before the merozoite has assumed an intra-cellular position.

When the sporozoites described above are compared with those described by METZNER (1903) and by WASIELEWSKI (1904) for *Eimeria stiedae*, it is observed that the sporozoites are shorter and broader than those of *Eimeria stiedae*, which, according to WASIELEWSKI, measure 9 μ to 16 μ, and have a width of 3 μ. The sporozoites may be considered as the end products of the exogenous cycle. With them also the endogenous cycle has its beginning, and will now be described, starting with the schizonts.

4. The schizonts.

Under the present heading will be described first those schizonts which result from the intra-cellular development of the sporozoites; and secondly, the asexual forms which result from the intra-cellular and extra-cellular growth of the merozoites. As has been stated above, the first generation of merozoites do not necessarily develop into the sexual elements, the gametocytes, but may form other schizonts which are in most respects, similar to those formed from the sporozoites. Just how long this endogenous cycle may continue without the intravention of the exogenous cycle it is difficult to say; but in no case observed by the writer have the sexual elements

been evolved sooner than ten days after the initiation of the infection. In some cases it appears that the schizogenous cycle may continue even after the exogenous cycle has been initiated and has come to an end.

Soon after the sporozoites have entered the cells, they may be seen as spherical cell-inclusions varying from 6 μ to 8 μ in diameter and, in stained preparations, manifesting a karyosome, surrounded by a large, pale nuclear halo (Pl. 1, Fig. 20). The texture is finely granular and shows no vacuoles. They are usually located at the base of the cell, not near the free surface as mentioned by SMITH (1910) for *Eimeria stiedae.*

The schizonts stain readily, taking strongly (with the exception of the karyosome) the cytoplasmic stains, especially eosin. The karyosome stains deeply black with iron-haematoxylin, and dark blue with DELAFIELD'S hematoxylin. Around the karyosome can usually be seen the pale halo of the achromatic nuclear material. The nuclear division frequently begins by the time the schizont reaches a diameter of 6 μ, and continues in the manner to be described.

5. The merozoites.

1. Origin. The eximination of a quantity of material in the form of both smear preparations and sections make it appear that there are two rather widely differing methods of merozoite formation. The first is more common, and has to do with the formation of a comparatively small number of merozoites, usually from six or eight to twenty within epithelial cells. The second method is more rare and has to do with the formation of an immense number of merozoites, in many cases several hundred in a single "merozoite cyst", frequently located in the mucosa.

As has been said, the nucleus of the sporozoite does not begin to divide until the organism has entered the epithelial cell. Hereupon the nucleus divides rapidly into eight or more daughter nuclei, which migrate to the periphery and assume a position in a ring in the outer layer. In many cases they appear to be imbedded in the outer surface of a spherical ball of cytoplasmic material (Pl. 2, Fig. 40) which, judging by its staining reaction, differs in its constitution from the plasm of the outer layer. It is often difficult to differentiate these daughter nuclei from chromatoid granules which sometimes lie about the outside of the schizont. The division of the cytoplasmic ball begins at the posterior end of the schizont

and extends gradually to the other, at which point the head-ends of the young merozoites are observed finally to be imbedded in a much reduced, spherical, or slightly flattened, residual body. This measures usually about 4 μ to 6 μ in diameter. The body usually appears vacuolated after the separation of the merozoites. This is probably accomplished either by their own contractions within the "merozoite cyst", or by the peristaltic contractions of the intestinal musculature of the host animal. The appearance of the merozoites during the process of their formation is similar to that of a split orange, in which the separate segments begin to separate gradually at one pole, but are still united at the other (Pl. 2, Figs. 41, 42). In smears of material containing many "merozoite cysts", some of them can usually be observed in this condition, but others show only one or two merozoites imbedded in the residual body, while the other merozoites lie scattered about in the neighborhood. Immature merozoites are pictured in Pl. 2, Figs. 56—60.

The second type of merozoite formation involves the production of an immense number of merozoites (Pl. 2, Fig. 43). In this case the earlier stages were not observed, but the merozoites were almost fully formed within the cysts at the time of observation. In these cases the "cysts" usually lie in the submucosa, a point the significance of which will be discussed in a subsequent publication. While the smaller merozoite cysts were usually oval or spherical, the larger variety may be extremely irregular in outline. Some are oval or spherical, but others are elongated, hemispherical, kidney- or bean-shaped. The average size is about 58 μ by 32 μ, although cysts 63 μ were seen. The first type of merozoite cyst described has usually only one center of segmentation, and consequently one residual body. In exceptional cases two segmentation centers were observed (Pl. 2, Fig. 42). The second variety, on the other hand, has several, even as many as six or eight. Frequently three or four appear in a single 7-micra section through one side of the cyst. These segmentation centers are formed by a single ball of cytoplasm varying in diameter from 5 μ to 7 μ. They usually are seen to lie in the midst of a clear zone, or halo, which extends about 1 μ beyond the central body. It is in the outer zone of this central ball, that the ends of the merozoites are imbedded. They apparently do not penetrate the central mass itself; its regular outline is unbroken (whether the body is inside the cyst, or without), after the liberation of the merozoites.

The location of the segmentation centers apparently determines

the direction or plane in which the longitudinal axes of the merozoites lie. Thus, in a single cross section through a large merozoite cyst, the merozoites may be separated into groups, each of which is characterized by having the longitudinal axes of the merozoites lying in a certain direction. Naturally, since some of the merozoites will be cut transversely, while others will be cut obliquely or longitudinally, the optical expression of the elements will have many forms. In many cases, if the transverse section of the merozoite has included the nucleus, all that is seen, is a small circle staining reddish, with a dark-staining center, the nucleus (or karyosome). The diameter of these elements is about 1,6 μ to 2 μ, thus indicating the breadth of the intra-cellular merozoites. The average length of those that appear fully developed and about to be liberated, is 9—10 μ. There is an apparent increase in size directly after liberation.

It is only in the still immature merozoite cysts that the regular arrangement about the segmentation centers described above can be observed. In older cysts a separation between the merozoites and the segmentation centers has usually occurred, with the result that the merozoites are more or less irregularly scattered about the interior of the "cyst". It is estimated that the large cysts contain from 200 to 350 merozoites.

In the division of the original mass of cytoplasm into several groups, thus forming as many segmentation centers for the developing merozoites, there exists a condition which is comparable, in a degree, to the division of the mass of reserve substance in the spore-formation of certain flagellates, especially *Trichomonas* (see BENSEN, 1909) in the encysted stage. Possible this analogy will not bear too close scrutiny, but it is suggestive of a related process of multiplication existing between these two groups of protozoan organisms.

In closing this section it may be noted that, notwithstanding the great size of these merozoite cysts, in the tissues they are often to be observed inside the epithelial or endothelial cells which are stretched to accommodate the inclusion. The nucleus of the cell can sometimes be seen pressed to a crescent against the merozoite cyst. Multi-nucleated cells were frequently observed surrounding the parasites in the mucosa (see SMITH, 1910).

