Growth of Eimeria alabamensis from Cattle In Vitro and the Changes in Fine Structure Associated with Schizogony

J. Robert Sampson
Utah State University

Follow this and additional works at: https://digitalcommons.usu.edu/etd

Part of the Biology Commons, and the Zoology Commons

Recommended Citation

This Dissertation is brought to you for free and open access by the Graduate Studies at DigitalCommons@USU. It has been accepted for inclusion in All Graduate Theses and Dissertations by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.
GROWTH OF *Eimeria alabamensis* FROM CATTLE IN VITRO

AND THE CHANGES IN FINE STRUCTURE ASSOCIATED

WITH SCHIZOGONY

by

J. Robert Sampson

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

DOCTOR OF PHILOSOPHY

in

Zoology

Approved:

Major Professor

Committee Member

Committee Member

Committee Member

Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

1970
ACKNOWLEDGMENTS

I extend my appreciation to Dr. John V. Ernst for providing the oocysts and to Mrs. Yoko Elsner for assistance in the tissue culture preparations. I wish to thank the members of my committee: Dr. Thomas L. Bahler, Dr. Paul B. Carter, and Dr. Rex S. Spendlove for their participation and assistance. To Dr. Hugh P. Stanley, who offered guidance and direction during the electron microscopy investigation, I am grateful. A sincere appreciation is extended to my major professor, Dr. Datus M. Hammond, who was most helpful during the experimental study and who offered an untiring effort during the writing.

A special thanks is offered Sue, Chris, and Susan, whose patience and moral support made this study more enjoyable.

The encouragement and assistance of Dr. Bronislaw M. Honigberg is gratefully appreciated.

J. Robert Sampson
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>REVIEW OF LITERATURE</td>
<td>2</td>
</tr>
<tr>
<td>Growth of <em>Eimeria</em> in Cell Cultures</td>
<td>2</td>
</tr>
<tr>
<td>Avian species of <em>Eimeria</em></td>
<td>2</td>
</tr>
<tr>
<td>Ruminant species of <em>Eimeria</em></td>
<td>9</td>
</tr>
<tr>
<td>Rodent species of <em>Eimeria</em></td>
<td>12</td>
</tr>
<tr>
<td>Fine Structural Aspects of Development in Tissue Culture</td>
<td>16</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>19</td>
</tr>
<tr>
<td>Electron Microscopy Techniques</td>
<td>21</td>
</tr>
<tr>
<td>RESULTS</td>
<td>24</td>
</tr>
<tr>
<td>Degree of Development in Various Cell Types</td>
<td>24</td>
</tr>
<tr>
<td>Sporozoites</td>
<td>27</td>
</tr>
<tr>
<td>Trophozoites</td>
<td>30</td>
</tr>
<tr>
<td>Sporozoite-shaped schizonts</td>
<td>30</td>
</tr>
<tr>
<td>Spheroidal schizonts</td>
<td>31</td>
</tr>
<tr>
<td>Merozoite formation</td>
<td>31</td>
</tr>
<tr>
<td>Host cell changes</td>
<td>34</td>
</tr>
<tr>
<td>Figures</td>
<td>35</td>
</tr>
<tr>
<td>Fine Structural Observations</td>
<td>41</td>
</tr>
<tr>
<td>Extracellular sporozoites</td>
<td>41</td>
</tr>
<tr>
<td>Intracellular sporozoites</td>
<td>41</td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS (Continued)

