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THE INCIDENCE AND LIFE CYCLE OF EIMERIA UTAHENSIS SP. N.

FROM KANGAROO RATS OF NORTHWESTERN UTAH

by

John V. Ernst

A dissertation submitted in partial fulfillment  
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Zoology

UTAH STATE UNIVERSITY  
Logan, Utah

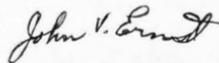
1967

#### ACKNOWLEDGMENTS

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John V. Ernst

TABLE OF CONTENTS

	Page
INTRODUCTION . . . . .	1
REVIEW OF LITERATURE . . . . .	2
Coccidia of heteromyid rodents . . . . .	2
Cytochemical observations of sporozoites . . . . .	8
METHODS OF PROCEDURE . . . . .	13
Collection of animals . . . . .	13
Care of animals . . . . .	14
Aging of animals . . . . .	16
Collection of inoculum . . . . .	17
Inoculation of animals . . . . .	18
Determination of prepatent and patent periods . . . . .	19
Study of oocysts and sporozoites . . . . .	19
Study of endogenous stages . . . . .	22
RESULTS . . . . .	24
Incidence of coccidia . . . . .	24
Exogenous cycle . . . . .	24
Endogenous cycle . . . . .	36
DISCUSSION . . . . .	54
LITERATURE CITED . . . . .	67
VITA . . . . .	72

LIST OF TABLES

Table	Page
1. Age and sex of <u>Dipodomys ordii</u> captured from September, 1966, through May, 1967 . . . . .	25
2. Age and sex of <u>Dipodomys microps</u> captured from September, 1966, through May, 1967 . . . . .	26
3. Occurrence of <u>Eimeria utahensis</u> , according to age groups, in <u>Dipodomys ordii</u> and <u>Dipodomys microps</u> . . . . .	27
4. Occurrence of <u>Eimeria utahensis</u> , according to age groups, in <u>Dipodomys ordii</u> during autumn 1966, and spring 1967 . . . . .	28
5. Occurrence of <u>Eimeria utahensis</u> , according to age groups, in <u>Dipodomys microps</u> during autumn 1966, and spring 1967 . . . . .	29

LIST OF FIGURES

Figure	Page
1. A view of the large cleared area where most of the kangaroo rats were captured . . . . .	42
2. A view of one of the smaller cleared areas where some kangaroo rats were captured . . . . .	42
3. <u>Eimeria utahensis</u> sp. n. from <u>Dipodomys ordii</u> . . . . .	44
4-9. Sporozoites of <u>Eimeria utahensis</u> . . . . .	46
4. Living sporozoite, bright field, X 1400 . . . . .	46
5. Living sporozoite, bright field, showing transverse striations in the anterior region, X 1600 . . . . .	46
6. Sporozoite, fixed in osmium tetroxide fumes, showing median rod-like organelles in the anterior region and nipple-like projection at the anterior extremity, X 1600 . . . . .	46
7. Sporozoite, protargol stain, showing median rod-like body in the anterior region, X 1600 . . . . .	46
8. Sporozoite, bromphenol-blue stain, X 1300 . . . . .	46
9. Sporozoite, Sudan black B stain, X 1400 . . . . .	46
10-14. Schizonts of <u>Eimeria utahensis</u> , iron-hematoxylin stain, X 1400 . . . . .	48
10. First-generation schizont . . . . .	48
11. Second-generation schizonts, showing prominent residual body (RB) . . . . .	48
12. Third-generation schizont . . . . .	48
13. Fourth-generation schizont . . . . .	48
14. Third (3) and fourth (4) generation schizonts . . . . .	48
15. Early gametocyte of <u>E. utahensis</u> , showing enlarged host cell nucleus. L, lumen of crypt, X 1400 . . . . .	48

Figure	Page
16-21. Gametocytes of <u>Eimeria utahensis</u> stained with iron-hematoxylin, Figures 16-20, X 1400, Figure 21, X 350 . . . . .	50
16. Young gametocyte in parasitized cell with flattened nucleus (HN) . . . . .	50
17. Young gametocyte in host cell apparently having two nuclei . . . . .	50
18. Two young gametocytes (P) in a host cell with a dividing nucleus (HN) . . . . .	50
19. Macrogametocyte showing plastic granules around periphery of parasite . . . . .	50
20. Young oocyst . . . . .	50
21. Gametocytes in lamina propria; microgametocyte showing microgametes in whorls at the periphery of compartments . . . . .	50
22-25. Microgametocytes of <u>Eimeria utahensis</u> , iron-hematoxylin stain, X 1400 . . . . .	52
22. Microgametocyte with nuclei in random arrangement . . . . .	52
23. Microgametocyte with nuclei arranged around compartments . . . . .	52
24. Microgametocyte with microgametes in whorl arrangement . . . . .	52
25. Microgametocyte with microgametes randomly arranged around the residuum . . . . .	52

ABSTRACT

The Incidence and Life Cycle of Eimeria Utahensis sp. n.  
from Kangaroo Rats of Northwestern Utah

by

John V. Ernst, Doctor of Philosophy

Utah State University, 1967

Major Professor: Dr. Datus M. Hammond  
Department: Zoology

A total of 176 kangaroo rats (151 Dipodomys ordii and 25 D. microps) were captured in northwestern Utah and examined for coccidia. Of these 176 rats, four D. ordii (2.6%) and four D. microps (16.0%) were infected with Eimeria utahensis, a new species of coccidia. Little seasonal difference was found in the infection rate of either species. The characteristics of the sporulated oocysts of this species were described. A sporocyst plug was reported for the first time in an eimerian oocyst.

Artificially excysted sporozoites were studied by various methods. Thirty living sporozoites averaged 22.5  $\mu$  in length by 4.5  $\mu$  in width at the anterior refractile body and 4.6  $\mu$  in width at the posterior refractile body. The refractile body was ellipsoidal and occupied almost half of the sporozoite. The refractile bodies were protein in nature. Living sporozoites exhibited gliding, flexing, pivoting, and probing movements. Subpellicular fibrils, anterior median rod-shaped organelles, and transverse striations of unknown significance were seen in living and stained sporozoites. In the vesicular nucleus the DNA was concentrated at the periphery and three to five chromatin clumps were present. Little, or no, glycogen was present. The test for lipids was inconclusive.

The mean prepatent period in experimentally infected D. ordii was 9.8 days. The discharge of oocysts continued for prolonged periods, evidently as a result of reinfection, although concerted efforts were made to prevent this.

The asexual endogenous stages were located in epithelial cells in the distal half of the villi of the small intestine. Four generations of schizonts were present. Mature first-generation schizonts were found 2 1/2 days after inoculation of the animals and contained 12 to 16 merozoites. Mature second-generation schizonts were found on the fourth post-inoculation day and also contained 12 to 16 merozoites. Mature third-generation schizonts were present on the fourth, fifth and sixth post-inoculation days and contained 4 to 8 merozoites. The third-generation schizonts gave rise to early sexual stages or to fourth-generation schizonts. Mature fourth-generation schizonts were found on the sixth and seventh post-inoculation days and contained 16 to 24 merozoites.

Young gametocytes were first observed on the fifth post-inoculation day. Shortly after the gametocytes entered the infected epithelial cells the cells became displaced into the lamina propria and the mature gametocytes were usually found in the latter location. The nuclei of infected host cells became considerably enlarged and modified in shape and position. In many host cells there appeared to be two or more nuclei in the parasitized cell; this was interpreted as an artifact of sectioning. However, in a few instances young gametocytes were observed in cells in which the host cell nuclei were undergoing division, indicating that some infected host cells might have been multinucleate.

Microgametocyte nuclei were randomly arranged in the microgametocyte

during the early stages of development. As the microgametocytes approached maturity the nuclei became arranged in whorls at the surface of compartments. At maturity the microgametes lost their whorl arrangement and became randomly arranged around a central mass of residual material. The mature microgametocytes averaged 63.9 by 48.3  $\mu$ .

The plastic granules of the macrogametes were slightly eosinophilic with hematoxylin and eosin stain, but did not stain with iron hematoxylin. The macrogametes measured 32.5 by 27.0  $\mu$  at the stage in which the plastic granules were at the periphery of the parasite but had not yet coalesced.

Eimeria utahensis caused no outward signs of coccidiosis in experimentally infected D. ordii, nor were any marked pathological changes observed in the tissue sections.

(81 pages)

## INTRODUCTION

Kangaroo rats are nocturnal animals which are restricted in distribution to the dryer regions of the western North American continent. Little is known concerning the incidence or pathogenicity of coccidia in kangaroo rats. Of the seven species of Eimeria described from these animals six are known only from the sporulated oocyst.

During a parasitological survey of kangaroo rats in northwestern Utah an area was found which contained a large population of kangaroo rats. A few of the animals captured in this area were observed to be passing an undescribed species of Eimeria. Preliminary observations showed that this particular species of Eimeria is well suited for studies on certain aspects of its biology, especially those relating to the sporozoite stage. The excysted sporozoites were found to be unusually large. Few cytochemical studies have been done on artificially excysted sporozoites. Using recently developed techniques, artificially excysted sporozoites can be obtained in large numbers for morphological and cytochemical studies.

The objectives of this investigation were (1) to determine the incidence of infection of this new species of Eimeria in a population of kangaroo rats, (2) to determine the life cycle and pathogenicity of this species of Eimeria in experimentally infected kangaroo rats, and (3) to determine the major cytological and cytochemical characteristics of the sporozoites of this Eimeria species and to compare these findings with those relating to other species of coccidia.

## REVIEW OF LITERATURE

Coccidia of Heteromyid Rodents

Eight species of Eimeria have been described from the family of rodents, Heteromyidae, to which the kangaroo rat belongs. These include E. mohavensis Doran and Jahn, 1949, from Dipodomys panamintinus mohavensis; E. dipodomys Levine, Ivens, and Kruidenier, 1957a, from Dipodomys phillipsi; E. scholtzsecki Ernst, Frydendall, and Hammond, 1967, from Dipodomys ordii; E. balphae Ernst, Chobotar, and Anderson, 1967, from Dipodomys ordii; E. perognathi Levine, Ivens, and Kruidenier, 1957b, from Perognathus intermedius; E. penicillati Ivens, Kruidenier, and Levine, 1958, from Perognathus penicillatus; E. liomysis Levine, Ivens, and Kruidenier, 1957a, from Iiomys pictus; and E. picti Levine, Ivens, and Kruidenier, 1957a, also from Iiomys pictus. The descriptions of the eight species are based on oocyst characters; only in E. mohavensis have the endogenous stages been described.

The oocysts of Eimeria mohavensis were first described by Doran and Jahn in 1949, but a more complete description was given later (Doran and Jahn, 1952). The oocyst was ellipsoid with a single, smooth, light brown wall, 0.7 to 0.9  $\mu$  thick. A micropyle, oocyst residuum, and polar granule were absent. The sporulated oocysts measured 21.5 to 26.0 by 14.0 to 18.5  $\mu$ , with a mean of 24.1 by 15.7  $\mu$ . The mean length-width ratio was 1.54. The sporocysts measured 6.0 to 10.0 by 5.5 to 8.0  $\mu$ , with a mean of 7.8 by 7.6  $\mu$ . The sporocyst residual body was oval, 4.0 to 4.5  $\mu$  in length.

Doran (1953) described the schizogonic part of the life cycle of E. mohavensis in detail. He found 2 generations of schizonts in the epithelial cells of the small intestine and cecum. The mature first generation schizonts were present from the third to the seventh day of the prepatent period. These schizonts measured 5.0 to 9.0  $\mu$  in diameter and contained 20 to 35 merozoites, which measured 6.5 to 8.5  $\mu$  in length by 1.5 to 2.0  $\mu$  in width. Larger schizonts, measuring 10.0 to 15.0  $\mu$  in diameter, were present on the sixth and seventh days of the prepatent period and the first and second days of the patent period. These later schizonts contained 50 to 75 merozoites, which measured 4.5 to 6.5  $\mu$  in length by 1.0 to 1.5  $\mu$  in width.

Gametogony in E. mohavensis was described by Doran and Jahn (1952) and by Doran (1953). The gametes and gametocytes were in the epithelial cells of the cecum, and were present during the last 2 days of the prepatent period and the first 6 days of the patent period. The macrogametocytes were round, 10.0 to 16.0  $\mu$  in diameter, and the ellipsoid microgametocytes were 17.0 to 18.5  $\mu$  in width by 21.0 to 22.5  $\mu$  in length.

Doran (1953) found E. mohavensis in 22 of 251 Dipodomys panamintinus mohavensis, collected over a 3 year period, in California. He noted that no natural infections were found in any of the rodents locally sympatric with D. p. mohavensis or in any of the other subspecies of D. panamintinus.

Doran (1953) successfully infected 3 other subspecies of Dipodomys panamintinus, namely, D. p. leucogenys, D. p. panamintinus, and D. p. caudatus. He also infected 11 of 12 D. merriami merriami, 11 of 12

D. nitratoides brevinasus, 5 of 6 D. heermanni morrensis, 6 of 6 D. h. tularensis, 1 D. h. swarthi, 1 D. deserti deserti, and 6 D. agilis agilis. He was unable to infect 8 Perognathus longimembris, 7 P. formosus mohavensis, 4 Peromyscus boylii, 12 P. maniculatus, 12 P. californicus, 7 P. truei, 5 Onychomys torridus, 4 Neotoma lepida, 8 Spermophilus leucurus, 4 Mus musculus, and 4 Rattus norvegicus. Doran (1953) concluded on the basis of these results that Eimeria mohavensis is probably genus-specific.

It is of interest that, although Dipodomys merriami merriami was readily infected in the laboratory, natural infections were not found in any of the 197 D. m. merriami trapped in the field. This was despite the fact that D. m. merriami is sympatric with D. p. mohavensis, that they have similar food habits and forage over the same range, that they have been taken in the same trap within half an hour, and that D. m. merriami frequently enters the burrows of other kangaroo rats, presumably including D. p. mohavensis.