A comparison of the merozoite cysts just described with those of *Eimeria stiedae* described by WASIELEWSKI (1904) shows several differences. The large "merozoite cysts" described by WASIELEWSKI had a diameter of only 12 μ to 20 μ. The largest merozoite cysts observed by the writer were more than twice this length. The

smaller cysts appear to be about the same size in both cases, i. e. 8 μ to 10 μ in diameter. It cannot be said at this time whether the difference in the size of the merozoite cysts represents only a difference in the power of growth, determined perhaps by favorable or unfavorable nutritive conditions, or whether there is concerned some more fundamental difference. Since JOLLOS (1909) appears to have established difference in the merozoites of *Adelea ovata*, it is not impossible that further investigations may show that, in the two varieties of merozoites described above, there are merozoites which continue, on the one hand, the asexual cycle, and, on the other, the sexual development, since we know that these two processes frequently proceed together in narrowly restricted areas.

2. The mature merozoites. The mature merozoites appear in fresh preparations as long, slender, slightly curved bodies, showing very faint granulations near the posterior end. In several cases the average length of the merozoites so observed was 10,5 μ while the extreme size limits were 9 μ and 14 μ. These merozoites, when placed on warmed slides, sometimes manifested a slight squirming motion, in which the anterior end moved from side to side, and the whole body progressed slowly. It is quite possible that the merozoites described above were immature, since, in another case, the average size was about 14 μ, and the largest 17 μ.

A clearer picture of the merozoites was obtained from smears stained by the ROMANOWSKY method. Here also the elements appeared as slender, sickle-shaped or slightly curved rods, which were more drawn out at the posterior end. In these stained preparations the average size of the merozoites was 1,7 μ by 9,5 μ. The extreme limits in breath were 1,3 μ and 3,1 μ, while the extreme limits of length were 7 μ and 16,7 μ. It thus appears that there is great variation in the size of the merozoites, and that the largest of them may be nearly twice as large as the smallest. The differences observed in the size of fresh and stained merozoites were probably due to the shrinkage consequent to fixation. It is possible, however, that there are present here two types of merozoites, the male elements and the female elements, as described by JOLLOS (1909) for *Adelea ovata*. Although there is some doubt on this point, the measurement of a large number of merozoites shows that there is a marked difference in shape and size. In view of the fact, however, that some of these differences appear to be determined by growth alone, there is as yet no basis in the present case for distinguishing male from female forms unless these are represented by the Figs. 46—50 in Pl. 2.

The inner structure of the merozoites is best shown in smears fixed in alcohol and stained by the ROMANOWSKY method. Such preparations show the following points: 1) That the cytoplasm does not stain at all evenly, but that the posterior third or the posterior half stains reddish and most intensely. The more blunt anterior tip of the merozoite stains more bluish than the posterior part, but not so deeply. It is, however more deeply stained than the remainder of the anterior third of the merozoite, which immediately surrounds the nuclear material. This nuclear material stains deeply reddish purple with the ROMANOWSKY stain. In freshly liberated merozoites it is usually ring shaped, less often solid, and the average size is 2 μ by 1,65 μ. Directly posterior to the nucleus is usually observed a single, spherical, dark-staining body 0,25 μ in diameter (Figs. 46, 47). Sometimes this body, which possibly represents the centrosome, is replaced by a group of small granules, some of which attain the size of 0,2 μ (Fig. 48). The position of this body, usually located about midway in the merozoite, marks the anterior limit of the dark-staining area occupying about the posterior half or third of the merozoite. Sometimes the line of demarkation is transverse and abrupt, but more often it is v-shaped, extending forward from the dark staining sphere to the lateral borders of the nucleus as shown in Pl. 2, Fig. 46. Occasionally there may be observed a second dark granule, about 0,22 μ in diameter, located in the anterior tip of the merozoite and surrounded by the more deeply-staining cytoplasm which occupies this region as shown in Fig. 47.

What may be the significance of this granule cannot be stated definitely at this time. It is true that it resembles the blepharoplast of certain flagellates, but it differs from this body in that, in the present instance, the single granule is often replaced by a group of granules. Its location would appear to preclude possible relation to flagella attachment unless it represents a rudimentary organ of phylogenetic significance. TYZZER has described such a granule for the extra-cellular coccidium, *Cryptosporidium muris* of the gastric glands of the mouse, and suggests that the granule in that instance may be a structure concerned in the development of the organ of attachment. Since an analogous granule is present, however, in nearly all of the merozoites of *Eimeria avium*, an intra-cellular coccidium, it is again indicated that the granule either is not to be regarded as having a connection with the organ of attachment of *Cryptosporidium*, or that, in *Eimeria avium*, it is a heritage from an older phylogenic form. Assuming a possible flagellate ancestry for

certain members of the class Sporozoa, it is conceivable that the extra-cellular forms, such as *Cryptosporidium*, are older than the intra-cellular; and that both have inherited this now rudimentary organ from a still earlier flagellated race.

The main differences to be observed in the merozoites are concerned with the structure of the nucleus. When the freshly liberated merozoites still clustered about the residual body, are examined in smears stained by the ROMANOWSKY method, the whole nuclear structure is frequently observed as a hollow ring, a semicircular disk, or some larger segment of a circle. The most common form is that shown in Fig. 51. Later in the development of the merozoite this hollow sphere, or ring-structure sometimes appears to be replaced by a more solidly staining body as shown in Fig. 46. This body is usually oval and appears to be slightly granular in texture. This is probably a preliminary to the first nuclear division which soon follows. It is only by the iron-haematoxylin method that the karyosome, separated from the other nuclear material, can be observed most clearly. In slides prepared by either method, however, it can be seen that the chromatic substance divides first into two bars or rods, which may extend longitudinally or lie transversely in the merozoite (Fig. 47). Each of these rods of chromatin divide again and form four daughter karyosomes within the area occupied by the still apparently undivided nucleus (Fig. 48). Whether or not further divisions of the daughter karyosomes, or of the nucleus itself, follow previous to the invasion of an epithelial cell, cannot now be stated; but they have not been observed by the writer. Various stages in the process of division are delineated in Pl. 2, Figs. 46—54. Here also are shown merozoites which have apparently undergone a partial development before they were able to penetrate the epithelial cells (Figs. 52—54).

