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The endogenous phases of the life cycles of Eimeria nieschulzi, Eimeria separata, and Eimeria miyairii coccidian parasites of the rat

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THE ENDODOGENOUS PHASES OF THE LIFE CYCLES OF
EIMERIA NIESCHULZI, EIMERIA SEPARATA, AND EIMERIA MIYAIRII
COCCIDIAN PARASITES OF THE RAT

By

Robert Lee Roudabush

A Thesis Submitted to the Graduate Faculty
for the degree of
DOCTOR OF PHILOSOPHY
Major Subject - Zoology

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Iowa State College
1936
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INTRODUCTION

Experimental work in parasitology, as in many other fields of biological endeavor, can be done effectively and judiciously only after the complete life histories of the organisms involved have been discovered. The knowledge derived from studying the life cycle may assist but little in direct control of a parasite, but the fundamental information thus derived is essential for intelligent experimentation on the best method of control. The rat is a laboratory animal that is well adapted in many ways for experimental work in coccidiosis. Furthermore, it harbors three species of coccidian parasites, whose endogenous cycles have never been delineated. An accurate comparative account of these cycles should constitute an important basic contribution to a program for the study of host-parasite relationships in coccidiosis.

It is because of the foregoing statements that a study of the life cycles of the three coccidian parasites of the rat has been undertaken.
HISTORICAL

Schaudinn (1900) was the first worker to elucidate the entire life history of a coccidium of the genus Eimeria. His work was an important step forward in the study of the coccidia, but was actually not complete in the light of what has since been uncovered concerning the histories of these parasites. Tyzzer (1929) and Tyzzer, Theiler and Jones (1932) have published most complete and understandable studies on the Eimeria of gallinaceous birds. These studies were concerned, in a large measure, with the life histories of the species of Eimeria found in the chicken and are at present the most authoritative works on the subject.

Dieben (1924) made some observations on the life-cycle of a rat coccidium, Eimeria nieschulzi, which he was describing. The work of this author, while in a general way correct as far as it went, was not so exhaustive as that of Tyzzer and his associates, carried out on the chicken coccidia.

The contributions of Tyzzer and Tyzzer, Theiler and Jones are stimulating and suggestive for the investigation of other species of Eimeria, but since their work was done on several species from the chicken it is felt that in the present instance there is no necessity to make too close comparisons between the developmental cycles in the two hosts. The fact that different hosts are involved is sufficient to necessitate individualiza-
tion of data. By no means, however, has their work been ig-
nored, since their papers have been used almost constantly as
a guide.
MATERIALS AND METHODS

All three of the known Eimeria of the rat were employed in this study. Each formed the basis for a distinct portion of the experimentation. No attempt was made to have all of the host animals from the same strain, since it was felt that the strain of rat would have little or no effect on the cycle of the parasite. It was known, however, that the rats had not been infected previous to the experimental infection. This precaution was necessary in order that no so-called "hold-over" infection could be present, and so that the animal would not be infected, experimentally, with less than the maximum number of parasites.

Infection, in most cases, was accomplished by injecting into the stomach through a rubber catheter a known number of sporulated oocysts. The number of oocysts given each rat varied with the length of time the host was required to live. For example, a much larger dose was given to animals intended to be killed on the first day of infection than to those intended to be used for study on subsequent days. This gradation in infective dose was made to insure, first, that the animal survived until the correct time, and second, that the infection be as heavy as possible in order to facilitate the study.

The oocysts used in the present work were derived from various sources. The oocysts of Eimeria nieschulzi were the progeny of the strain inbred by Hall (1934); oocysts of Eimeria
Eimeria miyairi were derived from the culture sent to Dr. E. H. Becker by Dr. A. C. Chandler; the Eimeria separata oocysts were collected by the author.

Material for study was secured from rats at twenty-four hour intervals and when necessary at intervals less than twenty-four hours. From each rat, five pieces of intestine were collected. These portions were taken from the upper small intestine, middle small intestine, lower small intestine, caecum and large intestine. The tissues were fixed in Zenker's, dehydrated, sectioned and stained with Goldhorn's polychrome methylene blue and eosin. Other stains were used, but, for the most of the work, it was found that the stain mentioned was the most useful.

At the same time that the tissues were fixed, living material was studied from the various levels of the intestine and smears were made from those portions of the intestine which showed parasites. In the case of early infections, smears were made not at levels where parasites were found, but at levels where experience showed the infection could be expected. This was done because some of the earliest stages in the life history were not observed in living condition. The smears obtained were fixed in Schaudinn's and stained with iron haemotoxylin and, in some cases, with Goldhorn's polychrome methylene blue and eosin.
Study of the microgametes was made using smears similar to those just described and also with two other types of preparations. The first of these was made by placing a drop of blood from the host on a clean slide and hurriedly smearing it with a piece of the intestine in which living gametes had been found. The slides were then dried and stained with Wright's blood stain in the usual manner. These preparations served to show the flagella to a distinct advantage. The second method consisted of mixing a little of the intestinal contents on a slide in a drop of two per cent aqueous Congo red. This drop was spread on the slide and allowed to dry. When thoroughly dry it was placed in ninety-five per cent ethyl alcohol to which several drops of hydrochloric acid had been added. This procedure stains the background black, while the flagella and body stand out as white objects on the black background.

Since the greatest difficulty presented by the study of the life cycles was the finding and identifying the various generations of merozoites, it may be well to explain the method employed.

Stained smears were studied and each type of merozoite found was drawn with the aid of a camera lucida. This study was made for each interval of time and for every rat infected. The resultant figures were all laid side by side and each merozoite from each rat matched with similar merozoites from
other rats. For example, if four distinct types of merozoites were found on the fourth day, three on the third, two on the second, etc., the similar merozoites found on the fourth, third and second days were all placed together, thus indicating a single type or generation. By this process of elimination the first type of merozoite to appear was discovered and designated the first generation, the second type derived was called the second generation, etc., until all possible types had been found. At the same time it was noted on what day each new type was first discovered, and thus it was found when each new generation appeared in the lumen of the intestine. The data accumulated were checked with the sections of the intestine. Specimens of each generation, on smears, were then drawn and measured to obtain the mean and range in size.