Eimeria dipodomysis was found in 1 of 3 Dipodomys phillipsi trapped in Mexico (Levine et al., 1957a). The ellipsoid oocyst had a wall composed of 2 layers, the outer 3.5  $\mu$  in thickness at the sides and 3.0  $\mu$  at the ends; the inner layer was 0.7  $\mu$  thick. The outer wall was yellowish brown, rough, and lacked a micropyle. Seventeen sporulated oocysts measured 47 to 61 by 38 to 42  $\mu$ , with a mean of 54.1 by 40.2  $\mu$ . The length-width ratios ranged from 1.2 to 1.5, with a mean of 1.35. The ovoid sporocysts measured 16 by 11  $\mu$ . A Stieda body was present. An oocyst polar granule was absent. The oocyst residuum was a large mass of homogeneous granules. In some oocysts these granules were few, large, and

loosely aggregated, while in others they were small and numerous, forming a compact mass. The sporozoites were embedded in a large amount of sporocyst residual material. The host animal was first given by Levine et al. (1957a) as Dipodomys ornatus but was later changed to D. phillipsi by Levine and Ivens (1965).

Eimeria scholtysecki was described by Ernst, Frydendall, and Hammond (1967) from Dipodomys ordii in Utah. The sporulated oocyst was broadly ovoid to ellipsoid, with a smooth wall composed of 2 layers; the outer approximately 1  $\mu$  thick and yellowish-brown, the inner about 0.5  $\mu$  thick and dark brown to black. A micropyle and oocyst residuum were absent. An ellipsoid polar granule, 2.2 by 1.5  $\mu$ , was present. One hundred and twenty-five oocysts measured 21 to 27 by 17 to 21  $\mu$ , with a mean of 24.6 by 19.6  $\mu$ . Their length-width ratios ranged from 1.1 to 1.4, with a mean of 1.25. The ovoid sporocysts had a small, flattened Stieda body. One hundred sporocysts were 10 to 14 by 7 to 10  $\mu$ , with a mean of 12.1 by 8.0  $\mu$ . The sporocyst residuum was composed of relatively coarse granules surrounded by a thin membrane. E. scholtysecki was found in 1 of 74 Dipodomys ordii. The prepatent period in 5 animals was 8.2 days. These animals discharged oocysts for 30 days or more, indicating the likelihood of reinfection.

Eimeria balphae was also described from Dipodomys ordii in Utah (Ernst, Chobotar, and Anderson, 1967). The sporulated oocyst was ovoid to broadly ellipsoid, with a smooth wall composed of 2 layers; the outer approximately 1  $\mu$  in thickness and pale yellowish-brown in color, the inner about 0.5  $\mu$ , and dark brown. A micropyle was absent. One hundred sporulated oocysts measured 15 to 18 by 13 to 15  $\mu$ , with a mean of 16.7

by 14.3  $\mu$ . Their length-width ratios ranged from 1.1 to 1.4, with a mean of 1.17. The oocyst residuum was usually a single, large, homogeneous body; sometimes 1 to 3 smaller granules were associated with it; rarely, it was composed of a compact mass of coarse granules. A spheroidal polar granule was present. The sporocysts were ovoid, with a Stieda body at the narrow end. The sporocysts were 8 to 9 by 5 to 7  $\mu$ , with a mean of 8.7 by 5.9  $\mu$ . The sporocyst residuum was composed of 7 to 14 round, clear granules. Eimeria balphae was found in 1 of 82 Dipodomys ordii collected in northern Utah.

Eimeria liomysis was first described from the painted spiny pocket mouse, Iiomys pictus, from Mexico (Levine et al., 1957a). The subspherical to ellipsoid oocyst had a wall composed of two layers, the outer 0.9  $\mu$  thick, pale yellow, slightly rough and pitted, the inner 0.3  $\mu$  and practically colorless. A micropyle was absent. Seventy-five sporulated oocysts, from 2 animals, measured 15 to 24 by 14 to 21  $\mu$ , with a mean of 19.5 by 17.7  $\mu$ . Their length-width ratios ranged from 1.0 to 1.3, with a mean of 1.10. The sporocysts were almost ellipsoid to ovoid, about 10 by 7  $\mu$ , with a small Stieda body. An oocyst polar granule was present by a residuum was absent. The sporozoites were usually at the end of the sporocysts, with relatively large residual granules between them. Four sporulated oocysts from Iiomys irroratus measured 15 to 23 by 14 to 20  $\mu$ , with a mean of 18.2 by 17.2  $\mu$ ; their length-width ratios ranged from 1.0 to 1.1, with a mean of 1.06 (Levine et al., 1957a). Ivens et al. (1958) measured 8 sporulated oocysts from 2 I. pictus; these oocysts were 18 to 23 by 17 to 21  $\mu$ , with a mean of 20.8 by 19.1  $\mu$ . The length-width ratios ranged from 1.0 to 1.1.

Eimeria picti, described from Liomys pictus (Levine et al., 1957a), had oocysts which were subspherical to ellipsoid. The oocyst wall consisted of 2 layers, the outer was brownish yellow, 1.3  $\mu$  thick, and rough and pitted. The inner wall was brownish yellow and 0.4  $\mu$  thick. A micropyle was absent. Fifty-three sporulated oocysts measured 22 to 32 by 19 to 28  $\mu$ , with a mean of 25.8 by 22.5  $\mu$ . Their length-width ratios ranged from 1.0 to 1.2, with a mean of 1.15. The broadly lemon-shaped sporocysts measured 11 to 12 by 8 to 9  $\mu$ . A Stieda body was present. One or 2 oocyst polar granules were present. The oocyst residuum was usually composed of a number of large, clear, irregular granules, but sometimes was a single large, granular mass. The sporocyst residuum consisted of few to many granules.

Eimeria penicillati was described from Perognathus penicillatus from Mexico (Levine et al., 1957a). The oocysts were subspherical, ellipsoid, or slightly ovoid with a single oocyst wall, 0.6  $\mu$  thick, which was smooth and pale brownish yellow or tan in color. A micropyle was absent. Six sporulated oocysts measured 16 to 20 by 14 to 16  $\mu$ , with a mean of 17.8 by 14.7  $\mu$ . The length-width ratios ranged from 1.1 to 1.3, with a mean of 1.18. The broadly lemon-shaped sporocysts measured 9 by 7  $\mu$ . The Stieda body was a small, rounded structure. The oocyst residuum was composed of one to several large, clear globules. A polar granule was present. The sporocyst residuum was composed of a number of large granules. Ivens et al. (1958) also described E. penicillati from Perognathus flavus from Mexico. Three oocysts of E. penicillati from this host measured 17 to 20 by 15 to 19  $\mu$ . These oocysts differed from those described from P. penicillatus by having either one or 2 polar granules

instead of a single one.

#### Cytochemical Observations of Sporozoites

Cytochemical investigations of coccidia have largely been limited to the endogenous stages which could most easily be studied in stained sections. Most of the observations reported on sporozoites have been done on smears of lumen contents, containing naturally excysted sporozoites, or on early intracellular stages, in which the sporozoite was still recognizable as such. Cytochemical observations on sporozoites have been done mainly on E. stiedae, E. magna, and E. intestinalis in the rabbit, on E. brunetti, E. acervulina, E. tenella, E. necatrix, and E. maxima in the chicken, and on E. bovis and E. auburnensis in the bovine.

#### Deoxyribonucleic Acid (DNA)

Pattillo and Becker (1955), using oocysts of E. acervulina which had been embedded in paraffin and then sectioned, found that the only element of the sporulated oocyst which was Feulgen positive was the nuclear ring at the middle of the sporozoite. Their illustration of this stage shows the sporozoite nucleus as a vesicular structure with 4 to 5 clumps of chromatin on the peripheral ring. The nucleolus was Feulgen negative. These investigators found the same type of nucleus was present in excysted sporozoites of E. brunetti and E. acervulina in smears of intestinal contents.

Ray and Gill (1955) reported that sporozoites of E. tenella encountered in the lumen and in epithelial cells always had DNA in the peripheral chromatin and around the nucleolus.

Cheissin (1960) observed that in smears of intestinal contents E. magna sporozoites showed Feulgen-positive material in a peripheral zone of the nucleus.

Hammond, Ernst, and Chobotar (1967) found that in excysted sporozoites of E. bovis and E. auburnensis the nuclei were vesicular, with the chromatin concentrated at the periphery.

### Proteins

The coccidian proteins, other than nuclear proteins, have been investigated by only a few workers. Pattillo and Becker (1955), using the mercuric bromphenol-blue test for protein (Mazia, Brewer, and Alfert, 1953), found that in smears of lumen contents the sporozoites of E. brunetti had a diffuse reaction with intense staining of the refractile bodies. In sectioned oocysts of E. acervulina the refractile bodies and a few granules within the sporozoites stained deeply. Predigestion for one hour in pepsin eliminated the reaction of the granules, but had little effect on that of the refractile bodies. Some of the refractile bodies were still positive after 16 hours digestion, but the greatest number reacted feebly or not at all.

Horton-Smith and Long (1963), also using the mercuric bromphenol-blue test, showed that the refractile bodies of the sporozoites of E. tenella stained intensely, and the cytoplasm stained to a lesser degree. The refractile bodies stained with eosin and light green, which are also good stains for non-nuclear proteins.

Hammond et al. (1967) observed that the nucleus, anterior portion of the body, and the refractile bodies of E. bovis and E. auburnensis sporozoites were deeply stained with mercuric bromphenol-blue.

### Lipids

Pattillo and Becker (1955) observed a weak positive reaction for lipids in the sporozoites of E. brunetti and E. acervulina in smears of lumen contents which had been stained with Sudan black B in 70 percent alcohol. In embedded and sectioned oocysts the sporozoites of E. acervulina gave a faintly positive color test for lipids by way of a slight graying of the refractile body and cytoplasm.

Cheissin (1958) found lipids in the sporulated oocysts of E. intestinalis; these were present as droplets in the residual body of the oocyst and sporocyst.

### Glycogen

Giovannola (1934), using the Best carmine method and the Bauer-Feulgen technique, with saliva-treated controls, found relatively little glycogen in the sporozoites within the oocysts of Eimeria stiedae and E. falciformis. Neither the amount nor the location of the glycogen was described.

Edgar, Herrick, and Fraser (1944), using iodine techniques, found that all sporulated oocysts of E. tenella showed no signs whatsoever of glycogen. However, after liberation of the sporozoites from the oocyst a small amount of glycogen was demonstrated in the central region of each sporozoite. These authors stated that the inability to demonstrate the small amount of glycogen in the sporozoite within the oocyst was further evidence of the extremely impermeable and resistant nature of the oocyst and sporocyst walls. They noted that the sporozoites need a small store of energy to enable them to survive in the lumen of the intestine after excysting and to penetrate the epithelial cells of the host.

Gill and Ray (1954), using the Bauer-Feulgen techniques on stages of E. tenella, with saliva-treated controls, observed that sporozoites in sections and smears of intestine made 34 hours after inoculation contained no glycogen. Very rarely, in some sporozoites which had just entered the host cells, a few red granules were seen around the refractile body. These authors stated that any glycogen which may have been present in the sporozoites was exhausted by the time they had invaded the host cell.

Horton-Smith and Long (1963) also studied the sporozoites of E. tenella. In stained smears of sporozoites harvested from the intestine of birds PAS positive material was present as large granules around the nuclear area, with smaller amounts sometimes occurring near one of the poles. No glycogen was detected after the sporozoites had reached the deep glands of the cecal mucosa.

Pattillo and Becker (1955), using the PAS technique with one-percent diastase digestion, reported that the sporozoites of E. brunetti and E. acervulina in smears of lumen contents and in sections of intestine contained no demonstrable glycogen. In contrast to this, sporozoites of E. acervulina in sectioned oocysts contained high concentrations of glycogen in the cytoplasm. According to these authors, the lack of glycogen in the excysted sporozoites was not surprising since their movements during and after excystation may have exhausted the supply; some of the glycogen may have been expended in protecting the sporozoite against the enzymes of the host.

Cheissin (1958) found considerable amounts of glycogen in the sporozoites of sporulated oocysts of E. intestinalis, using the PAS reaction with saliva treated controls. He stated that the glycogen

reserves in the sporozoites were used during excystation, movement in the intestinal lumen, and penetration into the epithelium.

Cheissin (1959), in discussing the cytochemical characteristics of different stages in the life cycles of three species of coccidia of the rabbit (E. magna, E. intestinalis, and E. media), observed that the zygote (oocyst) is filled with glycogen and fat. During sporogony a certain amount of glycogen is consumed, but the greater part is preserved in the cytoplasm of the sporozoites, as well as in residual bodies of the sporocysts, where it is dispersed among fat droplets. The sporozoites of the various species lost their store of glycogen in 18 to 20 months, at which time the sporozoites became inviable. In representatives of the genus Isospora, the loss of glycogen from sporozoites took place in 4 to 6 months.

The sporozoites of E. bovis and E. auburnensis, stained by the PAS-AO method, had numerous glycogen granules in their middle region, with small numbers occurring in the anterior and posterior regions (Hammond et al., 1967).

## METHODS OF PROCEDURE

Collection of Animals

All the kangaroo rats used in this study were collected within a one-square-mile area in the Great Basin desert 21 miles southwest of Snowville, Box Elder County, Utah (Range 11W, Township 13N, Section 9). The majority of the animals were captured in an area approximately 0.7 mile long and from 15 to 120 yards wide, which had been cleared of sagebrush several years ago (Figure 1). This cleared area was elevated approximately 25 feet above the surrounding land on one side and 15 feet on the other side. The predominant plant in the surrounding area was sagebrush (Artemisia tridentata), while the cleared region supported a variety of smaller plants, most of which are those typically growing in a disturbed area. The two most abundant plants in this cleared area were halogeten (Halogeten glomeratus) and cheat grass (Bromus tectorum); less abundant were tumbled mustard (Sisymbrium altissimum) and Russian thistle (Salsola kali). Other plants present, but in much smaller numbers, were sunflowers (Helianthus annuus), crucifers (Descurainia sophia, Lepidium perfoliatum, L. densiflorum, and Malcomia africana), composites (Erigeron pumilus), chenopods (Atriplex sp. and Kochia scoparia), and grasses (Agropyron cristatum, Sitanion hystrix, and Poa sp.). Most of the animals were captured near the edge of the sagebrush; they were probably foraging for food in the cleared area. A few animals were captured along the roads and in some smaller cleared spaces (Figure 2) within a half mile radius of the larger cleared area.