When the merozoites described above are compared with those of *Eimeria stiedae* as described by WASIELEWSKI (op. cit.), several differences are apparent both in point of size and structure. The size of the merozoites of *Eimeria stiedae* are, according to WASIELEWSKI, 12 μ to 13 μ in length and 2 μ to 2,5 μ in breadth. The writer has never observed fully matured merozoites, however, as small as 5 μ in length, as reported by WASIELEWSKI. Immature forms as small as this are pictured in Pl. 2, Figs. 56—58. There is also a very marked difference in the shape of the merozoites. As described and shown by WASIELEWSKI (op. cit.) the sharp pointed end of the merozoites is anterior, while the broader part of the

organism lies posteriorly. In the merozoite of the present coccidium, while the anterior end is pointed, the broadest part of the body of the merozoite lies anterior to the center (Pl. 2, Figs. 46—48). The posterior end becomes gradually attenuated. The examination of the nuclei of merozoites of *Eimeria stiedae* in smear preparation showed no sign of nuclear division into rods or four fragments as seen in the merozoites of *Eimeria avium*. It is possible, however, that they were not in the right stage.

6. The macrogametes.

1. Differentiation of the sexual forms. What factor starts the development of the sexual forms of the coccidium has not yet been determined. The only data that can be obtained from the observation of the life-histories of other sporozoa suggest that the change from the asexual to the sexual cycle is due to a definite necessity, which is imposed by the nature of the developing organism, and is not materially affected, at least to any great degree, by the conditions brought about by differences in location in the host animal, by consequent differences in nourishment, or by reactions (either serum or chemical) of the host. The best evidence for this hypothesis is the fact that, even in the same host-cell, three stages of development may frequently be found: namely, schizont, macrogamete, and microgametocyte. This evidence also controverts the view, which otherwise might gain some favor, that the different stages of development found in the same animal are due to the differences in the periods of infection, some having been earlier and some later. It must be admitted that when animals are so placed that many successive opportunities for infection are offered, the probability is greater that both the early and late stages of development of the coccidium would be found; but hardly in such close proximity that it could with justice be argued that a freshly liberated sporozoite had entered a cell in which a macrogamete and a microgametocyte were already developing. The view that, in such a case, the originally infecting elements entered the cell at approximately the same time, and probably from the same original group of merozoites, gains greater weight from the fact that the three cell-inclusions are apparently of the same age and manifest only such differences in size as might be ordinarily looked for in the three types mentioned. These considerations point strongly to the view that there are individual differences in the constitution of

the merozoites coming from the same merozoite cyst, or different cysts; and that these differences determine whether the merozoites shall continue to form the products of the asexual cycle, or begin to form the sexual products, the macrogametes and microgametocytes.

In case the merozoites did carry such potential differences in the chromatic substance, as suggested above, it might be expected that such differences would also be apparent either in the form of the merozoites, or in the first nuclear changes which take place within them, even before the schizont stage has been reached. That such differences actually exist among the merozoites of some coccidia has been shown by JOLLOS (1909) for *Adelea ovata*, in which he was able to observe two distinct forms of the merozoites, each with characteristic nuclear changes. With reference to the present coccidium these differences, so far as observable, have been mentioned in more detail in the section on the merozoites.

But notwithstanding the fact that even in the same narrowly restricted location the coccidia may belong to either the sexual or the asexual cycle, and thus give evidence of having arisen from groups of merozoites having, individually, different nuclear constitutions, the fact is also to be observed that in the majority of cases, the same locations (and by this is meant a single crypt, or group of adjoining crypts) contain coccidia, the vast majority of which are in the same cycle of development, and approximately in the same stage. The fact that the same smear preparation may contain many different stages, does not controvert this view, since the original material for such a preparation necessarily covered a large area of diseased tissue (i. e. a large number of crypts). In support of the above view it may be shown that in a certain section of the duodenum, for instance, the coccidia are practically all macrogametes and microgametocytes; or that in a section of the cecum, nearly all the coccidia, in an area involving perhaps several broken-down crypts, are the merozoite cysts. In other cases nothing but the young schizonts are to be found over wide areas, and sometimes even in many slides.

2. The young macrogametes. As has been said in the section dealing with the schizonts, it is difficult to distinguish between the macrogametes, microgametocytes and asexual schizonts when these are represented by elements less than 8 μ in diameter. While the young asexual schizonts and the young microgametocytes show, upon the examination of fresh preparations, fine granulations, the young macrogametes remain for a certain period clear and

homogeneous in structure. At first, in fresh preparations, no sign of nucleus is observable, and all that can be seen of the cytoplasmic material is scattered granules of varying sizes. The coccidium at this stage is usually spherical or oval, a further sign of the plasticity of the protoplasmic content.

Usually by the time the macrogamete has assumed a size of 8 μ to 12 μ the protoplasm begins to take on the form of more definite granules, which appear first in the center of the sphere surrounding the large clear nucleus; this they render evident by throwing it into strong relief. Gradually the granules become larger and coarser, possibly through fusion of the smaller ones, until they may be seen occupying the whole area of the macrogamete; the larger ones tend to gather about the periphery. At this stage the diameter of the coccidium is usually 12 μ to 14 μ or greater, and in its center can be seen the large pale nucleus, which has an average diameter of 5 μ. As development proceeds the granules become still larger and show a more definite tendency to accumulate about the periphery of the coccidium, which now begins to assume an elipsoid shape. During all these changes the macrogamete may remain enclosed within the host-cell, whose nucleus can usually be observed, pressed to a crescent against one wall, while the cytoplasmic portion, with the exception of a few granules, has been absorbed by the growing coccidium. The macrogamete is more rarely found in the mucosa.

a) Nuclear changes. While the changes in the outer form of the coccidium have been progressing, there have also been occurring nuclear changes of apparent importance. These changes now to be described were observed in smears stained by the ROMANOWSKY method. They appeared as peculiar modifications of the nuclear (or at least chromatic) material of young macrogametes, and involve apparent nuclear divisions in which the chromatic material frequently assumed the form of rings, crescents, strands, or loops. Some of these chromatic changes are pictured in Pl. 1, Figs. 21—27. All these pictures showed a definite organization of chromatic material which might possibly be interpreted as representing nuclear divisions. Although all the stages of such an assumed process were not observed, those which were seen suggest the following tentative explanation:

At a time when the cytoplasm assumes a strongly granular composition, the chromatic material which has gathered at the center of the sphere assumes the form of a ring and quickly divides (Pl. 1,

Fig. 21, 23 a, b) into daughter nuclei which are ring-shaped, or quickly assume this form. These daughter nuclei take positions diametrically opposite each other, and while one either undergoes disintegration, or divides again (Fig. 24 b; Fig. 25 b', b''), the other divides into two other elements similar to those first formed. One of these maintains the ball-like form (Fig. 24 a') while the other becomes more strandlike (Fig. 24 a''). The strand or ribbon-formation in both daughter nuclei gradually becomes less definite until it finally appears as if the nuclear material in these bodies was disintegrating. Several stages of this disintegration process could be observed. Finally, however, no further trace of the two daughter nuclei could be seen, while the third remained ball-shaped and distinct occupying a position either in the center, or slightly to one side, of the coccidium (Fig. 26). The last observable signs of the former nuclear elements were small chromatic granules scattered through the cytoplasm of the macrogamete. They were usually observed to occupy the spaces between the larger and more plastic food granules, where they sometimes seemed to fuse together to form larger granules. These chromatic elements are probably identical with what have usually been called the chromatoid granules (Pl. 2, Fig. 44).