Counting the number of merozoites in each group was done by counting the number of nuclei present only after the merozoites had been fully formed. This was useful especially when more than one section had to be examined to get the complete number in one group, because it prevented duplication in count as it was not likely that the same nucleus could occur on more than one section.
The first authentic description of a coccidium from the rat, *Eimeria miyairii*, was by Chira (1912). Since the article is written in Japanese and since it is rather difficult to obtain in this country, previous workers in the same laboratory as the author were not able to compare their material with the original description. They were guided in determining species entirely by Fukuhara's brief German abstract, and by the fact that Pérard (1926) accepted the above name for the parasite with which he worked. Recently, acting upon the advice of Dr. E. R. Becker, the author obtained a photostatic copy of parts of the original paper by Chira and, by comparing the figures with the material being used for the present study, found that the coccidium previously considered to be *E. miyairii* was not that described by Chira. A comparison of the macrogametocytes of the parasite described by Chira with the macrogametocytes of Pinto's (1928) *E. carinii* reveals that the latter is a synonym of the former. Therefore the coccidium discussed by Becker (1934) as *E. carinii* is actually *E. miyairii*. This correction leaves for the parasite considered by Becker (1934) to be *E. miyairii* the name *E. nieschulzi* Dieben (1924), the correct appellation according to the rules of nomenclature.

Parenthetically it should be noted that Becker and others in several papers (1932, 1934) noted the possibility that the names used were the incorrect ones, and also that it was Beck-
er who pointed out to the author that this study of the nomen-
clature should be made.

The correction made above does not change the status of
Eimeria separata Becker and Hall (1931) which remains valid as
described.

Eimeria halli Yakimoff (1935) remains an uncertain species
since the measurements are conflicting with the above species.
The method used in procuring the material and the lack of exper-
imental data also should cause some doubt as to its validity.
Endogenous Cycle of *Eimeria nieschulzi*

*Eimeria nieschulzi* Dieben (1924) is a parasite of the epithelium of the small intestine of the rat. The infections of this species are heaviest in the middle of the small intestine. The epithelial cells most affected are found along the base of the villi and in the glands, but usually not as far as the fundus of the gland. In several cases, however, schizonts were found at the base of the glands and even in the Paneth cells. The position of the schizonts is generally toward the distal end of the cell, but variation has been found in all of the generations of schizonts. The macrogametocytes are found in the vast majority of cases, toward the proximal end of the parasitized cell.

Dieben, in the description of *E. nieschulzi*, reports the prepatent period to be eight days, while Becker and Hall (1931) find it to be seven days. The latter is in accordance with the author's findings.

The original description of *E. nieschulzi* states that the parasite is found in the small intestine and further in the caecum. Since the present worker has been unable to find phases of the life history of this parasite in the caecum it is supposed that Dieben was working with a mixed culture of both
E. nieschulzi and E. separata, the latter being the form found in the caecum. However, since the majority of forms described by Dieben compare with those found to be parts of the cycle of E. nieschulzi, we are considering the form from the small intestine to be the parasite described by that author.

Becker (1934) gives the location of the coccidium under consideration as being in the small intestine, caecum and colon, and further, gives the present author credit for the information. The information given was intended only for the other two species in the rat.

Since the purpose of this paper is the discussion of the endogenous phases of the life cycle, the description will necessarily begin with the sporozoite after excystation in the intestine. Time, when mentioned, is the shortest period obtained, since the minimum time is the time desired in this type of life history survey.

From three to four hours after infecting a rat with E. nieschulzi the sporozoites may be found in the lumen of the small intestine. These sporozoites may remain in the intestine in infective condition up to four days. This statement may be substantiated by the fact that smears, made four days after a single infection, showed sporozoites in the lumen of the small intestine. Further, tissue from this rat showed very young first generation schizonts in some of the cells. Thus, it is
shown that a single infection per os actually means a continued infection for four days when the actual entrance of the sporozoite into a cell is considered. The continuous infection for four days accounts for the fact that the patent period is generally four days in length. Hall (1934) found, however, that in about fifty per cent of the infections carried on by her the patent period was five days in duration. Since the patent period is calculated from the appearance and disappearance of oocysts in the feces, it must be understood that it takes some time for the oocysts to be eliminated from the body. This time would be a factor in the apparent lengthening of the patent period.

Further, sporozoites may, possibly, live in some rats longer than four days, since they were present, at that time, in numbers great enough to allow them to be found on smears. A carmine suspension fed to rats with a stomach tube was not totally eliminated until slightly less than four days after feeding. (Pellets, taken at six hour intervals, and mashed in 70% alcohol gave a red tinge to the alcohol until almost the fourth day.) It is believed that motile sporozoites could remain in the intestine slightly longer than inert particles of carmine.

Hall (1934) suggests that the increased length of oocyst elimination with increase of the size of the infective dose might be because the greater number of oocysts being passed allowed them to be found later than usually. This may be a fac-
tor but it is not the entire explanation. In the light of the above discussion it is believed that the greater the number of sporozoites present the greater the chance that some of them might not contact a suitable host cell until a relatively late time, thus the patent period would be lengthened. Subsequent generations of merozoites may also have difficulty in reaching host cells, and thus lengthen the cycle.

The continued infection caused by the entrance of sporozoites over a period of time necessarily causes some complication in the life history. It has been shown that the cycle is beginning and ending over a period of approximately four days. Each generation of merozoites may be expected to be also found on a similar period of four days. This shows that on any single day several generations of merozoites may be present. The method of separating and identifying these generations has already been discussed.

Because the prepatent period is seven days, it should be noted that the oöcysts must leave the tissues at about six and one-half days in order to get to the outside on the seventh day. Therefore, the time for minimum endogenous development is six and one-half days, but the prepatent period ends at the time the oöcysts appear in the feces. Thus it is shown that the endogenous development does not end with the prepatent period, however, since it is the latter from which calculations have
been made by other workers, it is considered that the seventh day, or the time when the oöcysts first appear in the feces, is the termination of the cycle. For the period actually spent in the tissues, the name endogenous period is suggested in order to distinguish it from the prepatent period.

