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Increase in Size of *Eimeria separata* Oocysts during Patency

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INCREASE IN SIZE OF *EIMERIA SEPARATA* OOCYSTS DURING PATENCY

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ABSTRACT: Examinations of 18 consecutive fecal samples collected at 6-hr intervals from infected rats showed that oocysts increased in length and width by approximately 40% from the beginning to the end of the patent period. Unsporulated oocysts were first observed in the 3rd fecal collection, 84 to 90 hr postinoculation (PI) and 100 of these measured 9.9 to 14.3 by 8.8 to 12.1 μ (mean 11.7 by 10.1). Patency ended with the 15th fecal collection, 156 to 162 hr PI, and 100 unsporulated oocysts from this sample measured 14.3 to 17.6 by 13.2 to 15.4 μ (mean 16.3 by 14.2). Despite the size increase during patency, oocyst shape-index did not change. Sporulated oocysts did not differ significantly from unsporulated oocysts in length or width. The most significant increases in both length and width of the sporulated oocysts occurred 90 to 96 and 120 to 126 hr PI.

During studies on interspecific interactions between coccidian species, I observed that oocysts of *Eimeria separata* seemed to become larger as the infection progressed. Increase in the size of coccidian oocysts, although apparently unusual, has been observed by some investigators (Becker et al., 1955, 1956; Cheissin, 1947; Cordero del Campillo, 1959; Fish, 1931; Jones, 1932). It was interesting to note that Becker et al. (1932) stated, "It is evident here also that the oocysts [of *E. separata*] exhibit no tendency toward a larger or smaller size as the infection progresses." Since my observations appeared to conflict with those of Becker et al., the following experiment was conducted in order to determine if there was an increase in the size of oocysts during the course of the infection.

MATERIALS AND METHODS

Experimental animals

The hosts used in this study were 2 female, coccidia-free, SPF, Fischer 344 strain, inbred albino rats (National Laboratory Animal Company, Creve Coeur, Missouri). They were kept in a room with a relatively constant temperature (22 to 25 C) and were allowed food and water ad lib. Thus an attempt was made to standardize genetic, hormonal, age, and some environmental influences, all factors which may influence the course of an infection.

The *E. separata* isolate was obtained by inoculating rats with fecal material from an infected wild *Rattus norvegicus*. This infected fecal material was sent to me by Dr. J. V. Ernst, USDA Regional Parasite Research Laboratory, Auburn, Alabama. The specific status of this isolate was determined by measurement of 100 sporulated oocysts and comparison of observed characters with those from previously described species (Levine and Ivens, 1965). The species was further confirmed by observation of the living endogenous stages of the parasite in the cecum–colon using the technique of Marquardt (1966) and comparing these stages with those originally described by Roudabush (1937).

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Experimental design

The rats were caged individually for 1 week prior to inoculation to acclimate them to their surroundings. At the same time, pans containing 2.5% aqueous potassium dichromate ($K_2Cr_2O_7$) solution were placed under each cage to collect excretory products from each animal. The pans were large enough to cover the area under each hanging cage so that all material dropping through the mesh in the cage floor fell into the appropriate pan below. Fecal material from each rat was examined twice before the experiment to insure the animal was coccidia-free. No oocysts of other species were observed at any time during the course of the experiment.

Hosts were infected when they were 40 days old by administering approximately 7,000 recently sporulated oocysts of *E. separata* via stomach tube after each rat had been lightly anesthetized with ether. During the course of the experimental infections, the material in the pans was collected at 6-hr intervals starting 72 hr postinoculation (PI) and terminating with the 18th collection, 180 hr PI. The $K_2Cr_2O_7$-fecal material was homogenized for 10 sec in a Waring Blendor and then strained through 20-, 40-, and 60-mesh wire screens into a flask. Additional $K_2Cr_2O_7$ was used to wash each screen to insure that most of the oocysts were collected. The final volume in the collecting flask was 200 to 250 ml.