Although, as has been stated above, there usually appear to arise only three daughter nuclei, one of which remains as the functioning nucleus of the macrogamete, there are sometimes present four nuclear bodies or their remnants (Pl. 1, Fig. 25). This condition is perhaps explainable on the ground that both the daughter nuclei, which result from the first division, divide again. In this case three of them appear to disintegrate, while one remains as the permanent functioning nucleus of the cells.

In view of some divergence of opinion regarding such changes in chromatic material such as those described above; the view of other investigators upon phenomena which are probably analogous to those mentioned may be briefly stated. Siedlecki (1899) was the first to describe for *Adelea ovata* extrusions of chromatic substance from the nucleus. This phenomenon occurred almost coincidently with the differentiation of the microgametocytes, and was called by him "épuration". Dobell (1907), however, who worked especially with the microgametes of *Adelea*, figured no such forms. Jollos (1909) was also unable to observe them in *Adelea*, and states that they certainly are not to be regarded as the equivalent of reducing divisions.

More recently TYZZER (1910) has described for *Cryptosporidium muris* a peculiar chromatic material present in the macrogametes. This material, according to TYZZER is scattered through the cell, becoming distinctly granular in chraracter. Subsequently this material appears to fuse into irregular masses of globules, some of which appear ring-shaped in stained preparations. TYZZER believes that the behavior of this substance is not characteristic of degenerating chromatin, and concludes with the tentative assumption "that this material is of the nature of a specific granulation which is peculiar to this species".

Whether or not *Adelea* shows a true reducing division, or whether the chromatic material of *Cryptosporidium* is in reality nuclear substance, probably cannot be stated definitely at this time, although TYZZER's observations strongly support the view that the material is not nuclear chromatin. The observations made by the writer on *Eimeria avium*, however, suggest that here at least, there sometimes occurs a thrusting-out of chromatic substance, and that the process is characterized by nuclear divisions. The whole series of changes apparently takes place very rapidly, and this may explain why they have been so seldom observed. In conclusion it should be said that, whether this "épuration" is a true reducing division or merely an extrusion of chromatic substance as believed by JOLLOS, can not be decided until further studies have been conducted. The foregoing observations are, however, suggestive of the fact that the chromatic modifications observed in *Adelea* by SIEDLECKI, and in *Eimeria avium* by the present writer may be something more significant than irregularly occuring nuclear changes.

b) The granules. As has been stated, one of the most characteristic features of the macrogametes, whether they be observed in fresh preparations, in smears, or in sections, is the granules. These are of two sorts, the plastic food granules, and the so-called chromatoid granules, which have been referred to in the previous section. The two varieties may be differentiated from each other by their size and by their staining characteristics. Their position may be variable. The plastic food granules are much the larger. Their diameter may very between 1 μ and 3,5 μ. The size appears to be somewhat dependent upon the size of the macrogamete, and also upon its stage of development. The size is not constant for each individual. A characteristic picture of the granules as they appear in smear preparations and sections is shown in Pl. 1, Figs. 23—26. In the youngest macrogametes the plastic granules are altogether absent.

They appear first out of the cytoplasm and may then be scattered irregularly through it. As they increase in size and number, however, the larger granules assume a position about the periphery of the macrogamete where they may prevent observation of the nucleus which lies within, and where, later, they aid in the formation of the wall. In extreme cases, they produce slight bulgings from the otherwise evenly contoured walls of the coccidium and are highly refractive in fresh preparations. These plastic granules must be distinguished from the food granules of the host-cell, which are sometimes caught between the cell-wall and the parasitic inclusion. While, as has been said, the larger plastic granules of the macrogamete occupy the outer surface, the smaller granules are distributed throughout the interior (Figs. 25, 26). The shape of the granules is, in nearly all cases, spherical. In smears stained by the Romanowsky method, and in sections stained by iron-hematoxylin and eosin, the plastic granules are in all cases colored a pink or magenta.

The chromatoid granules, as has been said, make their appearance soon after the disintegration of the nuclear matter arising from the assumed primary divisions of the nucleus. They are in most cases much smaller than the plastic food granules, their diameter being from 0,5 to 2 μ. Moreover, while the plastic granules were always spherical, the chromatoid granules are sometimes irregular and sometimes bar-shaped. This irregularity in size and shape of the internal granules prevents their being confused with the other nuclear elements which are to form the new generation of coccidia. There is often present, however, about the margin of the macrogametes (seen in sections) a ring of these chromatoid granules. These are frequently very regular both in size and arrangement, and are easily confused with the daughter nuclei of the developing merozoites. The chromatoid granules take the nuclear stains deeply, and when colored with hematoxylin, appear either dark blue or black depending upon the intensity of the stain. In some cases the plastic food granules predominate, while in others the number of chromatoid granules appears to be greater. Wasielewski states that in *Eimeria stiedae* the chromatoid granules may be observed in young macrogametes which have a diameter of only 5 μ to 7 μ, and he believes that they leave the nucleus in drop-form ("daß sie den Kern in Tropfenform verlassen"). The plastic granules, according to this author, do not appear until later. The present writer has not observed that, as a rule, the chromatoid granules are the first to appear; later they become more or less obscured by the plasic

granules which cover them. It is not improbable that the size and time of appearance of the plastic granules depends largely upon the nutritive conditions surrounding the developing coccidium.

Nothing definite is known regarding the functional use of these granules. While the chromatoid granules, as has been suggested above, may possibly represent chromatic substance which was thrown off in the first nuclear divisions, which disintegrates, again forms globular aggregations, and is of no further use to the organism, the plastic granules doubtless represent substances which are being stored for the future use of the coccidium. These substances may be concerned either with the cytoplasmic material out of which will be formed later the sporoblasts, and subsequently the sporozoites; or with those substances which are to be used in the laying-down of the heavy cyst-wall, which begins to develop during the latter part of the maturation of the macrogamete. The possibility that there may be contained in the large plastic granules substances for the formation of the wall of the cyst was first suggested by SIMONDS (1897) and is accepted by WASIELEWSKI (1904). This hypothesis receives further support from the observations of the writer, which will be given in the following section.