Because kangaroo rats are nocturnal animals, all of the collecting was done at night. Through a process of trial and error it was found that the best time for collecting kangaroo rats was on nights when there was little moonlight. On moonlit nights attempts at collection were generally unsuccessful. Also, a strong wind seemed to keep the rats in their burrows. The greatest number of rats was captured on a moonless night during an intermittent rain. A pickup truck was driven back and forth over the area until a rat was seen in the light of the headlights. It was then pursued on foot and caught by the use of insect sweep nets. Such nets were found to be the best type for this purpose as they have a larger opening than a regular insect net; they also have a shorter handle, which makes them easier to wield.

A few attempts were made to capture kangaroo rats with live and snap traps. Much time was consumed in locating burrows and setting the traps near these; relatively few rats were captured, so the use of traps was abandoned.

#### Care of the Animals

As each kangaroo rat was captured it was placed in a wide-mouth pint mason jar which contained a thin layer of sawdust. If the jar contained no sawdust or sand the animal appeared ill and its coat became wet and rough within the few hours it took to return to the campus from the trapping area. At first, sand was used to absorb the moisture but the rat's fecal pellets, which were examined for coccidial oocysts, could not be easily found in the sand. When sawdust was substituted for the sand, it was found that the pellets could be easily located; the

sawdust was also satisfactory for absorbing the rat's moisture.

The lid of the jar consisted of two parts, a flat metal cover and a ring, which held the cover in place. Holes were punched in the metal cover for ventilation. When an animal was placed in a jar the lid was turned so that the sharp edges of the holes were up. This prevented the rat from cutting itself if it jumped against the lid, which it frequently did, and also indicated which jars contained animals.

The captured rats were brought back to the laboratory and put into individual cages. These cages were 7 inches wide, 9 1/2 inches deep, and 7 inches high; each had metal sides and roof, and wire bottom, back, and front. The front consisted of a door which swung out. The cages were kept on a 6-tiered rack. Each tier had two metal pans, each large enough to hold three cages. After the cages were placed in the pans the wire floor of each cage was covered with approximately 1/4 inch of sand. The metal pans were large enough so that any sand kicked out of the cages by the rats remained in the pans. The rack of cages was kept in a room which was infrequently used; thus, the animals were subjected to a minimum of noise and disturbance. As each kangaroo rat was placed in its cage the rat was numbered, tentatively aged, and its species determined; its sex was determined only if it was in breeding condition. The species of the kangaroo rat was easily determined, as there are only two which occur in northern Utah. The two, Dipodomys ordii and D. microps, can be differentiated on the basis of their lower incisors. D. ordii has lower incisors which are rounded across the front, whereas those of D. microps are flat across the front.

After these preliminary procedures the animals were handled only

when absolutely necessary. They did not live long in the laboratory if they were frequently disturbed, especially when this occurred soon after capture.

The kangaroo rats were fed a diet consisting of approximately 70% millet and 30% sunflower seed, occasionally supplemented with a piece of fresh lettuce. They were never given water, as kangaroo rats get all the water they need from their solid food. The animals were kept in a windowless room in which the lights were usually turned on at approximately 8 A.M. and turned off between 5 and 10 P.M. The temperature of the room was usually about 22 C.

Fecal pellets from each animal were recovered from the jars in which the rats had been brought to the laboratory and these pellets were examined for the presence of coccidial oocysts by the modified Sheather's sugar flotation technique (Levine, 1961). Thereafter, each animal was examined at irregular intervals for the discharge of oocysts, until it died or was used in experimental studies.

Each rat was necropsied as soon as feasible after its death. At this time a fecal sample was examined for coccidial oocysts. As each animal was necropsied, a careful notation was made of its number, sex, and species. After necropsy, the head of the animal was separated from its body, skinned, and placed in a dermestid colony for two weeks. After the skull had been cleaned by the insects it was used in a final determination of the age of the animal.

#### Aging of Animals

The captured kangaroo rats were aged by using the characters

described by Setzer (1949). Setzer considered Dipodomys ordii to be fully adult when the auditory bulla was shiny and translucent, the permanent P<sup>4</sup> teeth erupted and worn, and the tail fully striped and penicillate. By using the characters described by Setzer, the animals captured during this study were placed in one of three age groups, adult, young adult, or juvenile. The adult characters were as Setzer (1949) described them. The young adults were similar to the adults except that some indications of cusps were present on the fourth premolar teeth of the upper jaw (P<sup>4</sup>). The animals classified as young adults were probably less than a year old, but, since kangaroo rats of known age were not available for comparison, this cannot be stated with certainty. In older juveniles the tail is thin and the terminal tuft is short, the bulla is cancellous, and the permanent P<sup>4</sup> teeth are present but show little or no wear and the cusps are prominent. In young juveniles the deciduous P<sup>4</sup> teeth are still present.

The preliminary aging of the kangaroo rats, done when they were first brought into the laboratory, was based mainly on the size of the animal and the length of the tail tuft. Adults and young juveniles could easily be identified but the young adults and older juveniles were often confused with each other. The final aging of the animals was done only when the skull characters could also be used.

#### Collection of Inoculum

The oocysts used in the present study were originally derived from two naturally infected kangaroo rats (one Dipodomys ordii and one D. microps). When brought into the laboratory after capture these two

animals were found to be passing only oocysts of E. utahensis. They were then examined daily for three days to make sure that no other species was being passed. On the fourth and fifth days all fecal pellets passed by the animals were collected, macerated in a 2.5% potassium dichromate solution, and put in a thin layer in a petri dish at room temperature to sporulate. After one week the oocysts were examined and found to be sporulated. The sporulated oocysts were scrutinized to confirm that oocysts of E. utahensis only were present. Each of two parasite-free kangaroo rats (both D. ordii) were inoculated with 20,000 E. utahensis oocysts, which had been cleaned of dichromate solution by repeated centrifugation. These rats were examined daily for oocyst discharge; during the discharge period fecal pellets were collected and the oocysts were then allowed to sporulate for one week. The oocysts collected from these two rats were used as inoculum in the study of the endogenous life cycle and of the prepatent and patent period of E. utahensis. Each new batch of oocysts was carefully examined in order to avoid contamination with other species of coccidia. No inoculum was ever used which contained any species of Eimeria other than E. utahensis.

#### Inoculation of Animals

Kangaroo rats which were to be used for experimental work were examined at least four times over a two week period for coccidia. Only coccidia-free animals were used in the experimental studies. Only Dipodomys ordii were used as experimental animals; D. microps was caught in insufficient numbers for experimental purposes, and those collected usually died shortly after being captured. The animals used in the study

of the endogenous stages and in the determination of the prepatent and patent periods were inoculated with 10,000 to 20,000 oocysts. The oocysts were inoculated directly into the stomach of a lightly anesthetized animal by means of a 1 ml tuberculin syringe and a needle fitted with a piece of polyethylene tubing. Before inoculation, the potassium dichromate was removed from the inoculum by repeated centrifugation.

#### Determination of Prepatent and Patent Periods

Seven kangaroo rats were used to determine the prepatent and patent periods. After inoculation, each rat was examined daily for oocyst discharge. In an effort to determine the exact duration of oocyst discharge, each of three rats were put into a clean cage twice daily, beginning on the day of oocyst discharge. The clean cages contained new sand and food. The rats were transferred in the morning and at night, at intervals of approximately 12 hours. The other four rats were each placed in a cage containing a wire screen which was a few inches above the cage floor. The holes in the wire were large enough so that the animal's pellets would drop through. The cage of each of these last four rats was changed daily. Pellets from each animal were collected and examined each day for oocysts for at least 30 days during the patent period.

#### Study of Oocysts and Sporozoites

For the morphological study of the sporulated oocysts and the sporozoites, oocysts of E. utahensis were collected from rats used to determine the prepatent and patent periods. Fecal pellets collected

during the patent period were put in a 2.5% potassium dichromate solution, macerated with an applicator stick, mixed thoroughly, and allowed to sporulate in a thin layer in a petri dish at room temperature (approximately 22 C). To determine the sporulation time, samples of the fecal material were removed at various intervals, concentrated by sugar flotation, and observed for the stage of sporulation. After sporulation was completed, the fecal samples were placed in fresh potassium dichromate and stored in a refrigerator until used. The sporulated oocysts were studied with a Zeiss photomicroscope equipped with apochromatic objectives. The measuring of the oocysts was done with an ocular micrometer, and the oocyst was drawn to scale with the aid of the ocular micrometer and graph paper.

For the sporozoite study, sporulated oocysts of Eimeria utahensis were cleaned and concentrated by a modification of the method of Jackson (1964). In this technique the bottom part of a glass petri dish (90 mm X 15 mm) and the bottom part of a plastic petri dish were used. The plastic petri dishes were labeled as being 90 mm X 15 mm but they were slightly smaller than this so that they just fit inside the glass petri dish. Fifteen ml of oocyst suspension were mixed with 15 ml of Sheather's sugar solution in the glass petri dish bottom and the plastic petri dish was carefully set on top of the solution. The oocysts floated to the top of the solution and adhered to the plastic petri dish; the heavier fecal material settled to the bottom. After one-half hour the plastic petri dish was gently removed and the film of liquid adhering to it was washed into a beaker. The oocyst-sugar solution was again stirred thoroughly and the plastic petri dish gently set on the solution for another half hour. After removing the petri dish the second time the process was

repeated once more. The collected oocysts were then washed free of the sugar solution by repeated centrifugation and were ready to use for the study of the sporozoites.

Free sporozoites were obtained for observation by excysting oocysts with the method described by Doran and Farr (1962), except that oocysts were ruptured with a tissue grinder and bovine bile was used. Living sporozoites and sporozoites fixed with osmium tetroxide vapor were studied with a Zeiss phase-contrast microscope. A drop of sporozoite suspension was put on a cover slip and this was placed upside down for one minute over the top of a small Erlenmeyer flask containing a two percent solution of osmium tetroxide. The cover slip was then smeared lightly along its border with vaseline and placed face down on a slide for microscopic observation.

Fresh sporozoites were also stained with acridine orange and examined under a Zeiss fluorescence microscope equipped with apochromatic objectives, using a BG-12 exciter filter and a yellow barrier filter. One drop of 0.005% acridine orange in normal saline solution was mixed with one drop of sporozoite suspension on a slide just before the cover slip was added, and the preparation was then examined immediately.

Smears for permanent preparations were made by spreading a suspension of sporozoites on a cover slip and fixing with various reagents after partial drying. The smears were made on No. 0 thickness 22 mm square glass cover slips and stained in Columbia staining dishes. For convenience, the Columbia jars were placed in two rows of holes bored halfway through a 2" X 6" plank. This held the jars stable, preventing accidental disarrangement, and facilitated putting them away when they were not in use.

Sporozoites fixed in Bouin's fluid were stained with protargol according to Jensen's (1963) modification of the method of Honigberg and Davenport (1954). The Giemsa method was used as described by Kudo (1954) and modified by Jensen and Hammond (1964), except that commercial albumin adhesive was placed on the cover slip before making the smear instead of adding serum to the sample for this purpose. The periodic acid-Schiff method for polysaccharides with absolute alcohol fixation and the Feulgen method for DNA with Zenker's fluid as a fixative were used as described by Barka and Anderson (1963). To determine whether the PAS-positive granules were glycogen, smears were given a preliminary treatment with 1% diastase in 0.85% saline solution at 37 C for one hour. For studying the distribution of protein the bromphenol-blue method of Mazia, Brewer, and Alfert (1953) was used. The occurrence of lipids was investigated by the use of the Sudan black B method of Chiffelle and Putt (1951).

#### Study of Endogenous Stages

To determine the endogenous life cycle of Eimeria utahensis, 16 kangaroo rats (Dipodomys ordii) were inoculated with 10,000 to 20,000 oocysts by the method previously described. The animals were then killed at 12 to 24 hour intervals after inoculation by a blow to the head. The intestinal tract was removed and sections of the intestine were taken every five centimeters from the ileocecal valve to the stomach. Sections of the stomach, cecum, and three levels of the colon (upper, middle, and lower) were also taken. Tissues from the liver, kidney, spleen, and pancreas were fixed for eight hours in ten percent neutral buffered formalin. After fixation, the tissues were washed overnight in running

water, dehydrated in a graduated ethanol series, cleared in xylene, and embedded in paraplast. Sections were made at five to seven microns, stained with Heidenhain's iron hematoxylin, Harris' hematoxylin and eosin, and the Feulgen method, using the de Thomasi modification of Schiff's reagent (Barka and Anderson, 1963). Fresh intestinal contents and mucosal scrapings were obtained at the time of necropsy for study of living coccidia with phase-contrast microscopy. As a control, tissues from a parasite-free kangaroo rat were fixed, embedded, stained, and examined.

## RESULTS

Incidence of Coccidia

From September, 1966, through May, 1967, a total of 176 kangaroo rats (151 Dipodomys ordii and 25 D. microps) were captured and examined for coccidia. The dates of capture, sex, and age of D. ordii and D. microps are given in Tables 1 and 2. Of these 176 rats, 4 D. ordii (2.6%) and 4 D. microps (16.0%) were infected with Eimeria utahensis (Table 3). Little seasonal difference was found in the infection rate of D. ordii (Table 4). A higher percentage of infected D. microps was observed in the autumn than in the spring (Table 5), but the numbers of specimens were so small that no conclusions can be drawn from this finding. None of the juveniles of either species were infected.

Exogenous CycleDescription of the Sporulated Oocyst

The sporulated oocyst (Figure 3) was subspherical to broadly ellipsoid, with a wall composed of two layers. The outer wall was approximately 2.3  $\mu$  in thickness, rough and pitted, and yellowish-brown in color; the inner wall was about 0.8  $\mu$ , and bluish in color. The outer wall frequently had fecal material adhering to it so that it appeared rougher than it actually was. The inner wall usually remained intact when the outer one was broken by applying pressure to the cover slip. No micropyle nor thinning of the oocyst wall was observed. One hundred oocysts measured 37 to 45 by 33 to 41  $\mu$ , with a mean of 42.0 by

Table 1. Age and sex of Dipodomys ordii captured from September, 1966, through May, 1967.