Sporozoites of *E. nieschulzi* when excysted range in length from 9.9 - 12.15µ, the mean length being 11.35µ. Width, at the nucleus, varies from 1.15 - 2.25µ, with a mean of 1.8µ. When stained with iron haematoxylin the nucleus shows a well-defined central karyosome with a very light-staining nuclear wall. The sporozoite contains two globules which are extremely siderophilic; the anterior one being circular in outline, the posterior being elongate oval. These globules, when stained with Goldhorn's polychrome methylene blue and eosin, take an intense red color. One sporozoite was found in which the posterior globule was divided in two, each part being circular in outline. This particular specimen had a rather peculiar nuclear structure, the chromatin being divided in three pieces giving them the appearance of chromosomes. Since no other similar specimen was found it is considered that it was not a normal condition.

The sporozoite, after entering the host cell, forms the first generation schizont. This schizont may be distinguished from later generations by the presence of a large refractile globule. The two globules of the sporozoite evidently unite after the sporozoite has entered the cell, thus giving rise to
the characteristic globule. Like the two from which it is derived the globule of the schizont stains intensely red with eosin. The eosinophilic mass can be seen almost up to the time the merozoites are completely formed, but at some time before actual maturity of the merozoites it disappears. As long as the globule can be seen it is surrounded by the residual mass of the schizont. The first schizogony is complete at about thirty-six hours, and from the first schizont 20 - 36 merozoites are formed.

The merozoites formed are known as the first generation merozoites and have a range in length from 6.75 - 9.9 μ, mean length being 8.59 μ. They vary in width from 1.35 - 1.98 μ, mean being 1.64 μ. When seen on smears stained with iron haematoxylin these merozoites have a granular cytoplasm; the majority of them show a rather deeply stained anterior end. Tissues stained with polychrome methylene blue and eosin show these merozoites with two small eosinophilic globules - one anterior and one posterior to the nucleus. These globules are so strikingly similar to the globule of the sporozoite that one is led to think that they are made of the same material - perhaps these are derived from those of the sporozoite. Study, however, fails to find any trace of them on the smears stained with iron haematoxylin, so it is believed that they must be derived from some other source.
First generation merozoites, after breaking out of the host cell, migrate to and enter other intestinal cells. Here form the second generation schizonts. This schizont is characterized by having the red globules of the first generation merozoite imbedded in its cytoplasm. Both globules may sometimes be seen; at other times, only one; and some instances have been noted where neither is to be seen. The latter case may be because of the manner in which the section had been cut. The second schizont matures in about forty-eight hours after infection and gives rise to 10 - 14 second generation merozoites, which have a length of 12.6 - 16.2 μ, mean 14.36 μ, and a width varying from 0.9 - 1.4 μ, average being 1.24 μ. With iron haematoxylin they show a granular cytoplasm which stains relatively lightly in the region around the nucleus. Some groups of merozoites, evidently belonging to this same generation, when seen in sectioned material showed an eosinophilic mass near the posterior end. Only a few of these, however, have been seen.

Third generation schizonts are initiated when the second generation merozoites enter the epithelial cells. So far as can be determined no distinguishing morphological feature is present in this schizont which would differentiate it from the early fourth generation. The time of its appearance may assist in identifying it only in material in which it is certain
the fourth generation has not as yet begun. The difference in
the number of nuclei present in the older fourth generation
schizont is a feature which is not of much assistance. Third
generation schizonts give rise to 8 - 16 merozoites which break
out of the cells on the third day. These merozoites range in
length from 17.1 - 21.6 μ, the mean length being 19.0 μ; in
width from 1.0 - 1.35 μ with a mean of 1.19 μ. These mero-
zoites are generally the first noted in living material and on
smears because of their size. They are characteristically bent
and usually are seen shaped like a U or a J; this, however,
is not true of all specimens as some are straight. In the an-
terior end of each merozoite of the third generation there is a
densely granular portion. There are also, in the anterior por-
tion, along the median line, several larger granules - usually
three in number. These features along with size assist in
separating the third from the second generation.

Fourth generation schizonts, formed by third generation
merozoites possess, in their later development more nuclei than
any other generation. The fourth generation schizont gives
rise to merozoites on the fourth day of the infection. These
merozoites, from 36 - 60 in number, have a length range of 4.5 -
6.7 μ, average being 5.5 μ. Width varies from 1.0 - 1.8 μ,
with a mean of 1.35 μ. Size alone is a characterizing feature
of this generation of merozoites. It should be mentioned that
in the author's material these schizonts appeared on sections rather infrequently. Discussion of this point will be deferred until later.

Gamete formation begins when the fourth generation merozoites enter host cells. The young gametocytes may first be noticed at about five and one-half days but cannot be identified until some time later because the distinction depends upon the division of the nucleus of the microgametocyte. Each young gametocyte consists of a central mass of chromatin around which appears a crescentic area which seems to be filled with a fluid - at least granules are not found in that area. Surrounding this area is a region of rather densely granular cytoplasm which extends to the cell wall.

The nucleus of the microgametocyte undergoes a number of divisions, during which time the cytoplasm increases in volume. These nuclei, which are formed from the division, later migrate to the periphery of the gametocyte. During and after the migration to the surface the chromatin masses which have, up to this time, been circular in outline, begin to elongate and become roughly triangular in shape. This shape changes, and when the gametes are mature they have a general outline similar to that of a comma. When found on smears of intestinal contents their shapes vary considerably, depending upon the position they assumed at the time of fixation. The preparations described
under the section on materials and methods, along with some observations on living material, serve nicely to show flagella. The measurements were made from specimens on smears stained with Wright's. This fact is important because the size is probably influenced by the fact that they were allowed to dry.

The microgametes typically have two flagella which arise at the anterior end of the body. At the base of the flagella in a few specimens stained with iron haematoxylin a small round dark body was observed. This object, seen also by Dieben (1924), the author considers to be a blepheroplast. The remainder of the body of the gamete appeared in the majority of cases to be, for the most part, homogeneous. The body proper of the microgametes had a mean length of 4.38 µ and a width of 0.61 µ; the flagella averaged 9.18 µ in length.

The macrogametocytes of *Eimeria nieschulzi* occur in the same locality in the intestine as the asexual phases of the cycle. However, the macrogametocytes are usually found in the proximal end of the epithelial cells which they infect. The parasitized cells in infections with this species have never been found pushed out of the epithelial layer except when they contain the mature oocyst, and double infections with macrogametocytes have never been encountered. These are specific characters.

It was not deemed essential to try to measure the macro-
gametocytes because age would cause so much variation in size and because they can be measured much more correctly after they leave the animal as oöcyts.