3. Maturation phenomena, and the mature macrogamete. With the exception of the tentatively assumed nuclear changes, which have already been mentioned, the maturation phenomena include the following: 1) change in the shape of the macrogamete; 2) change in the wall and, 3) change in the arrangement of the cytoplasm.

a) The shape of the youngest macrogametes is nearly spherical. As development proceeds it becomes more elipsoid, and by the time the nuclear changes described on page 34 are completed, it has an average shape-index of 0,779.

b) The wall of the macrogamete does not begin to develop until the organism has attained nearly its full size. Otherwise the absorption of food materials would probably be inhibited by the thickening membrane, and further development prevented. The necessary preliminary to the formation of the cyst wall is the gathering of the red plastic granules about the periphery of the macrogamete (Pl. 1, Fig. 26). Here they remain for a time, the larger outside, and the smaller inside. Gradually it may be observed that those which lie next to the membrane wall, become flattened on the outer surface, and press closely against the membrane. This flattening continues until it is apparent that the material of the

granules is being applied to the formation of the wall (Pl. 2, Fig. 45). This is also apparent in the increasing thickness of the wall itself. From a thin membrane, without apparent thickness, it appears as a gradually thickening line, the color of which is the same as that of the granules. Finally the red, plastic granules wholly disappear, and the wall appears in nearly its full thickness, still staining faintly pink with eosin. Although this staining reaction remains during the life of the cyst, the wall gradually becomes more impervious to the stain, and never, at any subsequent time, stains so brilliantly as when freshly formed. When first formed the wall is elastic, but as the resistance to stains increases, it becomes at the same time more rigid and firm.

c) The changes in the cytoplasm of the macrogametes may be considered under two heads. Texture: The texture or consistency of the macrogametes has already been considered under the heading of "granules"; and it was there shown that while the young macrogametes were at first clear, the granulations (i. e. the red granules) in them gradually appeared and then again disappeared with the formation of the cyst wall. The cytoplasm which is left remains more finely granular but still contains a certain amount of dark-staining chromatic material.

Distribution: This cytoplasm, at the time when the cyst wall is nearly formed, is distributed evenly through the whole cavity, the nucleus at the center. As development proceeds, at first, irregularly, producing a slightly fimbriated margin; but finally culminates in the formation of a spherical ball of protoplasm with the large pale nucleus resting at the center. The different stages in this retraction are shown in Pl. 1, Figs. 28—32.

7. The microgametocytes.

Bodies which could certainly be recognized as microgametocytes have not been observed by the writer under 6 μ in diameter. These elements begin to develop from merozoites in the epithelial cells (occasionally in the mucosa), coincidently with the macrogametes, and can be distinguished from the latter as soon as the number of nuclear fragments is sufficiently large. It appears that, in many instances, the nuclear division begins even before the microgametocyte attains its maximum size, just, as the division of the merozoite nucleus may be observed even before the merozoite enters its host-cell.

The shape of the microgametocytes may be round, oval or irregular; the smaller bodies are more often round and regular in

outline, the larger bodies more often irregular. This stage is found both inside and outside the epithelial cells but the larger microgametocytes are found more often free in the tissues of the mucosa, in multi-nucleated cells, or in the lumina of the crypts In intracellular epithelial positions the appearance produced is the same as that which has been described for the intracellular schizonts and macrogametes. The number of microgametocytes developing at any one time is considerably less than the number of macrogametocytes. The numerical proportion as observed (in sections) in the organisms still included within the epithelial cells, and in smears made from the content of the cecum or intestine is 13 to 100.

In fresh preparations the microgametocytes vary in size from 10 μ to 36 μ. They are often characterized by a striated appearance of the surface, apparently brought about by the thin bodies of the microgametes lying packed nearly parallel to one another. It appears doubtful whether the flagella of the microgametes could aid in giving this appearance, which cannot be seen in freshly-stained preparations. The residual body, which occupies from one-half to three-fourths of the microgametocyte, cannot be seen in fresh preparations since it is covered by the layer of microgametes which lie between it and the periphery, and, in many cases, appear to radiate out from it or lie tangent to its surface. It is only in the early stage of development, however, that the radial arrangement of the "striations" can be observed; later, the microgametes appear to become detached from the residual body, and to lose to a greater or less degree their nearly parallel arrangement.

In stained preparations the striated appearance of the microgametocyte is usually lost, but the details of internal structure are made more clear. The picture varies, however, with the age and stage of the organism; dependent upon whether it is observed in sections, or in smears, and upon the staining process.

The best pictures of the microgametocytes are found in smear preparations fixed in methyl alcohol and stained by the ROMANOWSKY method, or in sections stained with iron-hematoxylin. Here the most common picture of the organism is that shown in Pl. II, Figs. 32—38. One sees in sections a lavender or pinkish-staining mass, round, oval, or irregular in shape, measuring from 7,82 $\mu \times$ 5,28 μ to 33 $\mu \times$ 20,04 μ in diameter, and dotted with the nuclei of the more deeply black-staining microgametes (Fig. 38). These nuclear fragments are either round or elongated, depending upon what stage of development has been reached. Usually they are distributed irregularly over the

surface of the residual body, and because of the manner of making the smear, often showing no definite arrangement. In exceptional cases, however, the microgamete nuclei are seen to radiate from the large residual body, somewhat like the petals of a sun-flower (Fig. 34). In still other cases the residual body is observed alone, but surrounded by a variable number of deeply purple- or blue-staining microgametes to be described later (Pl. 2, Fig. 37). The radial arrangement of the microgametes is more clearly seen in sections where their orientation to the residual body, and to one another, has not been disturbed.

In earlier stages of development than that mentioned above, the microgametocytes may reveal in smears very little structure. They are especially characterized by staining more definitely blue than the macrogametocytes, which, in the early stages, they much resemble. As development proceeds the nuclear fragments can be observed, at first in small numbers and indistinct, but gradually increasing both in number and in clearness.

In the majority of cases only one residual body occurred in a single microgametocyte (Pl. 2, Fig. 34). It may frequently happen, however, that there are two to ten (probably more) residual bodies, which serve as segmentation centers. In these instances one may be nearly twice as large as others, the smallest seldom attaining a diameter of over 7 μ. In such microgametocytes the microgamete nuclei cluster about the periphery of all the residual masses except sometimes at the plane of their union (Fig. 43). In this phenomenon we have a parallel in the "merozoite cysts" which possess two or more segmentation centers or residual bodies, as described on page 28. In some cases the residual body appeared to be deeply invaginated on one side, the invagination surface being bordered with microgamete nuclei (Fig. 35). The structure of the residual body is homogeneous, or frequently faintly granular. It appears to degenerate rapidly after the liberation of the microgametes.

8. **The microgametes.**

The microgametes have their origin in nuclear fragments, many of which are split off by the nucleus even before the microgametocyte has attained its full size. The fragmentation occurs coincidently with the maturation of the macrogametes, with which the microgametocytes can invariably be found. The number of daughter nuclei varies within wide limits. Some small microgametocytes appear to form not more than a dozen microgametes, while others form many hundred.