Date of Capture	Adult		Young Adult		Juvenile		Total
	Male	Female	Male	Female	Male	Female	
September 21, 1966	3	5	8	10	0	0	26
October 6, 1966	3	5	7	6	0	0	21
October 19, 1966	5	4	3	4	0	0	16
November 7, 1966	6	14	2	8	0	0	30
November 17, 1966	2	3	2	0	0	1	8
February 13, 1967	4	3	0	0	0	0	7
March 1, 1967	3	1	0	0	0	0	4
April 3, 1967	4	2	0	0	0	0	6
April 13, 1967	5	1	0	1	7	2	16
May 4, 1967	6	6	0	0	4	1	17
Total	41	44	22	29	11	4	151

Table 2. Age and sex of Dipodomys microps captured from September, 1966, through May, 1967.

Date of Capture	Adult		Young Adult		Juvenile		Total
	Male	Female	Male	Female	Male	Female	
September 21, 1966	0	2	0	0	0	0	2
October 6, 1966	4	2	0	0	0	0	6
October 19, 1966	1	1	0	0	0	0	2
November 7, 1966	2	3	0	0	0	0	5
November 17, 1966	0	2	0	0	0	0	2
February 13, 1967	1	0	0	0	0	0	1
March 1, 1967	0	0	0	0	0	0	0
April 3, 1967	0	0	0	1	0	1	2
April 13, 1967	3	0	0	0	1	1	5
May 4, 1967	0	0	0	0	0	0	0
Total	11	10	0	1	1	2	25

Table 3. Occurrence of Eimeria utahensis, according to age groups, in Dipodomys ordii and Dipodomys microps.

Age group	<u>Dipodomys ordii</u>			<u>Dipodomys microps</u>		
	Number of Specimens	Number Infected	Per Cent Infected	Number of Specimens	Number Infected	Per Cent Infected
Adult	85	2	2.4	21	4	19.0
Young Adult	51	2	3.9	1	0	0.0
Juvenile	15	0	0.0	3	0	0.0
Total	151	4	2.6	25	4	16.0

Table 4. Occurrence of Eimeria utahensis, according to age groups, in Dipodomys ordii during autumn, 1966, and spring, 1967.

Age group	<u>Dipodomys ordii</u>			<u>Dipodomys microps</u>		
	Number of Specimens	Number Infected	Per Cent Infected	Number of Specimens	Number Infected	Per Cent Infected
Adult	50	1	2.0	35	1	2.9
Young Adult	50	2	4.0	1	0	0.0
Juvenile	1	0	0.0	14	0	0.0
Total	101	3	3.0	50	1	2.0

Table 5. Occurrence of Elmeria utahensis, according to age groups, in Dipodomys microps during autumn, 1966, and spring, 1967.

Age group	Autumn			Spring		
	Number of Specimens	Number Infected	Per Cent Infected	Number of Specimens	Number Infected	Per Cent Infected
Adult	17	3	17.6	4	1	25.0
Young Adult	0	0	0.0	1	0	0.0
Juvenile	0	0	0.0	3	0	0.0
Total	17	3	17.6	8	1	12.5

39.3  $\mu$ . The length-width ratio ranged from 1.0 to 1.2, with a mean of 1.08. In freshly sporulated oocysts the oocyst residuum was a spheroidal body composed of many, relatively uniform, closely packed small granules, bounded by a thin membrane. Within a week after sporulation, the residual body was composed of many clear granules of varying sizes, with no bounding membrane.

The sporulated oocyst contained four elongate-ovoid sporocysts, each surrounded by a membrane of uniform thickness. A distinct Stieda body was present at the pointed end of the sporocyst. Immediately posterior to the Stieda body was a homogeneous flattened pyriform body bounded by a membrane. It was lighter in color than the Stieda body but darker than the adjacent contents of the sporocyst. This structure appeared to form a plug which blocked the narrow end of the sporocyst. Usually, three of the sporozoites were arranged lengthwise in the long axis of the sporocyst; the fourth was perpendicular so that it was viewed on end. One hundred sporocysts measured 18 to 23 by 12 to 14  $\mu$ , with a mean of 21.0 by 12.3  $\mu$ . The sporocyst residuum consisted of many, tightly packed, small granules forming an irregular mass in which the sporozoites were located. It was usually difficult to distinguish the outline of the sporozoites.

The sporozoites were elongate, with one end broader than the other. The broad end of one sporozoite was adjacent to the narrow end of the other. Each sporozoite had two refractile bodies, a larger posterior and a smaller anterior, but the smaller anterior one was usually partially or completely obscured by the residual granules (see upper sporocyst, Figure 3).

The only species of Eimeria from heteromyid rodents which E.

utahensis resembles is E. picti (Irvine et al., 1957a). E. utahensis differs from E. picti in having a much larger oocyst and larger sporocysts, as well as in lacking a polar granule. On the basis of these differences E. utahensis is considered as a new species of Eimeria from heteromyid rodents.

#### Sporulation of Oocysts

The first sporulated oocysts were found on the fourth day after the fecal specimen had been macerated in a 2.5% dichromate solution and put in a thin layer in a petri dish at room temperature. The maximum number of oocysts were sporulated on the seventh day. It was observed that in many of the samples large percentages of the oocysts remained unsporulated. In order to obtain further information about this phenomenon, ten different oocyst samples were each put in a thin layer of potassium dichromate solution and allowed to sporulate for seven days. Two hundred oocysts from each sample were counted and the percentage of sporulated oocysts determined. The number of sporulated oocysts ranged from 42 to 85%, with a mean of 60.2%. No attempt was made to determine whether variation in factors such as temperature might bring about an increase in the percentage of sporulated oocysts.

#### Morphological Studies of Sporozoites

Living sporozoites examined with the phase-contrast microscope had slender, slightly curved bodies. The most distinctive structures in the sporozoites were the refractile bodies. The nucleus was usually not very distinct; it normally appeared as a faint outline immediately posterior to the anterior refractile body. Small granules were seen around the nucleus

and in front of the anterior refractile body; these varied from 5 to 10 in number. Some indications of subpellicular fibrils could be seen. The anterior extremity of the sporozoite was distinctly darker than the rest of the body; a small nipple-like projection could be seen protruding from the anterior end.

Observations with bright-field microscopy gave results similar to those of phase-contrast, although the nucleus and the small granules appeared less distinct (Figure 4). Transverse striations were seen in the anterior region of the body of many sporozoites (Figure 5). Usually three to five of these striations were present, but in some specimens there were 10 or more, extending backward beyond the middle of the body. Occasionally the striations were obliquely oriented. They appeared to be at or near the surface of the body. In a few sporozoites median rod-shaped organelles could be distinguished extending from the anterior end almost to the anterior refractile body. The exact number of these organelles could not be distinguished with certainty, but there appeared to be between four and eight.

Thirty sporozoites measured 19.0 to 24.1  $\mu$  in length (mean, 22.5) by 4.0 to 4.6  $\mu$  (mean, 4.5) in width at the anterior refractile body and 4.0 to 5.2  $\mu$  (mean, 4.6) in width at the posterior refractile body. The anterior refractile body was usually round, but was sometimes ellipsoid, and measured 2.3 to 4.0 by 2.3 to 4.0  $\mu$ , with a mean of 3.0 by 3.0  $\mu$ . The posterior refractile body occupied almost half of the sporozoite. It measured 9.2 to 11.5  $\mu$  in length by 4.0 to 5.2  $\mu$  in width, with a mean of 10.6 by 4.6  $\mu$ .

Living sporozoites were very active after being excysted, exhibiting

gliding, flexing, pivoting, and probing movements. The flexing movements involved a bending of the anterior third of the body. This movement, invariably in the direction of the curvature of the body, continued until the anterior end was directed posteriorly, resulting in a J-shaped configuration of the body. After a few seconds the flexed portion of the body straightened out; this movement occurred more quickly than the flexion. The flexing movement was usually repeated many times by an individual sporozoite.

Usually the flexing movements were done while the sporozoites remained in one place, but sometimes the flexing sporozoite would begin gliding before resuming the unflexed shape; this resulted in the sporozoite following a circular path. More often, the sporozoite would undergo the gliding movement while the body was only slightly curved so that the course followed was a large arc. Frequently, the gliding movement was accompanied by apparent rotation of the body so that forward progression in a spiral path resulted.

The sporozoites often pivoted on their posterior ends in a clockwise or counter clockwise direction. The probing movements involved a lateral displacement of the anterior tip without any noticeable elongation or retraction.

The position and number of refractile bodies were examined in 100 live sporozoites stained with acridine orange and examined with the fluorescence microscope. All had a large posterior refractile body. Ninety-seven sporozoites contained one small anterior refractile body in front of the nucleus; two had no anterior refractile body, and one had two small anterior refractile bodies, both in front of the nucleus.

Nuclei of fresh specimens in acridine orange solution showed a positive reaction to DNA only at the periphery of the nucleus. The chromatin material was distributed in five to eight clumps, spaced more or less evenly around the margin of the nucleus; a thin layer was present elsewhere around the nucleus. Cytoplasmic granules similar to those seen in bright-field and phase-contrast microscopy fluoresced a light orange, which soon faded; the cytoplasm in the anterior end and around the nucleus reacted similarly. The refractile bodies showed a light green fluorescence, indicating that they contained DNA. However, this was shown to be an artifact of the staining technique, as the refractile bodies had no fluorescence with the acridine orange staining method outlined by Spendlove (1967). With the latter technique the only DNA positive material in the sporozoite was in the nucleus, which appeared the same as in the previous method; the presence of RNA was indicated by a diffuse fluorescence in the cytoplasm in the anterior end and around the nucleus. The extreme anterior end and the nipple-like projection showed no fluorescence by either method.

Sporozoites fixed in osmium tetroxide fumes and observed with the phase-contrast microscope were usually slightly curved, and the small nipple-like projection at the anterior end of each could be distinguished (Figure 6). Cytoplasmic granules similar to those observed in the live sporozoites appeared distinct in this preparation. Often a few granules were seen along the sides of the posterior refractile body, showing that at least in some specimens, the large refractile body does not occupy the entire width of the sporozoite. An indication of subpellicular fibrils could be seen, but not as well as in the living specimens observed with

phase-contrast. Approximately eight median rod-like organelles were seen in the anterior end of many of the sporozoites (Figure 6). Some of these organelles appeared to extend to, or around, the anterior refractile body, while the shorter ones extended only about half-way to the anterior refractile body. These organelles appeared to have a spiral arrangement.

In the protargol preparations the anterior end appeared as a dark area, with a nipple-like projection sometimes present. Often, the posterior end also appeared dark. The refractile bodies appeared lighter than any other structure in the sporozoites. The nucleus was vesicular, with a relatively thick, dark layer at the periphery. Cytoplasmic granules were difficult to distinguish. The cytoplasm of the anterior end had a coarsely granular appearance. A median rod-like body was present in the anterior end in many of the specimens (Figure 7). This rod-like body originated at the anterior tip of the sporozoite and ran posteriorly about half-way to the anterior refractile body. An indication of subpellicular fibrils could be seen indistinctly in many of the specimens. The surface layer over the posterior refractile body often had a beaded appearance.

In sporozoites stained with Giemsa, the refractile bodies stained pink and the cytoplasm stained reddish-purple. The extreme anterior end was a lighter color than the rest of the cytoplasm. The cytoplasmic granules stained dark reddish-purple. There were 2 to 4 granules at the anterior end, 5 to 10 around the nucleus, and 10 to 15 in front of the anterior refractile body. Often, there were a few granules along the sides of the posterior refractile body. The nucleus was difficult to distinguish because the chromatin masses stained the same color as the cytoplasmic granules.

In the Feulgen preparations only the nucleus gave a positive reaction. The marginal layer of chromatin appeared thinner in the Feulgen specimens than in fresh specimens stained with acridine orange.

In sporozoites stained with the periodic acid-Schiff procedure only one to five red granules, or none at all, were observed. When present, the stained granules were usually between the refractile bodies. Eimeria bovis, a species known to contain PAS-positive material in the sporozoites, was used as a control. In the diastase-treated slides no stained material was found in any of the sporozoites; the stained granules were thus shown to be glycogen.

In the preparations stained with bromphenol-blue (Figure 8), a dark staining reaction was observed in the refractile bodies of the sporozoites. The cytoplasm showed a light, diffuse reaction. The nucleus stained a little darker than the cytoplasm.

The refractile bodies stained a light gray with Sudan black B, indicating the presence of lipids (Figure 9). This staining was still present after the sporozoites had been subjected to a one-to-one mixture of chloroform and methyl alcohol at 50 C for 24 hours.

#### Endogenous Cycle

##### Prepatent and patent periods

Of the seven rats inoculated to determine the prepatent and patent periods, one began passing oocysts on the ninth day after inoculation and six began on the tenth day. Thus, the mean prepatent period was 9.8 days.

Of the three rats put into clean cages twice a day in an attempt to determine the duration of the patent period, one died on the fifteenth day

after it began discharging oocysts; it was still passing oocysts on the day of its death. The other two rats discharged oocysts for 30 consecutive days. The feces of these two rats were then examined at irregular intervals for oocyst discharge. One of the animals died 45 days after it had begun passing oocysts; its feces were still positive for E. utahensis oocysts on the day it died. The other rat was still discharging oocysts after 70 days, at which time this part of the study was terminated.

The four animals which were placed in cages with raised wire floors all died within four days after being put on the wire. All four were still passing oocysts at the time of their deaths.