The oöcyst wall begins to form early in the development of the macrogametocyte and since the construction of the wall is interesting, it is outlined below. The first change to take place in the development of the oöcystic wall is the formation of some granules in a circle around the nucleus. The first of these granules to develop are comparatively large and stain a dull red with eosin. The second are much smaller and stain an intense blue with methylene blue. The former granules are termed plastic granules; and the latter, haematoxylinophilic granules after Hosoda (1928). As the macrogametocyte grows older the plastic granules move toward the periphery of the cell and become flattened in a rather thick layer at the limits of the gametocyte. This layer forms the outer wall of the oöcyst. The haematoxylinophilic granules follow the plastic granules in their migration toward the limits of the cell, but do not reach the edge until the outer wall has been completely formed. Upon reaching the outer wall, these haematoxylinophilic granules begin to flatten out just as the plastic granules did. Since the former are smaller, the layer formed is much thinner. This layer derived from the haematoxylinophilic granules stains blue with methylene blue and eosin.
During the time the oöcyst wall has been forming another group of granules similar to the plastic granules arises around the nucleus. These begin to migrate toward the periphery but do not reach it; instead they remain dispersed in the cytoplasm. It is not at all unlikely that this latter set of granules, dispersed throughout the entire cyst, gives to the unsporulated oöcyst its vacuolate appearance. It is also probable that these granules may have some part in the subsequent formation of the sporocyst wall, since this wall is usually made in one layer and resembles the outside wall of the oöcyst.

In order to be certain that the oöcyst wall is composed of only two layers some oöcysts were placed on a slide and crushed with the cover-glass. Many of the oöcysts crushed had only the outside wall broken thus showing a distinct thick outer wall and a thin inner wall or membrane.

Fertilization spindles, etc. have not been seen but microgametes have been found in sections in close proximity to the wall of the macrogametocyte. Whether fertilization instigates oöcyst wall formation or not will be taken up subsequently.

When the oöcysts of E. nieschulzi are eliminated the host cell wall seems to break and the parasite is forced out. Only on rare occasions may mature oöcysts be found with a remnant of the host cell attached to it.

Henry (1932) pointed out that the oöcyst wall of Eimeria
was usually composed of two, and less frequently three, layers. She also noted that in the two layered walls the outer one was the thicker of the two. This is the same condition as noted above for the species under consideration. The figures of most authors represent the wall of oöcysts by a thin outer line and a thick inner one. The oöcysts appear as described but an explanation should be made for such appearance. The thin outer line is actually the interface between the outside medium and the first layer of the wall. The light area immediately inside of it must be interpreted to be the outer wall itself. Next, the heavy inner line must be the interface between the inner and outer layers. Thus the dark portions in most figures represent the interfaces and the light refracted from them and not the layers of the wall itself.

From the foregoing outline of the life history of the coccidium under consideration it would seem plausible to conclude that at least this species of the genus Eimeria has a self-limited life cycle. There is, however, a possibility that every host reacts exactly the same to infection, and so it would seem that the host actually limited the cycle in one way or another. This has already been proven incorrect by the transmission of merozoites as carried on by the author (Roudabush 1935). In the work mentioned the author infected a rat for five days and then transmitted the infection to a second rat, by means of the
merozoites. If the cycle is host limited the second rat should have thrown off oocysts on the seventh day after transmission; instead, the rat eliminated oocysts on the second day after transmission, thus showing that the cycle is limited not by the host but by the parasite itself.

It has been pointed out above that the fourth generation merozoites were not to be found in any great numbers and because of this fact two possibilities should be brought to mind. The first of these is the most likely and most obvious. The scarcity of the last generation merozoites may be only an apparent reduction in numbers because the material collected was not taken at the time of greatest production of these asexual phases. The other possibility is that instead of having a fourth generation of merozoites this small sized group is a sexually differentiated portion of the third generation. Should this be true the larger merozoites, which have been called third generation, would be the ones giving rise to the macrogametocytes while the smaller merozoites being fewer in number would give rise to the male elements. This, of course, is difficult to prove because if a merozoite is found in a cell it may be correctly placed in the group of merozoites to which it belongs but the product it is going to form cannot be assured. Conversely, when the product can be identified the merozoite from which it is derived cannot.
Before an accurate opinion is formed about each of the above possibilities consideration should be given to one other matter. This concerns itself with the number of oöcysts eliminated from a single infection of a known quantity. Hall (1934) in studies on the quantitative infections of this same species of parasite found that out of thirty-three attempts at single oöcyst infections only twenty-two were successful. From these data she concluded that two-thirds of the total number of oöcysts fed constituted the infective dose. This calculation, while probably mathematically correct, would seem to be biologically unsound. In order to be correct concerning the number of viable oöcysts in her cultures it would have been necessary to make all of the thirty-three infections on the same day from the same culture. Even then the possibility of the oöcyst remaining in the dilution pipette, or some other similar accident in technique, could not be guarded against sufficiently to be certain that every rat actually swallowed the oöcyst intended for it. Disregarding this latter statement and supposing that all of her attempts at single oöcyst infections were made from the same culture on the same day, to be correct about the remainder of the infections they would necessarily have had to be made at the same time from the same culture. This, of course, would be improbable. In order to attempt a correction of the data compiled by Hall and to correlate it with the present work
it is necessary to consider that every sporulated oöcyst fed is, disregarding the fact that cultures vary in viability, a potential infector. The author realizes that cultures vary in infectability because of age, environment, etc.; but since it is practically impossible to standardize each culture, if cultures are used when fresh each sporulated oöcyst does constitute at least a potential infector.

In view of the preceding statements a recalculation of the means for yields from various infective doses is given here. These calculations are on the basis that six oöcysts fed is a six oöcyst infection.

Yield per oöcyst fed

| 1 oöcyst fed | 62,000 |
| 6 oöcysts fed | 1,455,000 |
| 15 oöcysts fed | 1,389,000 |
| 75 oöcysts fed | 1,098,000 |
| 150 oöcysts fed | 1,029,666 |
| 2000 oöcysts fed | 144,150 |

From these data it will be seen that the highest mean yielded from a single oöcyst is 1,455,000. This number, of course, represents the number of macrogametocytes formed during the course of the infection.