The size of the original daughter nuclei is at first very minute, — usually less than 0,5 μ. They gradually increase, however, to form round nuclei having a diameter of 0,75 μ to 0,9 μ. These early stages of development cannot be observed at all in fresh preparations, and the first results of nuclear division can be seen only with difficulty in stained preparations. After these have undergone a certain period of growth, they may be observed as deeply blue-staining (or purple-, depending upon the success in staining) bodies against the lighter blue of the residual substance of the gamete, in which they appear to lie partly imbedded. Subsequently the round form of the microgamete nuclei is lost, and the bodies elongate to form lancet- or comma-shaped bodies. With further development this appearance becomes more marked until finally the nuclei become pointed at both ends and have a size of 2,5 μ to 3 μ. When the microgametes have attained this size they usually become separated from the residual body, or adhere to it only at their tip ends, and soon after they are probably liberated from the confining membrane. The nucleus apparently continues the process of elongation after liberation, and when the microgametes are liberated, the nuclear portion has a length of 3,8 μ to 4,5 μ, and a breadth of about 0,9 μ to 1 μ. The cytoplasmic portion of the microgamete, which lies at the posterior end, is seldom revealed upon staining, and it is doubtful whether very much cytoplasmic material encloses the microgamete nucleus. The fact that microgametes, seen in fresh preparations, have a maximum length of 6 μ to 7 μ, and a breadth of 1 μ to 1,1 μ, makes it apparent that there is a slight cytoplasmic elongation at one end, or at both ends, of the nucleus proper, although the microgamete is chiefly made up of nuclear material.

In smear preparations, in which the microgametes are scattered through the field, the form is generally sickle-shaped or crescentic as shown in Pl. 1, Fig. 20. Less often a compound curve in the bodies is observed.

That the microgametes possess two flagella has been shown by suitable methods of staining. It has proved difficult, however, to stain the flagella clearly, and at the present time no further data regarding their structure or mode of attachment are at hand. The length of the flagella is slightly greater than that of the microgametes. It has been impossible up to the present time to observe the flagella in fresh preparations.

A comparison between the microgametocytes and microgametes of the present coccidium with those of *Eimeria stiedae*, as described

by WASIELEWSKI (1904), and also as observed by the writer, show many similarities and few differences. The minimum size of the smallest microgametocytes (6 μ to 7 μ) is the same in both cases, but none attaining a diameter of 55 μ, as described by WASIELEWSKI for *Eimeria stiedae* were observed by the writer in *Eimeria avium*. The microgametes have the same form of development in the microgametocytes, and possess, in the adult stage, similar morphological features.

Fertilization of the macrogamete and subsequent development.

The mature macrogamete consists of an oval or egg-shaped double-walled membrane enclosing a spherical ball of cytoplasm of granular consistency, in the center of which lies the relatively large pronucleus. The appearance of the macrogamete at this stage is similar to that of the resting cyst stage. As the time of fertilization approaches, the cytoplasmic ball approaches one pole of the cell and a small cone is thrust out of the micropyle opening. In some instances there appears to be a mucilagenous film lying entirely about the macrogamete, or sometimes covering only the pole in which the micropile is present. Judging from the fact that various small particles adhere to this membrane, it seems probable that it is composed of a sticky substance, extruded from the interior of the cell. It was not seen in all cases and seems to remain only a short time. It is possible that its function is to attract and hold the microgametes. If this were true it could not, however, be analogous to the outer membrane which is extruded by some coccidia after the process of fertilization, and observed to harden into a protective "shell" (see LANCASTER, 1903). Indeed there is no such membrane enveloping the macrogametes of *Eimeria avium*, similar to that observed by the writer in the case of *Eimeria stiedae* unless the one described be considered as analogous. Nevertheless, further observations are required before a definite answer to this question can be given.

During the time that the cytoplasm moves toward the pole of the macrogamete, the nucleus migrates slightly forward toward the location of the micropyle. Here it rests until the fortunate microgamete, striking the projecting cone of cytoplasm (or some portion of the adhesive membrane described above), is drawn within the macrogamete. The further details of the fusion of the two nuclei have not been observed by the writer, although bodies which pro-

bably represent microgametes have been seen adhering to the outer surface of the ball of cytoplasm within the cyst. The next stage observed was that in which the cytoplasm had again gathered in the center of the cell, and the nucleus had stretched to form a spindle-shaped band across the cytoplasmic sphere. It appears probable that this has to do with the fusion of the male with the female pronucleus, and is merely a means of mixing the chromatin elements; for, shortly afterwards the nucleus again appears as a ball in the center of the cytoplasm. At this point the development rests, no further change taking place while the oöcyte is in the body of the bird. The conditions which have been found to favor development or non-development will be considered in a subsequent paper.

V. Conclusions.

The systematic position and specific relations of the coccidium under discussion remain to be mentioned. There exists at the present day no little confusion regarding what are actually the specific characters of many of the Coccidia, so that there is some difficulty in stating what are, and what are not, well defined and legitimately distinct species. One cannot review very much of the literature upon the Coccidia without being impressed with the view that organisms which appear to be the same species have been described many times under different names, the distinctions being, for the most part, based on such criteria as the size and shape of the cysts. Thus we have, for instance, *Psorospermium avium*, *Coccidium avium*, *Coccidium rivolta*, *Coccidium perforatum*, and *Coccidium tenellum*; also *Coccidium oviforme*, *Eimeria cuniculi* and *Coccidium perforans*. Of the last-named species alone there have been no less than six different varieties mentioned.

One reason for this confusion probably arises from the fact that one would not expect to find the same organism either present, or causing the same disease, in both birds and mammals; and thus many of the forms of coccidia found in animals have been ranked with *Coccidium cuniculi* (*Eimeria stiedae*) or with *C. oviforme*, while those found in birds have been classed with *Coccidium avium* (or *C. tenellum*). Another cause for existing uncertainity, is perhaps the fact that, in a great many instances, the slight variations in size or shape of the cysts (which has probably been often due to differ-

ences in age or technique) has been made to serve as the basis of specific distinctions.

As has been indicated in the preceeding pages, and the point cannot be too strongly emphasized, there exist very great differences in the shape and size of coccidia of the same species, not only when taken from different parts of the intestinal tract of the same animal, but also when coming from different animals. The marked variations which the writer has observed in *Eimeria avium* as determined by different hosts and by different conditions of nutriment has led him to the view that a re-examination of many coccidia now ranked as independent species would serve as a basis for the elimination of a large number which now receive mention in text-books on pathogenic protozoa.