#### Description of Endogenous Stages

The endogenous stages of Eimeria utahensis were located throughout the small intestine. The first-generation schizonts were most heavily concentrated in the anterior third of the small intestine; the succeeding generations of schizonts and the sexual stages were concentrated in the middle half of the small intestine. All of the schizogonous stages were located in epithelial cells in the distal half of the villus and usually were found above the host cell nucleus (i.e., toward the lumen side). None of the schizogonous stages caused much alteration in the host cell; however, in host cells harboring mature or nearly mature schizonts the nucleus was somewhat flattened on the side toward the parasite. The mature schizonts of all generations were usually spherical. In the following descriptions each measurement given represents a mean of 20 specimens in tissue sections fixed in Zenker's and stained with hematoxylin and eosin.

No parasites were found in tissue sections from the stomach, cecum, or colon; nor in sections of the liver, kidney, spleen, or pancreas. No

structures resembling any of the parasitic stages were found in the tissue sections from an uninoculated kangaroo rat.

The first schizogonous stages were found 2 1/2 days after inoculation. Stages ranging from unicellular trophozoites to schizonts with merozoites were present at this time. These mature schizonts were 9.7  $\mu$  in greatest diameter and contained 12 to 16 merozoites which measured 8.3  $\mu$  in length by 2.0  $\mu$  in width (Figure 10). It could not be determined whether the unicellular trophozoites and growing schizonts belonged to the first or second schizogonous generation. A refractile body, which is often found in early first-generation schizonts, was not observed in any of the growing forms or in the mature schizonts.

Second-generation schizonts, also containing 12 to 16 merozoites, were present on the fourth and fifth post-inoculation days. These schizonts measured 8.0  $\mu$  in greatest diameter. The merozoites were 8.0  $\mu$  in length by 1.2  $\mu$  in width. A compact, spherical, eccentric residual body, 3.4  $\mu$  in diameter, was present in these schizonts (Figure 11).

A few mature third-generation schizonts, 12.4  $\mu$  in greatest diameter, were present on the fourth post-infection day but were more numerous on the fifth and sixth days. These schizonts contained four to eight merozoites which were 12.6  $\mu$  in length by 2.3  $\mu$  in width (Figure 12). A few schizonts were found which measured approximately 14.0  $\mu$ , and contained 12 or more merozoites of this type. Further study is necessary before the significance of these larger schizonts can be interpreted.

On the fifth day very young sexual stages were present in the epithelial cells of the crypts of the small intestine (Figure 15). These young gametocytes were recognizable by their distinct nucleolus and by

their location below the host cell nucleus (i.e., away from the lumen).

The third-generation merozoites gave rise to the early sexual stages or to fourth-generation schizonts. The fourth-generation schizonts, 8.6  $\mu$  in greatest diameter, contained 16 to 24 merozoites which measured 8.0  $\mu$  in length by 1.2  $\mu$  in width (Figure 13). On the sixth post-infection day, third-generation schizonts, fourth-generation schizonts (Figure 14), and gametocytes were present. A few fourth-generation schizonts were present on the seventh day, but growing gametocytes were the most numerous parasitic stages present at this time.

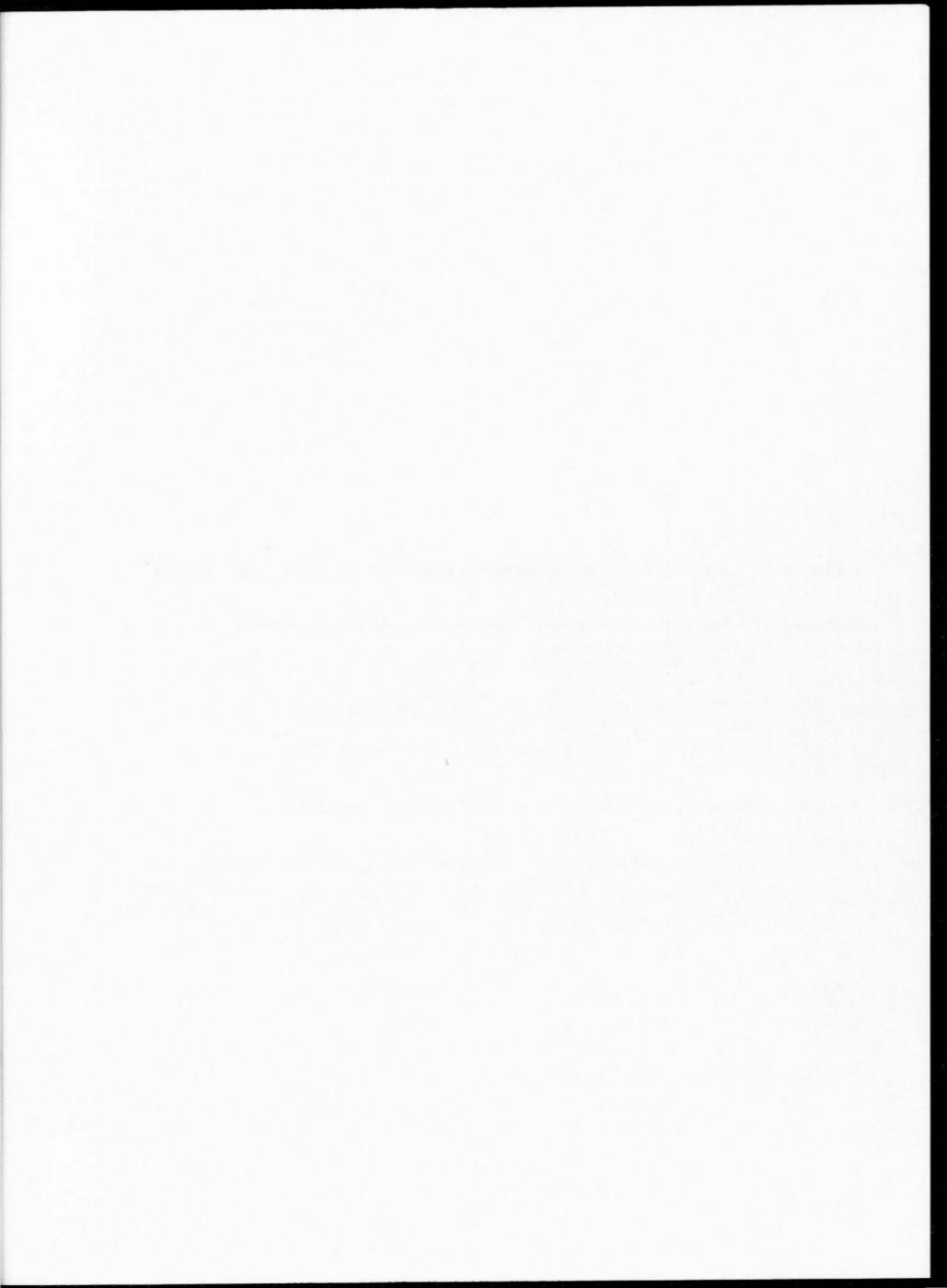
The gametocytes caused changes in the host cell, which became noticeable soon after penetration of the parasite. The host cell nucleus enlarged and became invaginated in the area adjacent to the parasite, resulting in a half-moon appearance (Figure 15). The host cell nuclei then became elongated and flattened, assuming a location near the periphery of the cells (Figure 16). This alteration of the host-cell nucleus was often so extreme that the nucleus encircled over half of the cell. In many host cells two or more nuclei were apparently present in the parasitized cell (Figure 17). This is considered to be the result of sections cutting through different portions of a single elongated, irregularly shaped nucleus. However, in a few instances young gametocytes were observed in cells in which the host-cell nuclei were undergoing division (Figure 18). This suggests the possibility that some of the infected cells may have been truly multinucleate. Shortly after the parasites entered the infected epithelial cells the cells became displaced into the lamina propria and the mature gametocytes usually were found in the latter location (Figure 21).

The nuclei of very young gametocytes were vesicular, with an eccentric nucleolus. At this stage the marginal ring of the nucleus was Feulgen-positive. As the parasite grew, the nucleolus enlarged, the margin became less distinct, and no DNA positive material could be observed. In these early stages the macrogametocyte could not be distinguished from the microgametocyte until nuclear division occurred. In microgametocytes with more than one nucleus these nuclei were Feulgen-positive. The nuclei were randomly arranged in the microgametocyte during the early stages of development (Figure 22). As the microgametocytes matured, the nuclei became arranged at the periphery of numerous compartments, which had lightly stained, homogeneous contents (Figure 23). The nuclei elongated and then appeared in whorls at the surface of the compartments (Figures 21, 24). At maturity the microgametes lost their whorl arrangement and became randomly situated around a central mass of residual material. (Figure 25). The mature microgametocytes were nearly twice as large as the macrogametes, and measured  $63.9 \mu$  by  $48.3 \mu$ . Some of the mature microgametocytes were spheroidal, but the majority were ellipsoid.

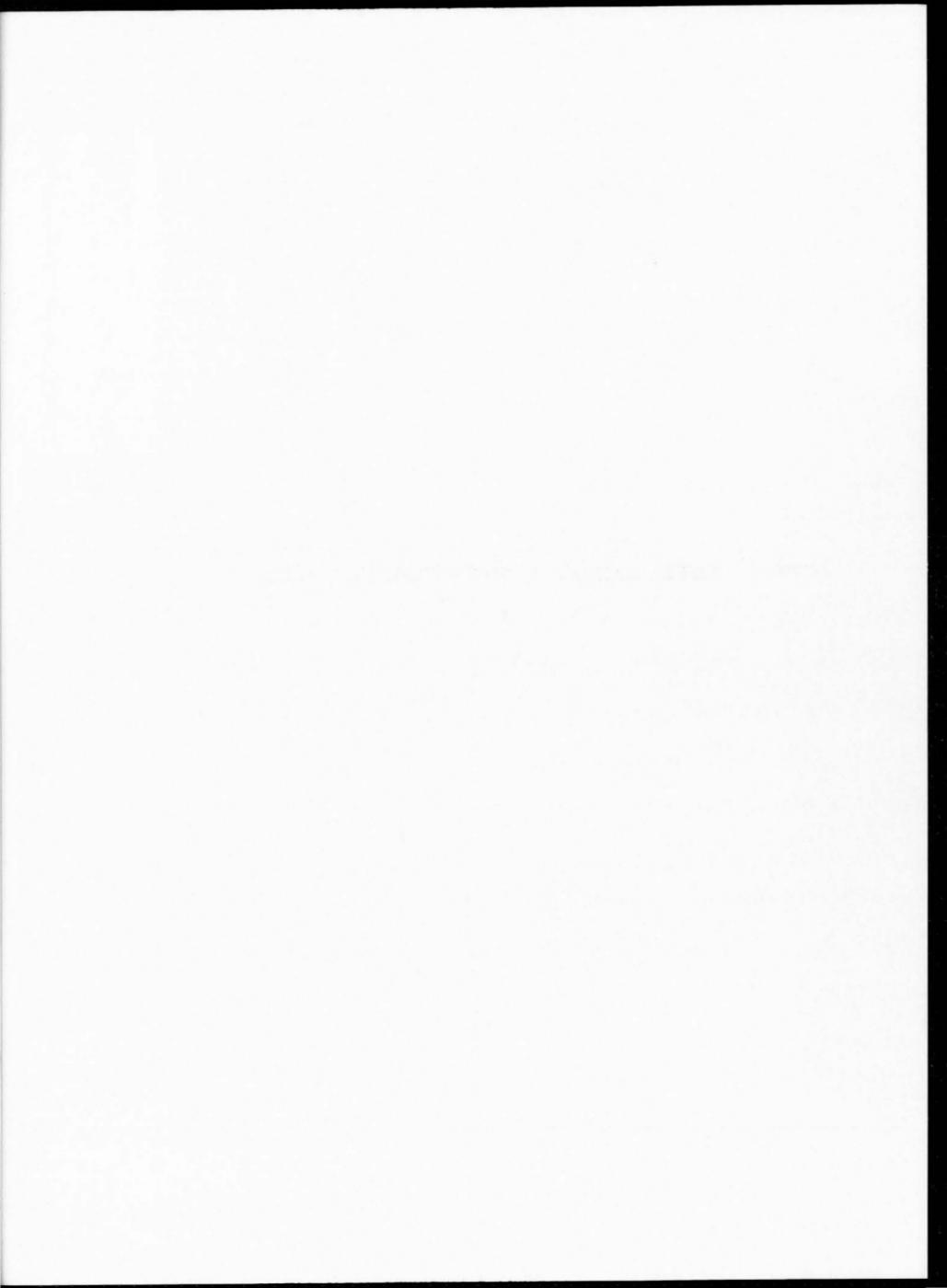
The plastic granules of the macrogametes were slightly eosinophilic in hematoxylin and eosin stain, but did not stain with iron-hematoxylin. In a few specimens, the plastic granules were distinctly eosinophilic. As the macrogametes matured the plastic granules moved to the periphery of the parasite and coalesced to form the oocyst wall. The macrogametes averaged  $32.5$  by  $27.0 \mu$  at the stage in which the plastic granules were located at the periphery of the parasite but had not yet coalesced (Figure 19). At this stage the nucleus was usually ellipsoid,  $6.3 \mu$  by  $4.6 \mu$ , but was sometimes spheroidal. The irregular margin of the nucleus was

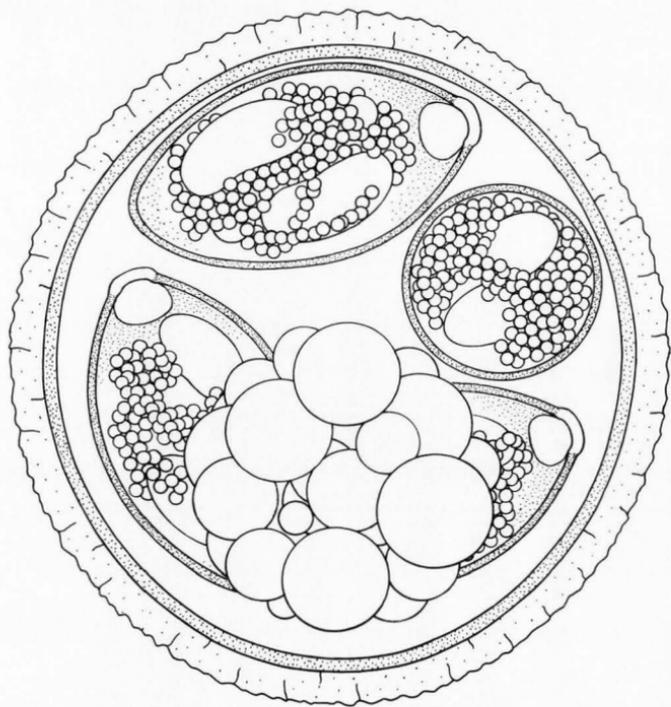
indistinct and appeared no darker than the nucleoplasm, which was homogeneous and eosinophilic in appearance. The distinct nucleolus, staining black with hematoxylin and eosin and with iron-hematoxylin, was spherical and measured approximately 2.3  $\mu$ . A distinct nucleolus was also present in the oocyst (Figure 20).