A 5-ml aliquot was then taken from each flask and concentrated for study by flotation in Sheather's

TABLE I. *Mean size (in μ) of* E. separata *oocyst length and width collected at 6-hr intervals throughout the patent period. Each mean value based on 100 oocysts with the range in parentheses.*

Collect. number	Hours PI	Length		Width		L/W ratio	
		Unsporulated	Sporulated	Unsporulated	Sporulated	Unsporulated	Sporulated
1	72–78	—	—	—	—	—	—
2	78–84	—	—	—	—	—	—
3	84–90	11.7 (9.9–14.3)	11.8 (9.9–14.3)	10.1 (8.8–12.1)	10.3 (8.8–12.1)	1.17 (1.10–1.50)	1.14 (1.00–1.33)
4	90–96	12.9 (9.9–16.5)	12.8 (11.0–16.5)	11.2 (8.8–13.2)	11.0 (9.9–12.1)	1.16 (1.00–1.33)	1.16 (1.00–1.50)
5	96–102	12.8 (9.9–16.5)	13.1 (9.9–15.4)	11.2 (8.8–13.2)	11.4 (9.9–13.2)	1.14 (1.00–1.36)	1.15 (1.00–1.30)
6	102–108	13.4 (11.0–16.5)	13.5 (9.9–16.5)	11.5 (9.9–13.2)	11.6 (9.9–14.3)	1.17 (1.00–1.40)	1.16 (1.00–1.50)
7	108–114	13.8 (11.0–17.6)	13.5 (11.0–15.4)	11.7 (9.9–14.3)	11.5 (9.9–13.2)	1.19 (1.00–1.33)	1.17 (1.00–1.33)
8	114–120	14.1 (11.0–16.5)	14.0 (11.0–16.5)	12.2 (9.9–13.2)	12.0 (9.9–13.2)	1.18 (1.00–1.40)	1.17 (1.00–1.30)
9	120–126	14.6 (11.0–17.6)	15.0 (12.1–16.5)	12.6 (9.9–14.3)	12.6 (11.0–14.3)	1.16 (1.00–1.33)	1.19 (1.08–1.40)
10	126–132	15.1 (12.1–17.6)	15.1 (12.1–17.6)	12.9 (11.0–14.3)	13.2 (11.0–14.3)	1.17 (1.00–1.33)	1.15 (1.08–1.33)
11	132–138	15.3 (12.1–17.6)	15.6 (13.2–17.6)	13.1 (11.0–15.4)	13.5 (12.1–16.5)	1.17 (1.08–1.27)	1.15 (1.07–1.27)
12	138–144	15.5 (12.1–17.6)	15.6 (13.2–17.6)	13.5 (11.0–15.4)	13.5 (12.1–15.4)	1.15 (1.00–1.27)	1.16 (1.08–1.36)
13	144–150	15.9 (13.2–17.6)	16.0 (13.2–17.6)	13.7 (11.0–15.4)	13.8 (11.0–15.4)	1.16 (1.08–1.33)	1.16 (1.07–1.40)
14	150–156	16.1 (12.1–17.6)	16.2 (14.3–17.6)	13.8 (11.0–15.4)	14.1 (13.2–15.4)	1.17 (1.07–1.45)	1.15 (1.07–1.25)
15	156–162	16.3 (14.3–17.6)	16.3 (13.2–17.6)	14.2 (13.2–15.4)	14.3 (12.1–15.4)	1.14 (1.07–1.33)	1.14 (1.07–1.33)
16	162–168	—	—	—	—	—	—
17	168–174	—	—	—	—	—	—
18	174–180	—	—	—	—	—	—
	Mean (N = 1,300)	14.4 (9.9–17.6)	14.5 (9.9–17.6)	12.4 (8.8–15.4)	12.5 (8.8–16.5)	1.16 (1.00–1.50)	1.16 (1.00–1.50)

sugar solution. If oocysts were not observed in 2 trials, the sample was discarded and considered negative. When oocysts began to appear in the fecal collections, 50 unsporulated oocysts from each rat were measured for each 6-hr collection period. The bulk of these fecal collections was then placed in individual 15-cm petri dishes (the depth of the solution not exceeding 1 cm) and incubated at 30 C for at least 24 hr, which allowed complete sporulation of *E. separata* oocysts. After sporulation, 50 oocysts from each rat were again measured for each 6-hr collection period in which oocysts were observed. In comparing measurements between unsporulated and sporulated oocysts or between the different collection intervals, the data from the 2 rats were combined because the measurements from each were more or less the same.