Another common source of error may be the mistake of associating certain species of coccidia with animals that are merely playing the rôle of incidental or intermediary hosts to the parasite. For example the writer has examined many rats (*Mus norvegicus*) and mice (*Mus musculus*) which contained, besides *Coccidium falciformis*, a coccidium, the cysts of which were in every way similar to the cysts of *Eimeria avium* as regards both their morphology and their manner of development into sporozoites. Similar coccidia have been found also in many of the rabbits kept at the rabbitry of the Experiment Station. More exceptionally, coccidia similar to the rabbit coccidium have been found in rats and mice. These facts can probably be explained without the need of assuming that the species of coccidia mentioned are commonly parasitic in all animals in which they change to be found. The scavengering characteristics of rats would, in addition to their chicken-killing propensities, easily account for the presence of *Eimeria avium* in their intestinal tract and in their excrement; and the presence of *Eimeria stiedae* or *Coccidium falciformis* in fowls can be explained in a like manner on the basis of the incidental ingestion of these parasites. It has not yet been proved that the coccidium common to one species undergoes any development when, by accident, it secures a lodging in the intestinal tract of another host. As stated above, the finding of certain parasites in what are in reality only incidental hosts, has probably, in many instances served to confuse our knowledge of the identity of many forms. This fact was partly instrumental in causing Cole and Hadley (1909) to refer to the coccidium found in poultry at the Rhode Island Station as *Coccidium cuniculi*. The name was then used to signify the morphological resemblance to

this organism; and it was not without some justification, for it is true that many of the coccidia observed in poultry actually belonged to this species, while many of those that did not, had many points of resemblance, as has been shown. It was not at that time assumed that the birds could, by any chance have taken in rabbit coccidia, since rabbit coccidiosis was not then known to exist among the rabbits at the Experiment Station. Since that time however, repeated examinations have shown the occurence of this coccidium, not only in the rabbits at the Station, but also in many wild rabbits shot in the vicinity. It has been one of the results of the investigations reported in this paper to show clearly that, although the coccidium of the rabbit has appeared frequently in fowls, the latter play merely the rôle of incidental hosts with respect to this organism; and, so far as has been ascertained up to the present time, the rabbit coccidia do not undergo any development within the bodies of birds. The reciprocal is true for the cysts of *Eimeria avium*, which have been found not infrequently in the intestinal tract of rabbits at the poultry department of the Rhode Island Agricultural Experiment Station. But notwithstanding these facts, the present morphological studies have clearly demonstrated the necessity of separating these two coccidia with regard to the part they play in the production of avian coccidiosis.

Shortly after this paper was completed, the writer was able to review the work of FANTHAM (1910) dealing with the coccidium studied in connection with the grouse disease. This species is undoubtedly identical with the organism that forms the subject of the present paper. Since the work of FANTHAM appeared to cover many points in the investigations here reported, it seemed at first advisable to withhold the present publication. Many of the facts presented here merely substantiate the work of FANTHAM, which is the first extensive exposition of the morphology, and to a lesser extent, of the biology, of *Eimeria avium*. Upon a closer reading of FANTHAM's paper, however, it was observed that many points taken up in the present communication might be regarded as supplementary to his data. For this reason the paper is published at this time, and further comment on FANTHAM's results will be deferred until a later date.

Kingston, R. I., U. S. A., Jan. 20, 1911.

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VII. Description of Plates.

Table of Measurements.

The following table of measurements, is compiled from data taken at the time when the coccidia were drawn. The dimension given is, in every instance, the greatest diameter, or length, of the figure.

Fig. 1	22 μ	Fig. 16	11 μ	Fig. 31	21 μ	Fig. 46	10,5 μ
2	21	17	10	32	22	47	9,5
3	22	18	9,1	33	14	48	9
4	23	19	9	34	16	49	9,5
5	21	20	8	35	20	50	10,5
6	22	21	21	36	30	51	8,5
7	8,1	22	22	37	26	52	8
8	9,6	23	20	38	27	53	8
9	10,3	24	21	39	8	54	7,5
10	11,5	25	22	40	8	55	7
11	12	26	23	41	14	56	3,5
12	12	27	24	42	15	57	4
13	11	28	23	43	58	58	4,5
14	11	29	21	44	17	59	5,5
15	9,2	30	20	45	21	60	6,7

Plate 1.

In Pl. 1, Figures 1—11, are drawn from developing cysts preserved in a 5 per cent solution of potassium bichromate. Figures 12—19 were drawn from smears of cecal or duodenal content, stained with the ROMANOWSKY malarial stain.

Figs. 1—6. Development of the cysts.

Figs. 7—11. Development of a single sporoblast.

Figs. 12—19. Sporozoites in different stages of development.

Fig. 20. Young schizont.

Figs. 21—27. Development of macrogamete.

Figs. 21—23. Reducing divisions (?) of nucleus.

Figs. 24, 25. Disintegration of chromatic substance and formation of chromatoid granules.

Fig. 26. Gathering of plastic granules about periphery of macrogamete.

Fig. 27. Granules forming wall of macrogamete.

Figs. 28—32. Maturation changes in macrogamete anticipating fertilization.

Plate 2.

In Pl. 2, Figures 33, 34, 35, 36, 38 and 43 are from sections stained either with iron-hematoxylin and eosin, or with DELAFIELD's hematoxylin and eosin. All other figures are from smears, stained with the ROMANOWSKY malarial stain.

Figs. 33—36. Stages in the development of the microgametocytes.

Figs. 33, 34. Young microgametes clustered about the residual body.

Fig. 35. Microgametocyte showing invagination into residual body.

Fig. 36. Microgametocyte with several segmentation centers.

Fig. 37. Young microgametes liberated from the residual substance but not yet freed from the microgametocyte.

Fig. 38. View of the surface of a young microgametocyte.

Figs. 39—43. Stages in the development of the merozoites.

Fig. 39. Early divisions of the schizont nucleus.

Fig. 40. Later divisions of the schizont nucleus; daughter nuclei clustered about the residual ball.

Fig. 41. Young merozoite-cyst of small type, within an epithelial cell.

Fig. 42. Young merozoite-cyst, of small type, containing two segmentation centers.

Fig. 43. Merozoite cyst, of large type, from the mucosa containing many merozoites grouped about several segmentation centers.

Fig. 44. Young macrogametocyte showing both plastic granules and chromatoid masses.

Fig. 45. Young macrogametocyte with the plastic granules grouped about the periphery, and forming the wall.