Multiplying the mean number of merozoites found in schizonts one, two, three and four and then multiplying that number by eight, the number of sporozoites in one oöcyst, it is
found that each oöcyst fed produces a mean total of 1,872,000
gametocytes. Subtracting 1,455,000 — the number of macrogametocytes
as found in Hall's revised data we have a total of 417,000
microgametocytes formed from one oöcyst. This number is slightly
over twenty per cent of the total number of gametocytes formed which is about the correct ratio as observed in sections of
the intestine. The extremes of infection may be calculated by
multiplying the minimum number of merozoites in each generation
by each other and that result by eight, the sporozoite number,
and by multiplying the maximum number in the same way. This
calculation gives a range of 460,800 - 4,819,200 gametocytes
produced from one oöcyst.

If the calculation, made from Hall's and the author's data
is correct it is plausible to propose to consider that the
small sized merozoites belong to a distinct generation, since
the average number in a single fourth generation schizont was
used in the calculation. The author for the present, at least,
considers that there are four complete generations of merozo-
ites - but points out the other possibility, merely because it
is a possibility.

Hosoda (1928) indicates in his work that fertilization
takes place before the oöcyst wall has actually begun to form.
Since fertilization has not been seen in the present work it is
impossible to verify Hosoda's observation. It has been observ-
ed, however, that the formation of the granules which ultimately constitute the oocyst wall takes place rather early. In addition to this all of the oocysts usually are eliminated and no hold-over macrogametocytes have been found. This would seem to indicate that either there is a one hundred per cent fertilization or else that all macrogametes after a time are thrown off by the host. It would seem rather improbable that in every infection there would be one hundred per cent fertilization so that the latter of the two suppositions would be the more plausible. This again allows several possibilities, the first of which would be that the unfertilized macrogametes are in some manner destroyed by the host. Another possibility is that the oocyst wall is formed without regard to fertilization and so some unfertilized oocysts are eliminated by the host. The latter may account for the number of oocysts which usually fail to sporulate.
Table I.

*Eimeria nieschulzi*

Data concerning the asexual phases

<table>
<thead>
<tr>
<th></th>
<th>Sporozoite</th>
<th>1st Generation</th>
<th>2nd Generation</th>
<th>3rd Generation</th>
<th>4th Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>9.9-12.15µ</td>
<td>6.7-9.9µ</td>
<td>12.6-16.2µ</td>
<td>17.1-21.6µ</td>
<td>4.5-6.75µ</td>
</tr>
<tr>
<td>Mean</td>
<td>11.33µ</td>
<td>8.59µ</td>
<td>14.36µ</td>
<td>19.08µ</td>
<td>5.56µ</td>
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<td><strong>Width</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.15-2.25µ</td>
<td>1.35-1.9µ</td>
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<td>1.35µ</td>
</tr>
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<td><strong>Number in one Schizont</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
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<td>10-14</td>
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<td>26</td>
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<tr>
<td></td>
<td>1.5 days</td>
<td>2 days</td>
<td>3 days</td>
<td>4 days</td>
<td></td>
</tr>
</tbody>
</table>
**Endogenous Cycle of Eimeria separata**

*Eimeria separata* Becker and Hall (1931) parasitizes portions of the epithelial layer of the caecum and large intestine of the rat. Infection is found to be heavier in the caecum. The cells attacked are found only in the surface epithelium of the caecum and large intestine, the epithelium of the glands never having been found parasitized. The gametocytes and schizonts are found below the nucleus toward the proximal end of the cell. Some stages, especially macrogametocytes, have been found apparently below the epithelial layer. This apparent location may be because of the plane at which the section was cut, and the parasite may actually be in the base of a cell, the long axis of which was at an angle to the plane of the section.

Becker and Hall (1931) report the prepatent period to be between five and six days. As pointed out for *E. nieschulzi* this period represents the time elapsed from infection until oocysts appear in the feces. The endogenous period for this species is four and one-half days.

Sporozoites of *E. separata* may be found in the caecum approximately six hours after infection. Some sporozoites, either because of later excystment or because they fail to enter host cells, have been found free in the lumen of the caecum at three days. This late infection, as in *E. nieschulzi*, complicates the life-cycle and also shows why the patent period
should be from three to four days as described by Becker and Hall (1931).

The sporozoites are similar in structure to the sporozoites of *E. nieschulzi* - differing only slightly in cytological composition. They possess an anterior and a posterior refractile globule, both of which are circular in outline. The posterior globule is the larger of the two. Sporozoites have a length range of 7.65 - 10.35 μ, the mean length being 9.45 μ. At the nucleus, the width varies from 1.8 - 2.7 μ with a mean of 2.27 μ. The nucleus is located at about the middle of the sporozoite and has a central karyosome. The refractile globules are siderophilic and the cytoplasm is granular, the anterior tip being densely granular.

The sporozoite enters a host cell and forms the first generation schizont. The spherical refractile globules may be found in the early schizont but disappear during the development. The nucleus divides and the cytoplasm with it until the schizont has the appearance of a morula. Each portion begins to elongate and form a merozoite. This process in no way appears as though the merozoites were budded off of a main body. No residuum has been seen. At about twenty-four hours after infection the first generation merozoites are completely formed and begin to break out of the cell.

These first generation merozoites have a range in length
from 10.8 - 13.05 μ, mean length being 11.89 μ. They vary in width from 1.8 - 2.7 μ with an average of 2.25 μ. Each group of first generation merozoites contains from 6 - 12 merozoites. Merozoites of the first generation have a nucleus which exhibits a central karyosome. The cytoplasm is granular, with a few large granules and, with the exception of the portion immediately surrounding the nucleus, takes an even blue stain with iron haematoxylin. The area around the nucleus stains more lightly than the remainder of the cytoplasm.

The first generation merozoites break out of their host cell, migrate to other cells, and enter them to form the second generation schizont. The early second generation schizont has a large circular area in its center which is not granular at all. The cytoplasm immediately surrounding this area is, in contrast, densely granular.

Schizogony is complete at about forty-eight hours, and the merozoites formed number from 4 - 6. These second generation merozoites have a length range from 6.3 - 9.45 μ; mean, 7.74 μ. In width they vary from 1.8 - 2.7 μ, average being 2.26 μ. The merozoites of this generation are characteristically short and broad. The cytoplasm is stippled and has several large granules scattered throughout. No special designating character except size has been found for this generation.