All measurements were made with a compound microscope using an ocular micrometer and a 100 × acromatic oil immersion objective. Oocysts were measured in the order in which they were encountered, so that there was no prejudicial selection as to size, and an oocyst was measured only when it became apparent that its maximum diameter was in view. Differences between lengths and between widths of sporulated oocysts of *E. separata* during successive collection intervals were calculated using Student's *t* test. All statistical data

TABLE II. *Standard deviation and standard error of the mean for the lengths and widths of sporulated oocysts of* E. separata *during each of 13 6-hr collection intervals throughout patency.*

Collect. number	Hours PI	Length		Width	
		Standard deviation	Standard error	Standard deviation	Standard error
3	84–90	1.084	0.153	0.770	0.109
4	90–96	1.176	0.166	0.737	0.104
5	96–102	1.498	0.212	1.040	0.147
6	102–108	1.564	0.212	1.022	0.145
7	108–114	1.084	0.153	0.865	0.122
8	114–120	1.248	0.177	0.920	0.130
9	120–126	1.037	0.147	0.974	0.138
10	126–132	1.234	0.174	0.927	0.131
11	132–138	1.043	0.147	1.025	0.145
12	138–144	1.133	0.160	0.833	0.118
13	144–150	1.281	0.181	0.946	0.134
14	150–156	0.973	0.138	0.840	0.119
15	156–162	1.141	0.161	1.144	0.162

were compiled using the IBM 360 computer at the University of New Mexico.

RESULTS

Measuring the length and width of 50 unsporulated and 50 sporulated oocysts during 13 6-hr intervals gave a total of 52 mean values from each of the two rats. The difference between corresponding mean values for the two rats during any 6-hr collection interval never exceeded 0.8 μ and in 13 of these 52 instances it was < 0.1 μ. These differences were not considered significant and, therefore the data from the two rats were combined (Table I). There is a direct correlation between time into patency and increase in oocyst length and width. The total increase in either length or width was about 40% during the 3-day period of patency. Despite the increase in size of the oocysts during patency, there was no change in the shape-index.

Sporulated oocysts did not differ significantly from unsporulated oocysts in length or width, and since coccidian species are best distinguished by the structure of the sporulated oocyst, further statistical analyses were limited to data from the sporulated oocysts of *E. separata*. Standard deviation and standard error of the mean were determined for oocyst length and width during each of the 13 6-hr collection intervals in which oocysts were observed (Table II).

Because oocyst size increased strikingly during patency, I was interested in ascertaining the period(s) of most significant increase. Table III shows the differences which exist between lengths and widths of sporulated oocysts during successive time intervals. Since length and width constantly increased during patency it is obvious that if significant differences exist between either of these parameters

TABLE III. *Statistical differences between lengths and between widths of sporulated oocysts of* E. separata *during successive time intervals.*

t-test between collection groups	Hours PI	*t*-values	
		Length	Width
3–4	90–96	4.182‖	4.379‖
4–5	96–102	1.389*	2.319‡
4–6	96–108	2.543§	3.456‖
5–6	102–108	N.S.	N.S.
5–7	102–114	1.346*	N.S.
5–8	102–120	3.271‖	2.902‖
6–7	108–114	N.S.	N.S.
6–8	108–120	2.021‡	1.912†
6–9	108–126	5.795‖	4.958‖
7–8	114–120	2.352‡	2.821‖
7–9	114–126	7.145‖	6.089‖
8–9	120–126	4.210‖	3.261‖
9–10	126–132	N.S.	2.997‖
9–11	126–138	2.652‖	4.610‖
10–11	132–138	1.926†	1.801†
10–12	132–144	2.136‡	1.884†
10–13	132–150	3.323‖	3.406‖
11–12	138–144	N.S.	N.S.
11–13	138–150	1.695†	1.450*
11–14	138–156	3.174‖	2.828‖
12–13	144–150	1.365*	1.717†
12–14	144–156	2.718‖	3.288‖
13–14	150–156	N.S.	1.364*
13–15	150–162	1.549*	2.420§
14–15	156–162	N.S.	1.315*

* $P < 0.10$.
† $P < 0.05$.
‡ $P < 0.025$.
§ $P < 0.01$.
‖ $P < 0.005$.

during any two successive intervals, then the difference will be of equal or greater significance in all subsequent collection intervals. Highly significant ($P < 0.005$) increases in length occurred during the 90- to 96- and 120- to 126-hr collection intervals. Similarly significant increases in width occurred during the 90- to 96-, 114- to 120-, 120- to 126-, and 126- to 132-hr collection intervals. Less significant increases between successive and later collection periods are also shown in the table.