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trophozoites</td>
<td>46</td>
</tr>
<tr>
<td>Multinucleate spheroidal schizonts</td>
<td>49</td>
</tr>
<tr>
<td>Multinucleate sporozoite-shaped schizonts</td>
<td>50</td>
</tr>
<tr>
<td>Merozoite formation in spheroidal schizonts</td>
<td>51</td>
</tr>
<tr>
<td>Merozoite formation in sporozoite-shaped schizonts</td>
<td>52</td>
</tr>
<tr>
<td>Merozoites</td>
<td>54</td>
</tr>
<tr>
<td>Host cell changes</td>
<td>55</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>66</td>
</tr>
<tr>
<td>Fine Structural Observations</td>
<td>72</td>
</tr>
<tr>
<td>Sporozoites</td>
<td>72</td>
</tr>
<tr>
<td>Trophozoites</td>
<td>77</td>
</tr>
<tr>
<td>Multinucleate schizonts</td>
<td>80</td>
</tr>
<tr>
<td>Merozoite formation</td>
<td>81</td>
</tr>
<tr>
<td>Host cell changes</td>
<td>85</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>87</td>
</tr>
<tr>
<td>VITA</td>
<td>98</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table                                                                 Page
1. Average numbers of *Eimeria alabamensis* endogenous stages found daily after inoculation into cultures . . . . 25
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sporozoite which has just entered host cell. Note clear pathway in cell cytoplasm (BEint, 6 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>2.</td>
<td>Intracellular sporozoite (BEint, 24 hours). X1280</td>
<td>38</td>
</tr>
<tr>
<td>3.</td>
<td>Four sporozoites around the host cell nucleus (MDBK, 4 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>4.</td>
<td>Intracellular sporozoite with refractile granules forming at the surface of refractile body (MDBK, 48 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>5.</td>
<td>Sporozoite with anterior and posterior refractile bodies (MDBK, 48 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>6.</td>
<td>Trophozoite with enlarged nucleus and nucleolus (BEint, 48 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>7.</td>
<td>Schizont with two nuclei in binucleate host cells (BEint, 48 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>8.</td>
<td>Schizont with five visible nuclei and large refractile body (MDBK, 48 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>9.</td>
<td>Schizont of small type in early stage of merozoite formation (BEint, 48 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>10.</td>
<td>Schizont of small type in intermediate stage of merozoite formation (MDBK, 48 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>11.</td>
<td>Schizont of small type, with immature merozoites, each having a nucleus and a refractile body (MDBK, 72 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>12.</td>
<td>Schizont of small type with merozoites attached to a multinucleate central residual body (MDBK, 72 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Schizont of small type, with nearly mature merozoites, each with a prominent anterior refractile body (MDBK, 72 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>14.</td>
<td>Mature schizont of small type with merozoites arranged in parallel in a degenerating host cell (MDBK, 72 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>15.</td>
<td>Free merozoite of long type (MDBK, 72 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>16.</td>
<td>Sporozoite-shaped schizont with cytoplasmic outpocketings (arrows) (BEint, 24 hours). X1000</td>
<td>38</td>
</tr>
<tr>
<td>17.</td>
<td>Binucleate sporozoite-shaped schizont with one nucleus in lateral outpocketing (arrow) (BEint, 24 hours). X1000</td>
<td>40</td>
</tr>
<tr>
<td>18.</td>
<td>Intermediate schizont of large type in early stage of merozoite formation (MDBK, 48 hours). X1000</td>
<td>40</td>
</tr>
<tr>
<td>19.</td>
<td>Schizont of large type with nearly mature merozoites (BEint, 48 hours). X1000</td>
<td>40</td>
</tr>
<tr>
<td>20.</td>
<td>Schizont of large type with developing merozoites attached to a central residual body (MDBK, 48 hours). X1000</td>
<td>40</td>
</tr>
<tr>
<td>21.</td>
<td>Free merozoite of short type with anterior and posterior refractile bodies (BEint, 48 hours). X1000</td>
<td>40</td>
</tr>
<tr>
<td>22.</td>
<td>Large schizont with nearly mature merozoites, some of which have two nuclei (arrows) or a dividing nucleus (MDBK, 72 hours). X1000</td>
<td>40</td>
</tr>
<tr>
<td>23.</td>
<td>Sporozoite entering a host cell nucleus. Note constriction of sporozoite body at site of entrance (MDBK, 24 hours). X1280</td>
<td>40</td>
</tr>
<tr>
<td>24.</td>
<td>Intranuclear sporozoite (MDBK, 48 hours). X1000</td>
<td>40</td>
</tr>
<tr>
<td>25.</td>
<td>Intranuclear trophozoite (MDBK, 48 hours). X1000</td>
<td>40</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>26.</td>
<td>Intranuclear multinucleate schizont (MDBK, 72 hours).</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27.</td>
<td>Intranuclear schizont with immature merozoites (MDBK, 72 hours).</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28.</td>
<td>Four second-generation trophozoites within a host cell nucleus (MDBK, 96 hours).</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29.</td>
<td>Longitudinal section of sporozoite (5 minutes). Note numerous polysaccharide granules and absence of nucleolus.</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30.</td>
<td>Longitudinal section of anterior end of sporozoite (24 hours). Note annuli (arrows) and spirally arranged fibrillar substructure of conoid (arrow).</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31.</td>
<td>Longitudinal section of sporozoite (24 hours) with enlarged nucleus, nucleolus, and active micropore.</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32.</td>
<td>Longitudinal section of anterior portion of sporozoite (48 hours). Note position of anterior organelles.</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33.</td>
<td>Longitudinal section of trophozoite (48 hours). Note anterior organelles and elongate nucleus with centrioles.</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34.</td>
<td>Transverse section of centrioles (48 hours), each with nine peripheral tubules and one central tubule.</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35.</td>
<td>Longitudinal section of an active micropore of a sporozoite (24 hours).</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36.</td>
<td>Longitudinal section of an active micropore of a sporozoite (24 hours) with enlarged inner portion.</td>
<td>59</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>37</td>
<td>Longitudinal section of an active micropore of a sporozoite (24 hours) with elongation of inner portion and an adjacent food vacuole. X52,000</td>
<td>59</td>
</tr>
<tr>
<td>38</td>
<td>Longitudinal section of an enlarged micropore and a food vacuole in a sporozoite (24 hours). X30,000</td>
<td>59</td>
</tr>
<tr>
<td>39</td>
<td>Transverse section of a trophozoite (48 hours) with apparently dividing Golgi complex and enlarged nucleus. X20,000</td>
<td>59</td>
</tr>
<tr>
<td>40</td>
<td>Longitudinal section of trophozoite (48 hours) with elongate nucleus and partial disappearance of inner membrane. X22,000</td>
<td>59</td>
</tr>
<tr>
<td>41</td>
<td>Partial section of trophozoite (48 hours) with centrioles and thickened inner membrane (arrow). X24,000</td>
<td>59</td>
</tr>
<tr>
<td>42</td>
<td>Oblique section of trophozoite (48 hours) with several refractile bodies and extension of inner membrane into the interior. X16,000</td>
<td>61</td>
</tr>
<tr>
<td>43</td>
<td>Section of binucleate schizont (48 hours) with two refractile bodies and a single limiting membrane over most of the surface. X16,000</td>
<td>61</td>
</tr>
<tr>
<td>44</td>
<td>Longitudinal section of sporozoite-shaped schizont (48 hours). Note the three nuclei and anterior organelles. X15,000</td>
<td>61</td>
</tr>
<tr>
<td>45</td>
<td>Longitudinal section of transforming sporozoite-shaped schizont (48 hours). Note the presence of two nuclei and the partial disappearance of inner membrane and anterior organelles. X17,500</td>
<td>61</td>
</tr>
<tr>
<td>46</td>
<td>Partial section of schizont (60 hours) with conoid adjacent to thickened inner membrane. X24,000</td>
<td>63</td>
</tr>
<tr>
<td>47</td>
<td>Longitudinal section through multinucleate schizont (60 hours) showing merozoite anlagen and inner membrane extensions. X20,000</td>
<td>63</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Magnification</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>48</td>
<td>Longitudinal section of sporozoite-shaped schizont (60 hours). Note slender immature merozoites. X17,500</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>Stage in transformation of sporozoite-shaped schizont to spheroidal schizont (60 hours). Note the two immature merozoites. X16,000</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>Partial section of schizont (72 hours) with stubby merozoites forming at surface. X20,000</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td>Multinucleate schizont (72 hours) with immature stubby merozoites growing outward at surface. X20,000</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>Schizont with forming merozoites of slender type (72 hours). X17,250</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>Longitudinal section of three mature merozoites of slender type (72 hours). X10,000</td>
<td></td>
</tr>
</tbody>
</table>
ABSTRACT