Upon entering other cells, the second generation merozoites form third generation schizonts. The early schizont of this
generation looks very similar to other schizonts. The third generation merozoites are mature at about seventy-two hours after infection. In each group there are 2 - 6 merozoites formed. These have a length range from 12.60 - 15.30 µ; mean, 13.62 µ. In width they vary from 2.02 - 3.15 µ and average 2.63 µ. This generation not only contains the largest merozoites of this species, but they have one distinguishing character: the tip of the anterior end of each stains an intense red with methylene blue and eosin. With iron haematoxylin this same area stains a rather brownish yellow; the latter color probably is derived from the iron alum used to destain the preparations. No residuum occurs as a result of this schizogony.

Gametocytes are formed when the third generation merozoites infect the epithelial cells. The macro- and the microgametocytes resemble each other in their early development but when the nucleus of the microgametocyte divides it can be definitely distinguished. The early gametocytes have an oval vacuolated area which contains an eccentric chromatin mass.

The development of the macrogametocyte parallels that of E. nieschulzi, but differs in some details. The granules which go up to make up the oöcyst wall arise in the same way, but the haematoxylinophilic ones seem to reach the periphery before the plastic granules. The former do not flatten out, however, until the latter have passed between and have formed the outer
oöcyst wall. The haematoxylinophilic granules then flatten out to form the thin inner membrane of the oöcyst. Meanwhile, a second set of plastic granules forms and migrates to just within the inner wall. They remain in this position when the oöcyst is thrown out of the tissues. Their further development has not been traced but as in *E. nieschulzi* they probably contribute toward the formation of the sporocyst wall at the time of sporulation.

The microgametocyte undergoes its nuclear divisions to form the microgametes without any outstanding variation from *E. nieschulzi*. The chromatin afterwards migrates to the periphery of the cell, begins to elongate, and becomes comma shaped. Finally, in the last stage it loses its heavy anterior end and becomes rather evenly elongate. This is the mature condition and is the motile phase. These microgametes, because they break out in the caecum and mix with the bacterial content, are rather difficult to find on smear preparations. For this reason the measurements of these gametes are based on specimens found on sections of the caecum. The body of the microgamete varies from 1.8 - 2.7 μ in length; the width is estimated to be somewhat less than 0.5 μ.

Actual fertilization in this species has not been observed, but since microgametes have been found in the tissues around macrogametocytes, it is probable that it takes place in the
tissues during the early oocyst wall formation.

The outline of the endogenous cycle indicates that this species has a self-limited cycle. No attempt has been made, however, to check this by transmission of merozoites.

The author does not consider that the fact that *E. separata* has only three generations of merozoites contradicts in any way the data obtained for *E. nieschulzi*. The difference in number of generations is probably only a specific difference, i.e., not generic.

Becker and Hall (1931) observed that the numbers of oocysts eliminated in an infection of *E. separata* were relatively low. In accordance with this observation several facts should be noted. First, the fact that this species attacks only the surface epithelium of the caecum and colon would tend to make the number of available cells extremely low, as compared with the area attacked by *E. nieschulzi*. Second, the reduced number of generations of merozoites and third, the reduced number of merozoites produced in each generation together contribute to considerable reduction in the possible number of gametocytes formed.

To elaborate further on this multiply the mean number of merozoites formed in each generation by each other and then by the number of sporozoites in one oocyst and the resultant 1,536 represents the number of gametocytes formed from one oocyst.
The extremes of infection may be found by multiplying the minimum number in each generation by each other and then by eight and by multiplying the maximum numbers by each other and by eight. The range calculated in this manner is from 384 to 3,456 gametocytes per oöcyst fed. These figures not only show the reason for the lowered oöcyst production, but also point out just where the reduction actually takes place.

The prepatent period for *E. separata* is shorter than that for *E. nieschulzi*. The lowered number of generations of merozoites assists in understanding just why this period should be so short.
### Table II.

**Eimeria separata**

Data concerning the asexual phases

<table>
<thead>
<tr>
<th></th>
<th>Sporozoite</th>
<th>1st Generation</th>
<th>2nd Generation</th>
<th>3rd Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>7.65-10.35μ</td>
<td>10.8-13.05μ</td>
<td>6.30-9.45μ</td>
<td>12.6-15.30μ</td>
</tr>
<tr>
<td>Mean</td>
<td>9.45μ</td>
<td>11.89μ</td>
<td>7.74μ</td>
<td>13.62μ</td>
</tr>
<tr>
<td><strong>Width</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.8-2.7μ</td>
<td>1.8-2.7μ</td>
<td>1.8-2.7μ</td>
<td>2.02-3.15μ</td>
</tr>
<tr>
<td>Mean</td>
<td>2.27μ</td>
<td>2.25μ</td>
<td>2.26μ</td>
<td>2.63μ</td>
</tr>
<tr>
<td><strong>Number in one</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizont</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>6-12</td>
<td>4-6</td>
<td>2-6</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>8</td>
<td>5.5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><strong>Time of</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maturing</td>
<td>1 day</td>
<td>2 days</td>
<td>3 days</td>
<td></td>
</tr>
</tbody>
</table>
Endogenous Cycle of *Eimeria miyairii*

*Eimeria miyairii* Ohira (1912) passes its endogenous cycle in the epithelium of the mucosa of the small intestine of the rat. (Ohira probably had a mixed culture of this species and *E. separata*. At least, he figured some merozoites of the latter species.) The parasite is found almost exclusively in the epithelium of the villi, but has been found, on a few occasions, down in the glands. One most striking difference between this species and *E. nieschulzi* is the former's habit of pushing cells parasitized by it out of the epithelial layer toward the tunica propria. This behavior was noted by Ohira in the description of the species, and it is largely upon this basis that the correct determination of the specific name has been made. In addition, this species frequently causes double infections of cells, especially in the later stages of the cycle.

Becker (1934) reported the prepatent period for this species to be six days. The endogenous period is, therefore, about five and one-half days.