DISCUSSION

The oocyst is the most available stage in a coccidian life cycle because in most circumstances host animals do not lend themselves to laboratory study. For this reason, the majority of coccidia are known only by their oocysts, which have been and continue to be most commonly used to identify the various species. In general, the oocyst is thought to have a high degree of dimensional constancy, and biometric data on oocysts, usually collected at one time, have been considered valid for that coccidian species.

Boughton (1930), working with sparrow coccidia, first cast doubt on the constancy of oocyst size limits when he found a great deal of variation in the size and volume of oocysts of *Isospora lacazei* over a 2-month period. Although it appears he was working with two species of coccidia, he did consider the possibility of oocysts changing greatly in size during patency. Fish (1931) reported the first documented change of oocyst size during patency when he noted that oocysts of *E. tenella* from chickens became longer and broader as the infection developed. He also noted that the range of length and width tended to diminish as the infections progressed, but that the shape-index remained more or less constant. Somewhat similar results were observed in this study (Table I).

Jones (1932) contributed one of the most elaborate statistical studies concerning factors which (might) influence variation of oocyst size during patency. After analyzing size differences in the oocysts of *E. acervulina*, *E. maxima*, and *E. tenella* from chickens following numerous experiments, she concluded that oocyst size was independent of the length and severity of the infection and of the breed and age of the host.

Cheissin (1947) studied the variability of shape and size of the oocysts of *E. magna* from rabbits. He found that oocysts appearing earliest in the infection were smaller and lighter in color than those observed later during patency. In contrast to the results of Jones (1932), he noticed that size of the oocysts of *E. magna* decreased when he increased the size of the inoculum.

Becker et al. (1955), in their biometrical study of the oocyst of *E. brunetti* from chickens, concluded that small oocysts were produced early in the infection and that larger ones succeeded them. Becker et al. (1956) found similar results in their measurements of *E. necatrix*, also from chickens. In that study they noticed a slight, though significant, increase in oocyst size after the first 3 days of the patent period and then a slight, though equally significant, tendency for the oocysts to shorten near the end of patency. In both papers by Becker et al. (1955, 1956) as well as in other studies (Jones, 1932; Cheissin, 1947) oocysts from different host specimens, although collected on the same day PI, showed much variation in size. As stated, such variability in oocyst size was not observed in the two rats used in this study.

Apparently the only recorded finding of rodent coccidia increasing in size during patency was by Cordero del Campillo (1959). He made a biometric study of the dimensions of over 1,500 oocysts of *E. falciformis* (from the common house mouse) during a 20-day period. The oocysts tended to increase in size from the 1st to 5th or 6th days of patency and then decreased in size for the remaining 4 to 5 days of the patent period.

In their study of *E. miyairii* (= *E. nieschulzi*) and *E. separata* in rats, Becker et al. (1932) stated that *E. separata* oocysts did not change in size during patency. However, they infected three rats with 1,500 sporulated oocysts of *E. separata* over a 5-day period. Then on days 6 to 11 they collected fecal material and measured 50 unsporulated (?) oocysts. Therefore, during patency some oocysts resulting from the early infections might be mixed with those resulting from the late infections. This design would tend to obscure differences in size.

The number of oocysts present at any one

time during patency might also have been important to the conclusion of Becker et al. (1932). From previous work (Duszynski, 1970), I observed that during an *E. separata* infection with approximately 7,000 oocysts, host animals shed the largest number of oocysts in their fecal material during the 2nd day of patency. Thus, many of the oocysts which they observed on day 6 probably represented the 2nd day of oocyst production from inoculation No. 1; many of those observed by them on day 7 probably represented the 2nd day of oocyst production from inoculation No. 2; and so on. Based on data from *Eimeria* species in other hosts the first inoculation may have had a greater effect than successive ones, but the above must still be one of the factors considered. When their results are interpreted in this manner, it is obvious why they concluded the oocysts of *E. separata* did not increase in size during patency.

Various studies have been concerned with the morphology of rodent (Levine and Ivens, 1965) and other coccidian oocysts (Levine, 1961; Pellérdy, 1965). In many instances the descriptions of oocyst length–width dimensions of a single species from the same host vary greatly when described by different authors. This study has demonstrated that the time during patency when oocysts are measured is important in obtaining an accurate range of oocyst size for certain coccidian species and that several samples of oocysts collected on different days of the patent period should be measured if representative values are to be obtained.

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