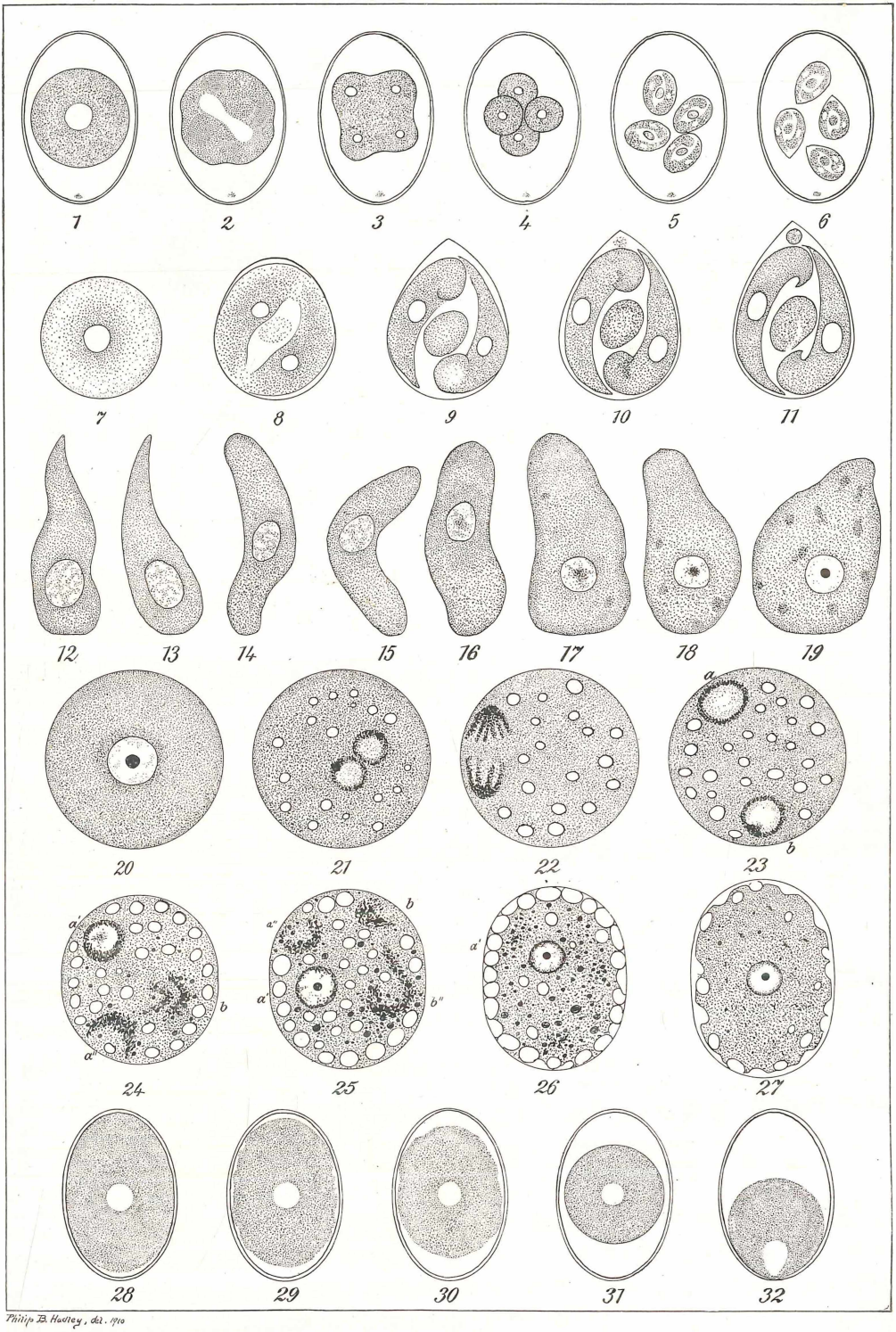
Figs. 46—60. Merozoites in different stages of development.

Figs. 47, 48, 51. Merozoites showing nuclear divisions.

Figs. 49, 50. Merozoites of peculiar form, possibly representing a sexual type.

Figs. 52—55. Merozoites which have undergone a limited development outside of cells, or have been pressed out of host-cells after development had begun.

Figs. 56—60. Young immature merozoites broken away from the residual body, and showing several atages of development.



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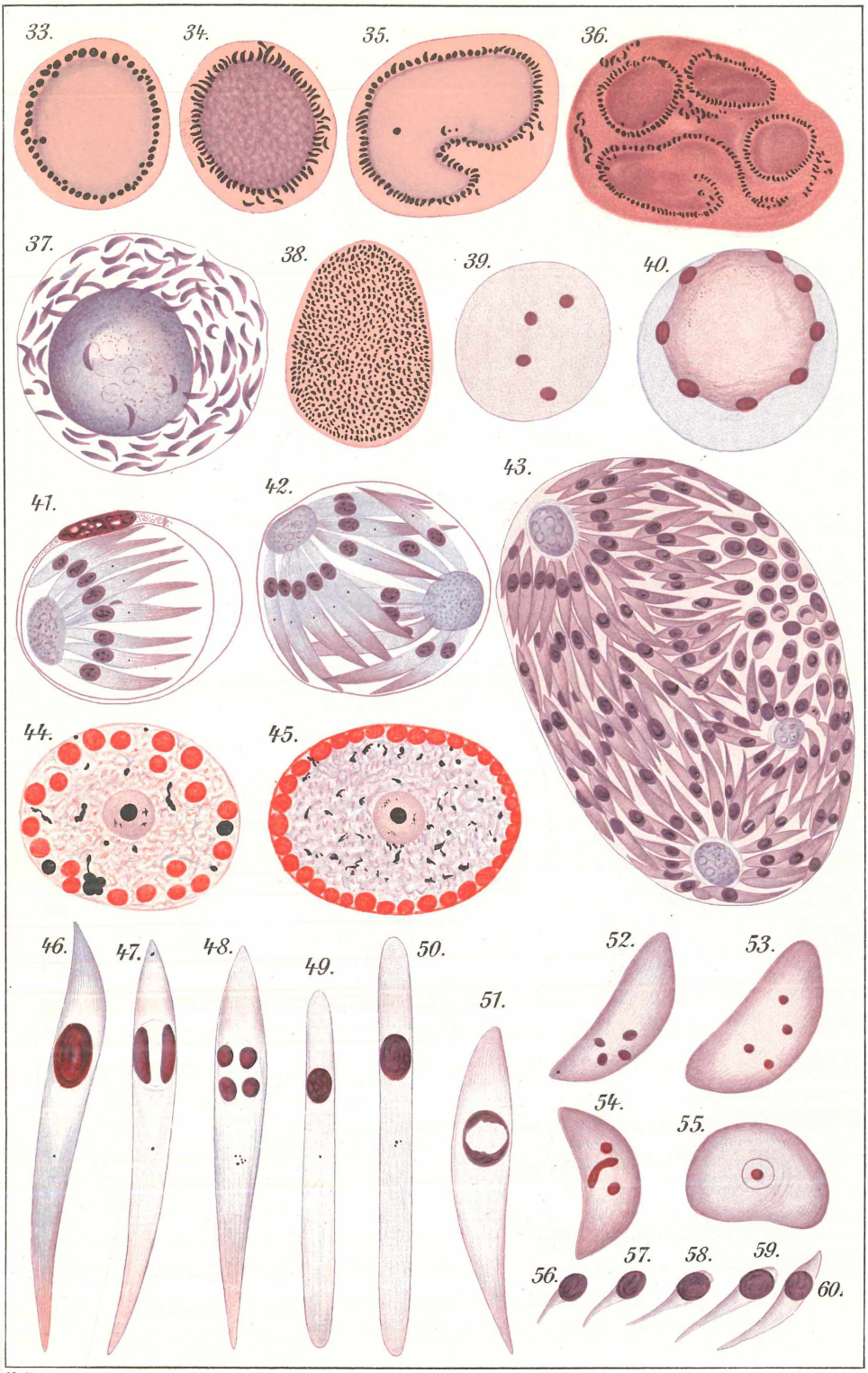
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