Sporozoites of *E. miyairii* are found most abundantly in the small intestine about twelve hours after infection. A series of infections with this species failed to show any sporozoites in the lumen of the small intestine after two days, but since the time of excystment is so long and since it is probable that they will live in the intestine for four days, the
Sporozoites of this species have two refractile globules—one anterior and one posterior to the nucleus. As in the other species, the posterior globule is the larger of the two. The posterior globule is an elongate bar rounded at both ends, while the anterior is circular in outline. The sporozoites have a length range of 12.15 - 16.65 µ; mean, 14.53 µ. Width varies from 2.25 - 3.15 µ with an average of 2.65 µ. The nucleus is situated toward the anterior end of the sporozoite, and shows a marginal chromatin ring with a central karyosome. Both refractile globules are siderophilic and the cytoplasm is granular, the anterior end being densely granular.

The sporozoite upon entering a cell forms the first generation schizont. Nothing, as far as has been determined, is distinctive of this schizont. (No early schizonts have been observed and only one sporozoite has been found in an intestinal cell.) When the first generation merozoites are fully formed a residual mass may be seen in the center with the merozoites arranged in a circle around it. The merozoites leave the host cell in about two days after infection.

The first generation merozoites have a range in length from 5.58 - 7.20 µ; mean length, 6.58 µ. Width, in these merozoites, varies from 1.06 - 1.57 µ, with a mean of 1.27 µ. From
12 - 24 merozoites are formed from each first schizont. The nucleus of the first generation merozoites is central and has a central karyosome. The cytoplasm is evenly granular, except for a lighter area surrounding the nucleus.

First generation merozoites break out of their host cells and enter other cells to form the second schizont. Since the host cells are situated below the epithelial layer, in most cases, the merozoites get out into the lumen of the intestine by migrating through spaces left by the injured cells. In the cells which do not leave the epithelial layer the parasites are located toward the proximal end of the cell. The second schizonts are, in their early development, similar to other schizonts and so no distinction may be made between them.

Schizogony is completed on the third day of the infection and 8 - 16 merozoites are formed in each schizont. A residuum is formed in this schizont. The merozoites formed are the second generation merozoites. They have a length range of 8.10 - 11.25 μ; mean length, 9.15 μ. Width varies from 1.00 - 1.80 μ with a mean of 1.44 μ. Second generation merozoites are the largest formed in this species. They may be distinguished from other merozoites by the fact that the nucleus is located in the posterior one-fourth of the body. The cytoplasm takes an even stain, except for the area around the nucleus, which, as in other merozoites, takes a lighter stain. These merozoites
usually have one or two large granules situated anterior to the nucleus.

The second generation merozoites enter cells and form the third generation schizont which is similar to other schizonts of this species. This third generation merozoites formed mature on the fourth day, leaving a large residual body. These merozoites range from 20 - 24 in number. They have a length range of 3.60 - 5.13 μ, with a mean of 4.37 μ; in width they vary from 1.00 - 1.57 μ, average being 1.23 μ. Merozoites of this generation have the usual light area surrounding the nucleus, the remainder of the cytoplasm being evenly granular.

Third generation merozoites infect other cells and form the gametocytes. The microgametocytes and macrogametocytes are indistinguishable before the microgametocyte undergoes its first division. These early gametocytes have a central vacuolated area in which an eccentric chromatin mass is located.

The development of the macrogametocyte differs slightly from the other two species discussed. The plastic granules are extremely large while the haematoxylinophilic granules are very small. The plastic granules migrate to the border of the cell and fuse to form the outer wall of the oocyst. The fusion is not normally complete and consequently the outer wall of this species is marked with radial striations. In some cases, however, the plastic granules fragment upon reaching the margin of the cell and then fuse. This latter instance along with the
crowded condition of double infections probably explains the occasional smooth walled oocysts of this species. When the oocysts of E. miyairii are eliminated from the intestinal wall, the epithelial layer is at least temporarily broken by the passage of the oocyst through it.

The microgametocyte in its development resembles the microgametocytes of the other species previously described. The chromatin migrates to the periphery of the cell and then elongates to form the microgametes. Typically the microgametes have two flagella attached at the anterior end of the body. The body of the microgametes has a mean length of 3 μ, width being approximately 0.75 μ. A large residual mass is present when the microgametes have reached maturity.

Microgametes have been found in the tissues around the macrogametocytes so that fertilization probably takes place while the latter are still in the tissues.

Again it is felt that the endogenous cycle as outlined is indicative of a self-limited life cycle.

Becker (1934) indicated that the number of oocysts eliminated by a rat infected with E. miyairii was not so great as in the case of E. nieschulzi, but greater than in the case of E. separata. The present results point toward the same conclusion. To compare this with the other two species, the mean number of merozoites in each generation should be multiplied by
each other, and then by the number of sporozoites in one oocyst. This calculation gives a mean number of 38,016 gametocytes formed per oocyst fed. The extremes of infection are gotten by multiplying the extremes of the numbers of merozoites by each other and then by eight. This gives a minimum number of 25,360 and a maximum of 73,728 gametocytes per oocyst fed. These figures show the possible extremes and the average infection.

The double and triple infections in single cells by *B. miyairii* are interesting in view of the recent work by Beach (1936) on *Plasmodium vivax*. He found that multiple infections probably arose by the division of the schizont after infecting the cells. This is not true for the *B. miyairii* under consideration. It has been shown that each macrogametocyte is formed from a third generation merozoite and each third generation schizont is formed from a second generation merozoite. Since many of the doubly infected cells contain both macrogametocytes and third generation schizonts it is evident that the infecting form for each differed, and that they were not formed by the division of one merozoite after entering the host cell.
### Table III.

_Eimeria miyairii_

Data concerning the asexual phases

<table>
<thead>
<tr>
<th></th>
<th>Sporozoite</th>
<th>1st Generation</th>
<th>2nd Generation</th>
<th>3rd Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>12.15-16.65μ</td>
<td>5.58-7.20μ</td>
<td>8.10-11.25μ</td>
<td>3.60-5.13μ</td>
</tr>
<tr>
<td>Mean</td>
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<td>9.15μ</td>
<td>4.37μ</td>
</tr>
<tr>
<td><strong>Width</strong></td>
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</tr>
<tr>
<td>Range</td>
<td>2.25-3.15μ</td>
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<td>1.00-1.80μ</td>
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<tr>
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<td>2.65μ</td>
<td>1.27μ</td>
<td>1.44μ</td>
<td>1.23μ</td>
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<td><strong>Number in one Schizont</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>12-24</td>
<td>8-16</td>
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<tr>
<td>Mean</td>
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<td><strong>Time of Maturing</strong></td>
<td></td>
<td>2 days</td>
<td>3 days</td>
<td>4 days</td>
</tr>
</tbody>
</table>
The endogenous cycles of the three species of Eimeria studied indicate several important principles concerning infections with coccidian parasites. First, as emphasized before, the fact that the cycles are definite and exact indicates that they are limited not by the host but by the parasites themselves. Second, it would appear that no sexual dimorphism is present in the asexual phases. The merozoites heretofore described by Dieben (1924), Hosoda (1928) and others as sexual dimorphic forms are probably different generations of merozoites. Third, this study shows that localization of infection is characteristic for each species. Fourth, each species has an individualistic life history which need not be exactly like that of any other coccidium.
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Hosoda, S.