Eimeria utahensis caused no outward signs of coccidiosis in experimentally infected D. ordii, nor were any marked pathological changes observed in the tissue sections.



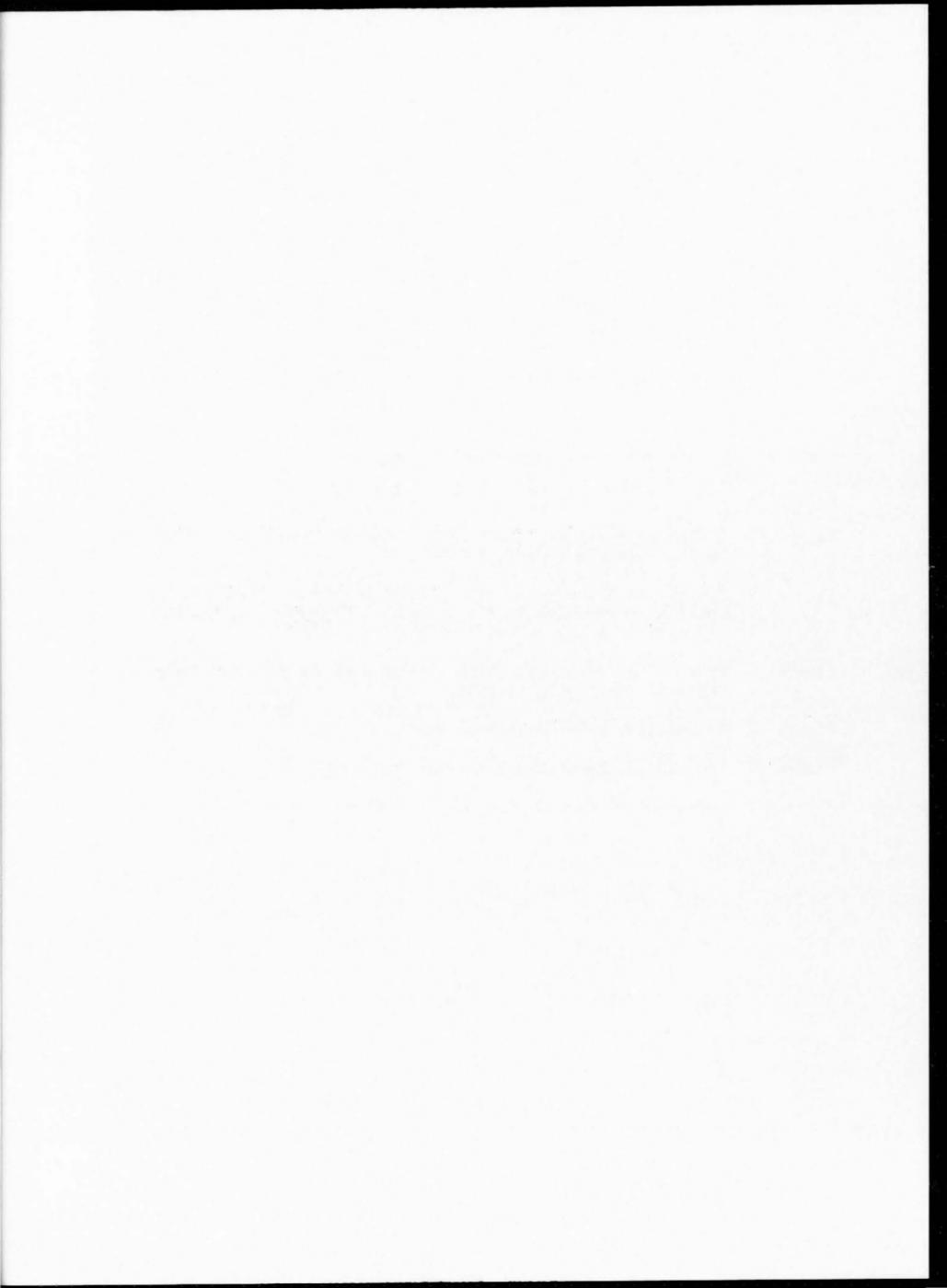


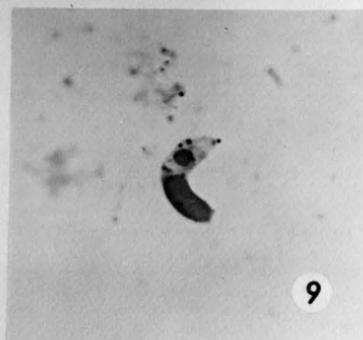


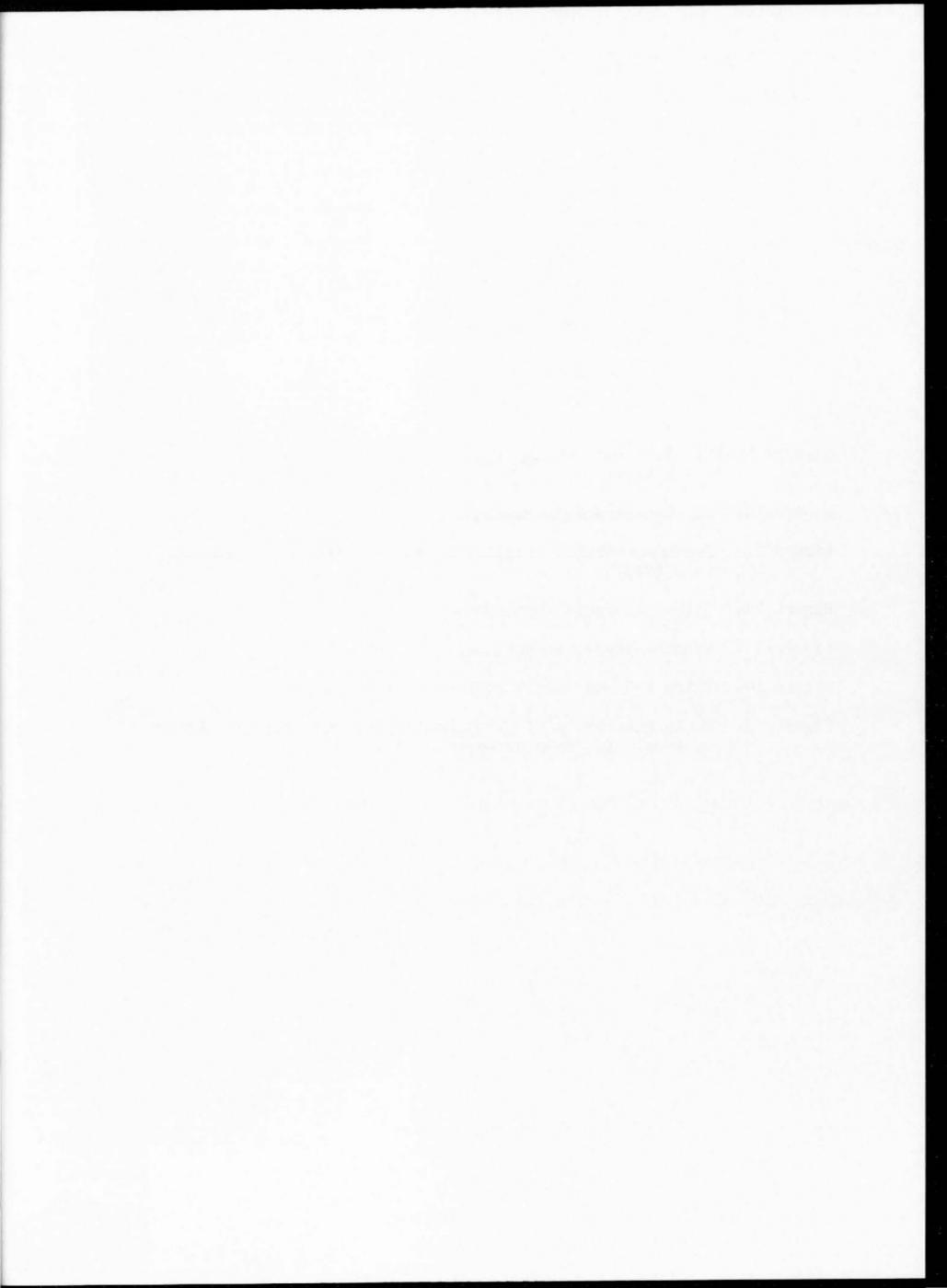


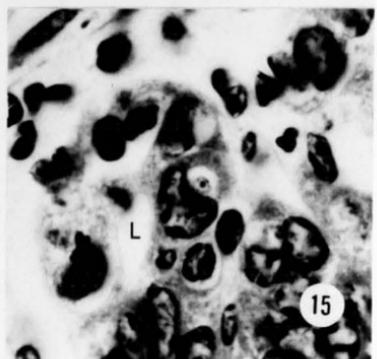
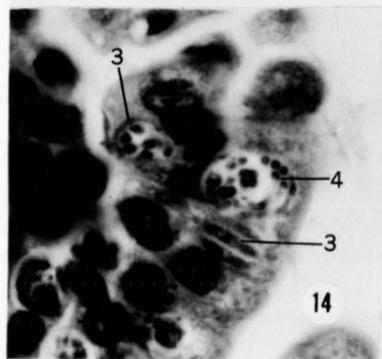
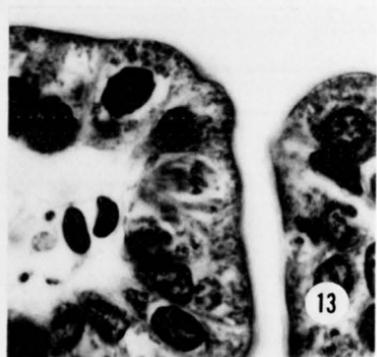
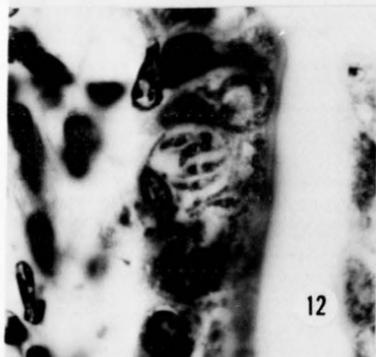
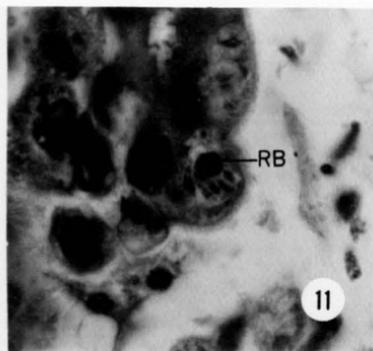
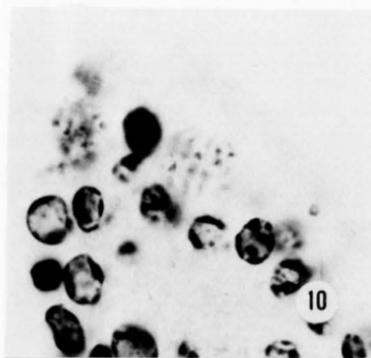
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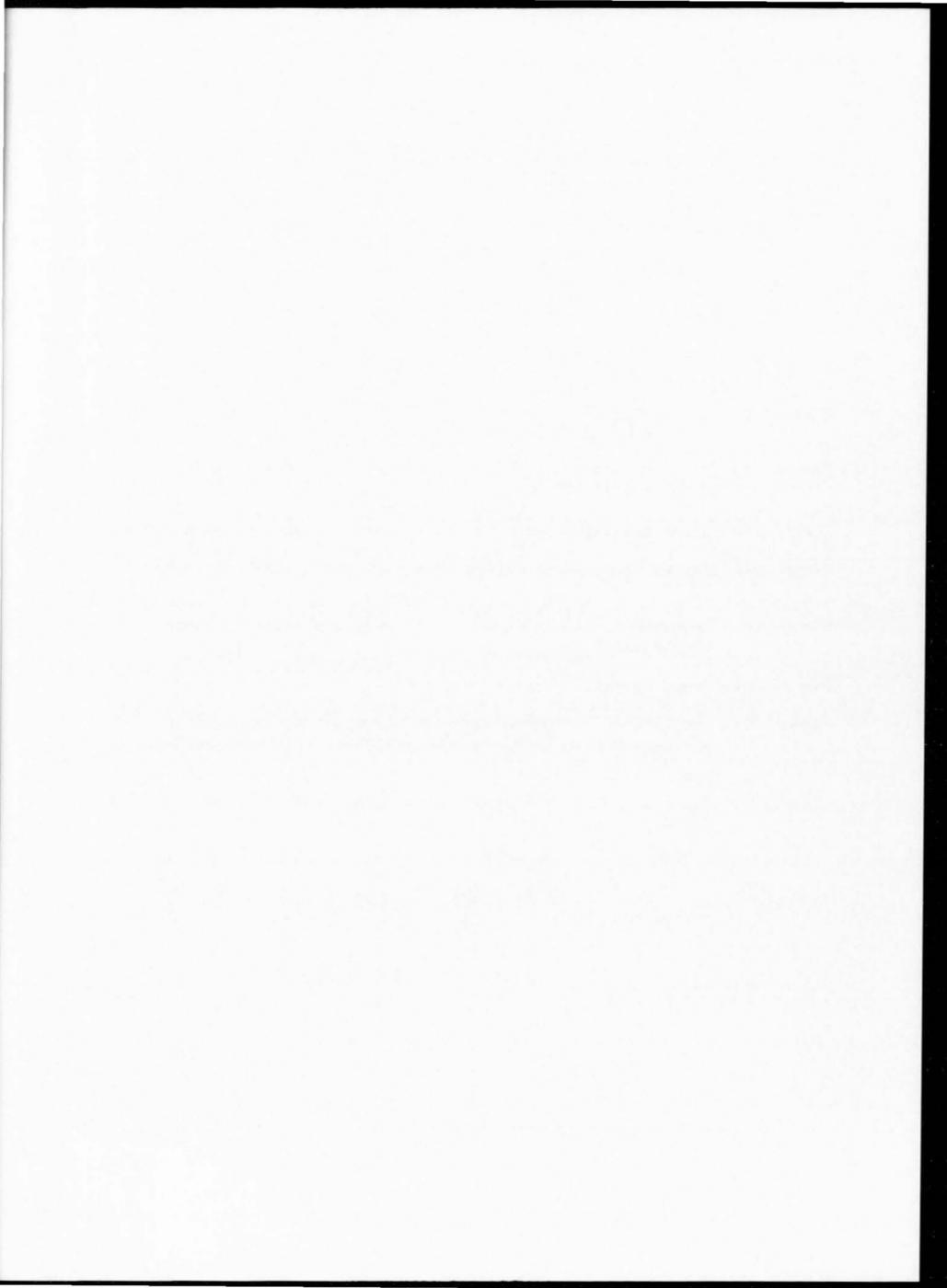
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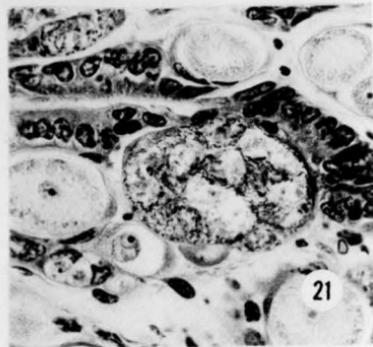
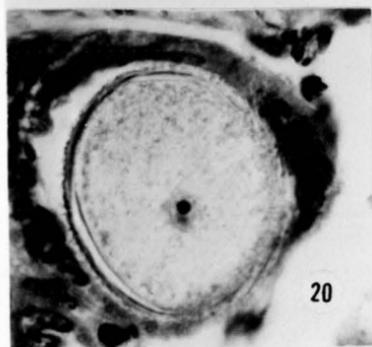
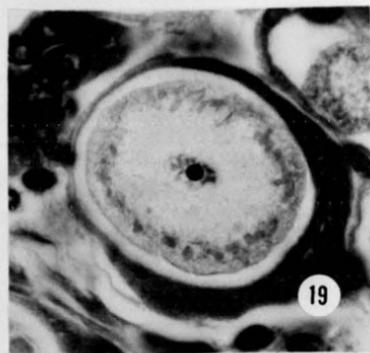
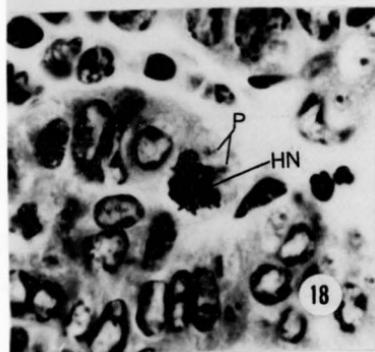
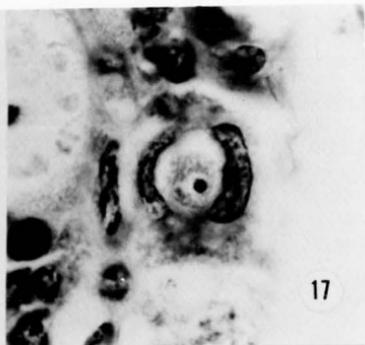
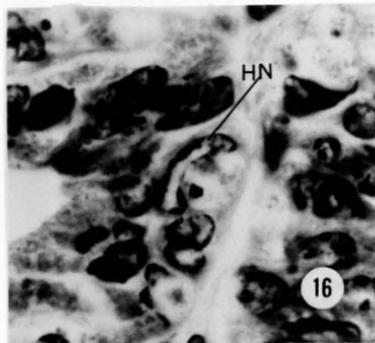