Ohira
Pérard, Ch.

Pinto, C.

Roudabush, R. L.

Schaudinn, F.

Tyzzer, E. E.

Tyzzer, E. E., Hans Theiler, and E. Elizabeth Jones

Yakimoff, W. L.
Table IV.

Comparison of the Three Species of Coccidia in Rats

<table>
<thead>
<tr>
<th>Name applied by Becker 1934</th>
<th>E. nieschulzi</th>
<th>E. miyairii</th>
<th>E. separata</th>
<th>E. carinii</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Range in size of oocyst</td>
<td>16.2-26.4μ x</td>
<td>12.8-19.4μ x</td>
<td>16.8-29μ x</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13.4-21.3μ</td>
<td>11.2-17.2μ</td>
<td>16.1-26μ</td>
<td></td>
</tr>
<tr>
<td>*Mean size of oocyst</td>
<td>22.5μx17.8μ</td>
<td>16.06μx13.85μ</td>
<td>24.38μx22.12μ</td>
<td></td>
</tr>
<tr>
<td>*Character of the wall</td>
<td>Smooth or granular</td>
<td>Smooth</td>
<td>Radial striated, rough</td>
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</tr>
<tr>
<td>*Prepatent period</td>
<td>7-8 days</td>
<td>5-6 days</td>
<td>6 days</td>
<td></td>
</tr>
<tr>
<td>Endogenous period</td>
<td>6.5 days</td>
<td>4.5 days</td>
<td>5.5 days</td>
<td></td>
</tr>
<tr>
<td>Number of merozoite generations</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Part of intestine affected</td>
<td>Small intestine</td>
<td>Gaecum and large intestine</td>
<td>Small intestine</td>
<td></td>
</tr>
</tbody>
</table>

*Data taken from Becker 1934 with permission of the Collegiate Press, Ames, Iowa.
Explanation of Life History Graphs

The numbers on the abscissa indicate the day of the infection. Each solid line beginning at zero, one, two, etc., days represents the length of the prepatent period for the progeny of each sporozoite. Since sporozoites may infect on any of the first four days, the lines are found beginning up to and including the fourth day. Infection, however, begins at any time during that period, therefore the lines projected below more correctly illustrate the continuity of the infection. These projected lines show graphically when each phase of the life cycle may be found and at the same time show exactly what forms may be found in the intestine at any day of the infection.
Graph 2.
E. miyairii

Graph 3.

- Sporozoite
- Merozoite
- 2
- 3
Plate I.

Eimeria nieschulsi
x1470

All drawings made with the aid of camera Lucida. Unless otherwise stated all motile phases are stained with iron haematoxylin, those in cells with methylene blue and eosin.

1. Sporozoite.
2. Sporozoite with divided posterior globule.
3. Sporozoite after entering host cell.
4. First generation merozoites in cell; merozoites shown in cross section. Note the refractile globule.
5. Group of first generation merozoites.
6. First generation merozoite.
7. First generation merozoite after entering host cell. (Iron haematoxylin)
8. Second generation schizont.
10. Group of second generation merozoites.
Plate II.

*Eimeria nieschulzi*

All drawings made with the aid of camera Lucida. Unless otherwise stated all motile phases are stained with iron haematoxylin, those in cells with methylene blue and eosin.

1. Group of third generation merozoites.
2. Third generation merozoite.
3. Division in fourth schizont.
4. Division in fourth schizont.
5. Group of fourth generation merozoites.
6. Fourth generation merozoite.
7. First division in microgametocyte.
8. Later stage in microgamete development.
10. Microgamete.
11. Early macrogametocyte.
Plate II.
Plate III.

*Eimeria nieschulsi*

x1470

Development of oocyst wall. Plastin granules shown as white bodies with a solid outline. Haematoxylinophilic granules shown as large black dots.

1. Early development - showing migration of granules.
2. Plastin granules have almost reached the periphery. The second group of plastin granules remains close to the nucleus.
3. Plastin granules have reached the edge and begin to flatten out. Haematoxylinophilic granules begin to elongate.
4. Outer wall formed from the plastin granules. Inner wall forming from the haematoxylinophilic granules.
5. Inner wall almost fully formed.
6. Mature oocyst inner wall shrunken away from the outer because of dehydration. Second set of plastin granules shown in the cytoplasm.
Plate III.
All drawings made with the aid of camera Lucida. Unless otherwise stated all motile phases are stained with iron haematoxylun, those in cells with methylene blue and eosin.

1. Sporozoite.
2. Sporozoite in host cell.
3. Sporozoite after rounding up.
4. First division in first schizont.
5. Early first generation merozoites.
7. First generation merozoite.
8. First generation merozoite after entering cell.
9. Early second schizont (double infection).
10. Group of second generation merozoites.
13. Third generation merozoite.
14. Third generation merozoite after entering cell.
15. Microgametocyte.
17. Young macrogametocyte.
18. Later macrogametocyte.
19. Almost mature macrogametocyte.
Plate IV.
Plate V.

Eimeria miyairii
x1470

All drawings made with the aid of camera Lucida. Unless otherwise stated all motile phases are stained with iron hae-matoxylin, those in cells with methylene blue and eosin.

1. Sporozoite.
2. Sporozoite in host cell.
3. Group of first generation merozoites.
4. First merozoite.
5. Schizont (probably second generation).
7. Second generation merozoite.
8. Group of third generation merozoites.
10. Microgametocyte.
11. Microgamete.
12. Double infection, with young macrogametocytes shown below epithelium.
14. Figure copied from Ohira (1912) showing typical macrogametocyte.