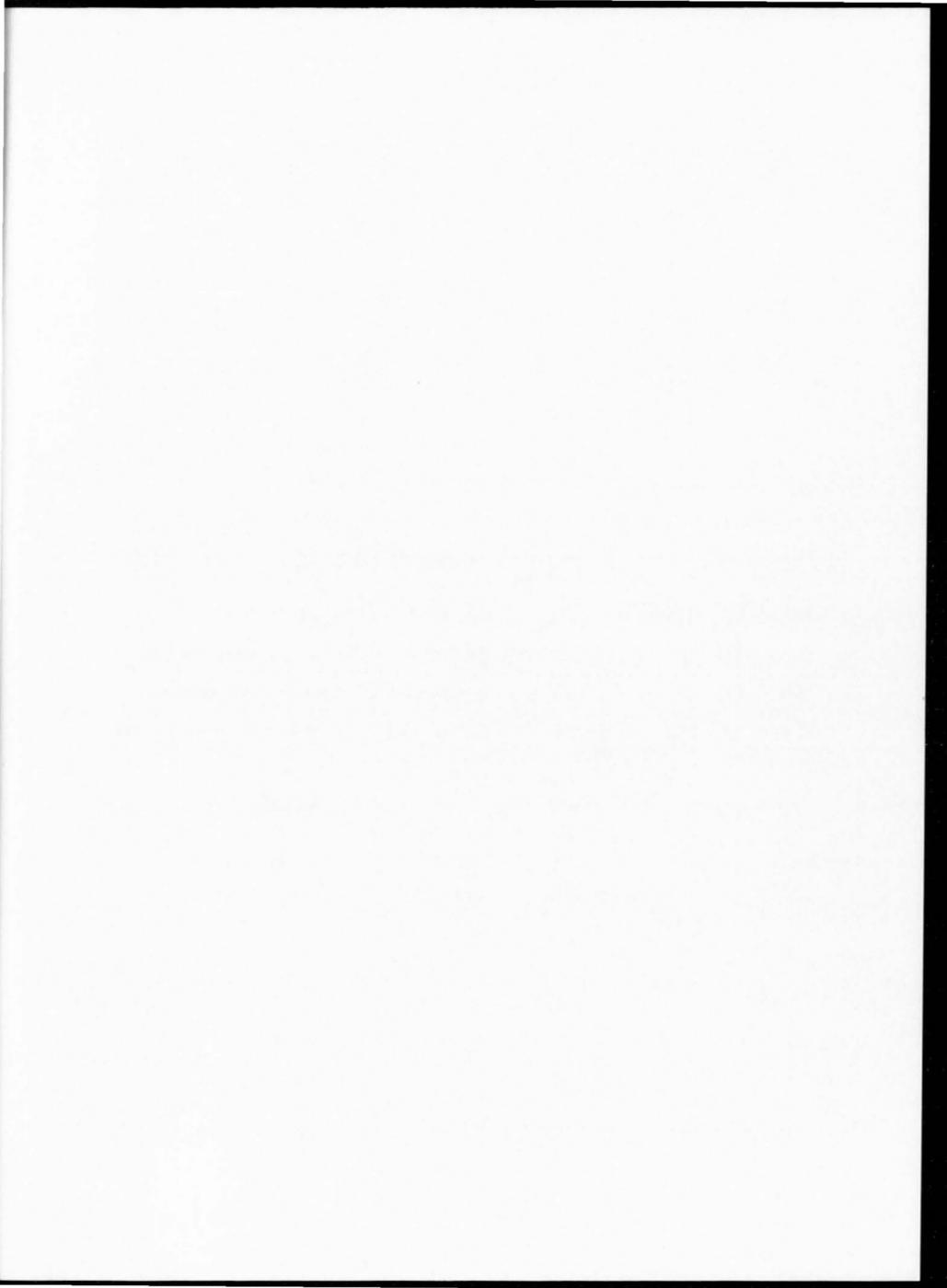


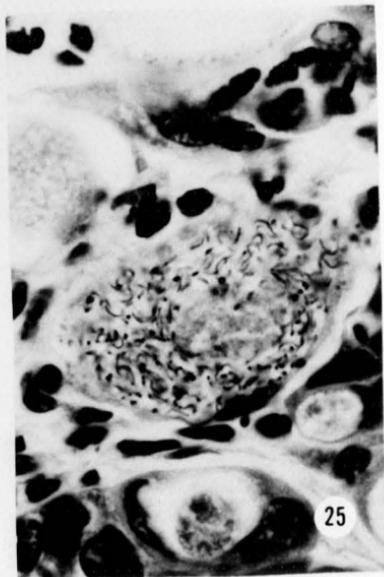
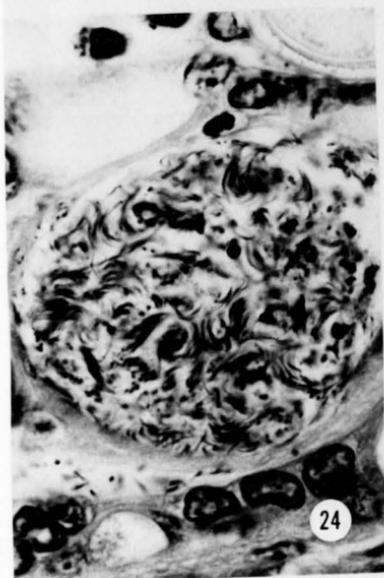
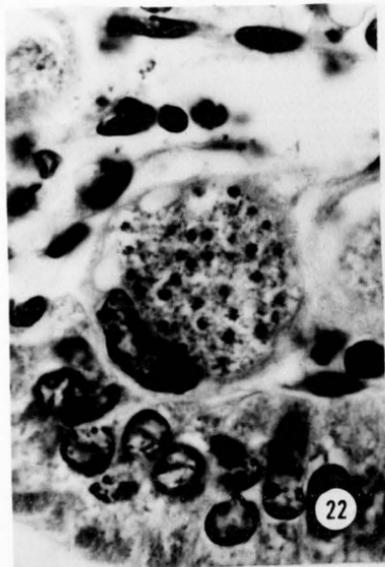












## DISCUSSION

The kangaroo rats used in this study were not good laboratory animals. All the Dipodomys microps died within a few days after being brought into the laboratory. Approximately half of the D. ordii died within the first two weeks of captivity, one-fourth died within a month, and the remaining one-fourth lived for a prolonged time; these were used for experimental studies. The younger D. ordii adapted much better to captivity than did the older animals. Once the animals had become used to being in the laboratory they were gentle and usually could be handled without fear of being bitten.

This study indicates that the incidence of infection of Eimeria utahensis in kangaroo rats in northwestern Utah is low. Doran (1953) found E. mohavensis in 8.7% of 251 Dipodomys panamintinus mohavensis from the Mohave desert in California. He reported that the low incidence of infection was probably due to the infrequent association between the kangaroo rats, and to the sensitivity of the oocysts to the high temperatures and low relative humidity of the desert soil surface. He stated that the self-limited life cycle and the susceptibility of oocysts to freezing may also contribute to the low incidence of infection. Of the physical factors present in the desert which are harmful to coccidian oocysts probably the most lethal is the low relative humidity. Marquardt, Senger, and Seghetti (1960) reported that only 2 to 12% of Eimeria zurnii oocysts survived a relative humidity as low as 25%.

Doran (1953) suggested that infection of kangaroo rats with

E. mohavensis possibly took place in the kangaroo rat burrows. He stated that if the oocysts of E. mohavensis were deposited in a burrow it would be highly probable that the oocysts would sporulate, remain viable, and serve as a source of infection for a longer time than they would if deposited on the soil surface, because of the lower temperatures and higher humidities below the surface of the ground. These factors are probably important, but the concentration of the pellets in a restricted area (the burrow) is also an important factor. Because of the wide dispersal of kangaroo rats in the study area and their aggressiveness toward each other, the chances of an animal becoming infected with E. utahensis from a fecal pellet on the soil surface is probably slight. The most likely source of infection would be the feces in the burrows, where the animal's food is stored. Accidental contamination of the food would be the most plausible means of reinfection or of passing the oocysts from one animal to another.

One of Doran's (1953) most interesting findings was that none of 197 Dipodomys merriami merriami, which was sympatric with D. panamintinus mohavensis, were infected with E. mohavensis even though it was demonstrated in the laboratory that D. m. merriami was more susceptible to infection with E. mohavensis than was D. p. mohavensis. In this study both species of kangaroo rats occurring in northwestern Utah were infected with E. utahensis.

The differences between rates of infection of D. ordii (2.6%) and D. microps (16.0%) is difficult to explain. Since only 25 D. microps were examined for coccidia, further trapping should be done to confirm this finding as to rate of infection. If this is verified it might be

concluded that D. microps is the original host animal or that it is more susceptible to infection with E. utahensis. The relative susceptibility of these two kangaroo rat species to infection with E. utahensis was not determined. A possible reason for the low incidence of infection in D. ordii may be that these rats do not enter burrows other than their own, thus decreasing their chances of acquiring an infection. During this study it was often observed that an animal could be captured, although it was in the vicinity of a burrow entrance; the kangaroo rat would try to hide on the surface instead of entering the burrow. Apparently the burrow was that of another animal. On approximately six different occasions while pursuing a rat, the animal entered a burrow but immediately came back out, and was captured. Another kangaroo rat, or a predator, may have been in the burrow, but the animal being pursued came back out so quickly that it was concluded that the rat had entered a burrow which was not its own.

Because of the small numbers of infected animals in this study, no conclusions can be made as to the relationship between age of the animal and incidence of infection. The low incidence of Eimeria stiedae in older rabbits reported by Bull (1958) in New Zealand and Myktowycz (1962) in Australia is associated with immunity, which the rabbits acquire by exposure to oocysts as younger animals. The immune responses of the rabbit to the intestinal species of coccidia are less well known (for a review of immunity to coccidiosis in rabbits see Pellerdy, 1965). Todd and Hammond (1967b) reported that the incidence of infection in the Uinta ground squirrel, Spermophilus armatus, with E. larimerensis was similar in adult and juvenile animals. They were able to cause repeated patent

infections in the host animals; one individual of S. armatus was subjected to 23 infections over an 11 month period and still passed viable oocysts after a normal prepatent period. No variation in the prepatent period, viability of the discharged oocysts, or severity of infection was noted in the animals that had been repeatedly infected. Todd and Hammond (1967a) reported that S. armatus could be subjected to repeated experimental infections with E. callospermophili unless the animals were in a torpid condition that resembled hibernation. In the present study, D. ordii remained infected for long periods of time after it was inoculated, which shows there is little, if any, immunity to this species of coccidia.

Doran (1953) was able to circumvent natural infections with E. mohavensis by changing the cages of infected rats twice a day. In this study the rats were put into clean cages twice a day, but the animals still remained infected. Ernst, Frydendall, and Hammond (1967) changed the sand twice daily in cages containing Dipodomys ordii infected with E. scholtyssecki, but the rats still evidently became reinfected and had prolonged oocyst discharges. Coprophagy has not been reported in kangaroo rats, nor was it observed during this study. Even if coprophagy occurred, it would not provide a suitable explanation for reinfection of those animals put into clean cages twice a day, as the minimum sporulation time of the oocysts was four days. A more plausible explanation for reinfection is that sporulated oocysts could occur in the fur around the anal region, and the animals might become infected by ingesting these sporulated oocysts. While cleaning themselves, kangaroo rats were often observed licking the fur around their anal regions. Eisenberg (1963) in describing the cleaning of the body carried out by Dipodomys noted that the mouth was

used to lick and nibble the fur of the body, including the anal region.

All four animals placed in cages with raised wire floor died within four days after being placed on the wire. Doran (1953) stated that kangaroo rats could not be raised on wire because they lost their food in attempting to store it. In the present study the rats were given food twice a day in a small container; much of the food was lost, but some of it was eaten immediately. The animals were also given daily a piece of lettuce which was too large to fall through the wire. Therefore, it seems likely that these animals died of causes other than starvation.

Dorney (1966) reported the incidence of four species of Eimeria in eastern chipmunks and red squirrels, and cited data given by Dorney (1965) and by Vetterling (1964) as to incidence of seven species of Eimeria in two other Neartic sciurid genera (Marmota and Cynomys). Dorney (1966) stated that all of the species with a high percentage of occurrence (E. perforoides, E. monocis, E. ludoviciani, E. vilasi, E. tamiasciuri) in these four rodent genera were small, clear, and thin walled, whereas the species having a low incidence (E. tuscarorensis, E. os, E. cynomysis, E. larimerensis, E. wisconsinensis, E. toddi) were large, yellow to brown, and (with one exception) thick walled. According to Dorney, these data indicate an interesting parallel between percentage of occurrence and oocyst structure of the eimerians within these four host genera. My findings do not agree with this concept. Ernst, Frydendall, and Hammond (1967) found E. scholtzsecki, which has small, smooth walled oocysts in 1 of 74 Dipodomys ordii and Ernst, Chobotar, and Anderson (1967) reported the occurrence of E. balphae, another species having smooth, thin walled oocysts, in 1 of 82 D. ordii. In the present study E. utahensis, a rough,

thick-walled oocyst, was found in 4 of 151 D. ordii and 4 of 25 D. microps. All of the kangaroo rats of these 3 studies were captured in the same area. Therefore, these findings indicate that the most common species of Eimeria in kangaroo rats (E. utahensis) of this area has rough, thick-walled oocysts.

The homogeneous structure posterior to the Stieda body in the sporocysts has not previously been described in an eimerian oocyst. Schwalbach (1959) described a similar structure in the sporocysts of several species of Isospora from birds. He called this structure a micropyle plug (Micropylenpropf); he also called the Stieda body a micropyle. Both of these terms were erroneously used, as the micropyle is a structure of the oocyst. An appropriate term for this structure is sporocyst plug. Schwalbach (1959), using the iodine technique, reported that the Stieda body and sporocyst plug were glycogen, but this appears questionable, because the Stieda body in E. acervulina and E. brunetti (Pattillo and Becker, 1955) and in E. tenella (Horton-Smith and Long, 1963) are protein in nature. The composition and function of the sporocyst plug in E. utahensis have yet to be elucidated.

The sporozoites of E. utahensis had four kinds of movement, gliding, often with an apparent rotation of the body, flexing, pivoting, and probing. Each of these movements was about equal to the others in frequency. The gliding movement has also been reported from sporozoites of E. bovis, E. ellipoidalis, and E. auburnensis of cattle (Nyberg and Hammond, 1964), and E. nieschulzi of rats (Marquardt, 1966). Doran, Jahn, and Rinaldi (1962) stated that the movement of Eimeria acervulina of chickens was helical with a corkscrew movement. Vetterling (1966) observed

that the most common movement of E. deblicieki sporozoites was probing, in which the anterior end would stretch and retract periodically. This contraction and elongation was also made by E. adenceides sporozoites, as were also the flexing, gliding, and pivoting movements (Clarkson, 1958). Vetterling (1966) found that gliding and flexing movements of E. deblicieki were sporadic but not uncommon. Probing movements were also made by E. utahensis but the anterior end moved laterally instead of forward and backward. Fayer and Hammond (1967) observed movement of E. bovis sporozoites in cell cultures. These sporozoites were seen to flex, glide, and pivot on the posterior end. These authors stated that the significance of the flexing and pivoting movements was unknown. The gliding movements of E. utahensis sporozoites probably help the organisms move along the intestinal tract as well as in penetration of the parasite into the host cell.

Subpellicular fibrils were observed in only a few specimens of living and stained E. utahensis sporozoites. Marquardt (1966) estimated that 15 to 18 longitudinal fibrils were present in E. nieschulzi sporozoites examined with phase-contrast microscopy. Colley (1967) reported 25 fibrils (microtubules) directly beneath the pellicle in the sporozoites of E. nieschulzi studied with the electron microscope. He stated that these microtubules may aid in the support and locomotion of the sporozoite. Eird and Baker (1963) also found peripheral fibrils in an electron microscopic study of sporozoites of Eimeria stiedae. The presence of subpellicular fibrils in the motile stages of numerous other sporozoan species (Sheffield and Hammond, 1966) provides support for the hypothesis that they are the structural basis of locomotion in these stages (Garnham,

1966).

The presence of a conoid in E. nieschulzi sporozoites was demonstrated by Colley (1967) using the electron microscope. A conoid was also seen in electron micrographs of Eimeria stiedae (Bird and Baker, 1963). In the sporozoites of E. nieschulzi observed with phase-contrast microscopy the presence of the conoid could only be inferred by the dark tip of the sporozoites (Marquardt, 1966). This darkened tip was seen in the sporozoites of E. utahensis in phase-contrast and stained specimens. It is doubtful whether a structure such as the conoid can be seen with light microscopy and the dark appearance of the anterior end of the sporozoites could be associated with any one of several other structures or with the narrowing of this body region.

The median rod-shaped organelles seen most clearly in specimens of E. utahensis sporozoites fixed with osmium tetroxide vapor probably represents the structure seen as a single rod-shaped body in protargol preparations. These may represent a multiple paired organelle. The electron micrographs of the sporozoites of E. nieschulzi (Colley, 1967) show structures probably representing paired organelles having more than two members, some of which appear to be branched. Bird and Baker (1963) were not able to identify a paired organelle in the sporozoites of E. stiedae observed with the electron microscope.

The transverse striations observed in the anterior end of sporozoites of E. utahensis have not previously been described and their nature and significance is unknown.

Eimeria utahensis differs from E. tenella (Horton-Smith and Long, 1963), and E. bovis and E. auburnensis (Hammond et al., 1967) by having

little glycogen in freshly excysted sporozoites. The sporozoites of E. utahensis might need a relatively small supply of glycogen. The kangaroo rat has a much shorter intestine than either the chicken or bovine; thus, the sporozoites of E. utahensis may spend less time in the intestinal lumen than do those of E. tenella, E. bovis, or E. auburnensis.

It would be interesting to speculate that the lack of glycogen is correlated with the possession of a large posterior refractile body. Todd and Hammond (1967a) did not find any PAS positive material in another sporozoite (E. callospermophili) which also has a large posterior refractile body. This would seem to substantiate the above interpretation. However, the sporozoites of E. larimerensis, which have a large posterior refractile body, contained PAS granules (Todd and Hammond, 1967b). Further studies are needed before any conclusions can be reached as to the relationship between stored reserve material and the possession of large refractile bodies.

The refractile bodies of E. utahensis were shown to be protein in nature, as were the refractile bodies of sporozoites of E. brunetti and E. acervulina (Pattillo and Becker, 1955) and E. tenella (Horton-Smith and Long, 1963). Pattillo and Becker (1955) also observed a weak reaction for lipids in the refractile bodies of E. acervulina, which was not present in sporozoites treated with a hot methyl alcohol-chloroform solution. E. utahensis refractile bodies gave a positive reaction for lipids but this reaction could not be eliminated with the methyl alcohol-chloroform solution. Schwalbach (1959), using an iodine staining technique, reported that the refractile bodies of several species of Isospora were paraglycogen. This observation has not been substantiated in any other

investigation. The chemical composition of the refractile bodies will probably have to be determined by more precise chemical means than was employed in this and other investigations.

The refractile bodies, often called refractile globules or eosinophilic globules, are characteristically present in the sporozoites of coccidia and have been reported to be present in developing first-generation schizonts in many species, including E. tenella (Tyzzer, 1929) and E. necatrix (Tyzzer, 1932) in the chicken, E. adenocoides (Clarkson, 1958) and E. meleagridis (Clarkson, 1959) in the turkey, E. nieschulzi and E. separata (Roudabush, 1937) in the rat, and E. bovis (Hammond et al., 1946) in cattle. Recently, the refractile bodies have been observed in trophozoites and/or schizonts grown in cell cultures in E. tenella (Patton, 1965), E. acervulina (Strout et al., 1965), E. meleagridis (Doran and Vetterling, 1967), and E. bovis (Fayer and Hammond, 1967). Roudabush (1937) stated that the globule found in first-generation schizonts of E. nieschulzi was evidently derived from the union of the two globules of the sporozoite, which occurred after the sporozoite had entered the cell. Hammond et al. (1946) reported that the spherical body found in young and intermediate schizonts of E. bovis broke up into two or more smaller bodies and finally disappeared during the later development of the schizont. They stated that this spherical body was probably composed of some kind of reserve material used in growth. Colley (1967) observed in electron micrographs of E. nieschulzi that some toxonemes are closely associated with the anterior paranuclear body (refractile body) and suggested on the basis of this finding that the refractile bodies may be secretory in nature. More work is needed to determine the function and

fate of the refractile bodies.

The findings concerning the nucleus of E. utahensis agree in general with those of other investigators (Pattillo and Becker, 1955; Ray and Gill, 1955; Cheissin, 1960; and Hammond et al., 1967) except that larger granules were present in the peripheral ring of chromatin.

In Giemsa-stained specimens the nucleus was surrounded by granules, which made the nucleus difficult to distinguish. These granules were also found in the anterior end; a few were also present in the posterior end. Granules of the same type were seen in bright-field, osmium vapor-fixed, and protargol specimens. The granules seen by the various methods are likely identical, but this is not certain. Colley (1967) found similar osmophilic bodies in electron micrographs of E. nieschulzi. Identification of these granules will have to await further electron microscope studies.

Eimeria mohavensis from Dipodomys panamintinus mohavensis is the only coccidian species of kangaroo rats other than E. utahensis for which the endogenous stages have been described (Doran and Jahn, 1952; Doran, 1953). The asexual stages of E. mohavensis were found in the small intestine and cecum; the sexual stages were in the cecum. The entire endogenous life cycle of E. utahensis took place in the small intestine of experimentally infected D. ordii. Doran (1953) described a small first-generation schizont and a larger second-generation schizont. On the basis of the theoretical yield of oocysts and the number actually obtained, Doran stated that there must be more than two generations of schizonts and merozoites. Since the small schizonts were present for a longer time than the large ones, he considered that the additional one or two generations

were probably of this type. E. utahensis had four distinct schizont generations, each of which were easily identifiable on the basis of the size of the schizont, the presence or absence of a residual body, the number of merozoites present, and the size of the merozoites.

Scholtyssek (1959) reported that as the gametocytes of Eimeria maxima grew, they were displaced from the epithelial layer into the mucosa, and, in a few cases, into the muscularis mucosa. The gametocytes of E. utahensis were displaced very early from the epithelial layer into the lamina propria, although a few of the parasites remained in the epithelial layer. Tyzzer (1929) observed that this same phenomenon occurred in the second generation schizonts of E. tenella.

Cheissin (1965) described microgametogony of E. magna and E. intestinalis in the rabbit. In the early stages, the nuclei were randomly arranged, but in later stages they moved to the periphery of the microgametocyte as well as around cytoplasmic masses, which were called cytomeres, where additional nuclear division took place. Microgametocytes of E. magna had several cytomeres; E. intestinalis usually had only one, and rarely two, of these cytomeres. At maturity the microgametes appeared in a whorl arrangement. Microgametogony of E. utahensis resembled that of E. magna except that the nuclei remained dispersed in the microgametocytes of E. utahensis for a longer period of time and the microgametes of E. utahensis lost their whorl arrangement when they were mature.

It is questionable whether Cheissin's use of the term cytomere in describing microgametogony in E. magna and E. intestinalis is correct (for a discussion of the term cytomere see Hammond, Ernst, and Miner, 1966) and the term compartments was used for these structures in E. utahensis.

The multinucleate appearance of the host cell containing gametocytes needs further investigation. The nuclei of infected cells became so abnormal in shape and size that study of serial sections or other methods would be necessary to determine whether in all cases the apparently separate nuclei are different portions of a single nucleus. The occurrence of young gametocytes in a host cell in which the nucleus is dividing may indicate that some of the cells apparently having two or more nuclei are in reality multinucleate. Infected dividing cells were not observed often enough to account for the many parasitized cells that were apparently multinucleate.

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## VITA

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