Growth of *Eimeria alabamensis* from Cattle in vitro and the Changes in Fine Structure Associated With Schizogony

by

J. Robert Sampson, Doctor of Philosophy

Utah State University, 1970

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

Monolayer primary cultures of cells from bovine embryonic intestine (BEInt), kidney (BEK), spleen (BES), and thyroid (BET) and cell line cultures of embryonic bovine trachea (EBTr) and synovium (BESy) as well as established cell line cultures of Madin-Darby bovine kidney (MDBK), human intestine (Int 407), and Syrian hamster kidney (BHK-21) were inoculated with freshly excysted sporozoites of *Eimeria alabamensis* and observed for 4 days. Sporozoites penetrated all cell types during the first 24 hours in culture. Numerous intracellular sporozoites, trophozoites, and binucleate schizonts were seen in all cell cultures except Int 407. The best development occurred in BES and MDBK cells. Mature schizonts were first found at 2 days after inoculation in all cell types except for BHK-21 and Int 407 cells in which they were first observed at 3 days. Large, 14.2 microns (11-18-5) by 10.2 microns (8.5-11), schizonts with 6-15 short, stubby merozoites, each with
two refractile bodies, were found at 2 and 3 days in all cells except BESy, Int 407, and BHK-21. Small, 9.7 microns (5.5-13) by 6 microns (5-8.5), highly refractile, compact schizonts with 6-10 long, slender merozoites, each with two refractile bodies, were found at 3 days after inoculation in all cell types. All merozoites had left the schizont by the end of the third day in culture; few parasites were seen thereafter.

Intracellular merozoites were found at 4 days. Some second-generation trophozoites were seen in host cell nuclei. Development within the host cell nucleus, the normal development site in the host animal, was observed infrequently in cell cultures. Intranuclear sporozoites were not seen until 2 days after inoculation. Thereafter, developmental stages similar to those occurring in the cytoplasm were observed in the nucleus. Small intranuclear schizonts, having 6-10 merozoites each with two refractile bodies, were found at 3 days.

Freshly excysted sporozoites and intracellular stages, found from 5 minutes to 72 hours after inoculation of sporozoites into cultured cells, were studied with the electron microscope.

Extracellular sporozoites were similar to intracellular sporozoites observed 5 minutes after inoculation. The numerous polysaccharide granules present in these disappeared during the first day of intracellular existence. Some sporozoites transformed into trophozoites which ultimately lost the inner membrane and anterior organelles; others retained these organelles and became sporozoite-shaped schizonts with
2-4 nuclei. Centrioles, each with nine peripheral tubules and one central
tubule, were observed in association with intranuclear spindle fibers in
dividing nuclei within trophozoites or schizonts. The sporozoite-shaped
schizonts later transformed into spheroidal schizonts which gradually
lost the inner membrane and anterior organelles.

Merozoite formation occurred in two ways. In sporozoite-shaped
schizonts, anlagen of merozoites appeared in the interior of the cell.
Extensions of the inner membrane of the parent cell penetrated deep into
the cytoplasm. Such internal membranes evidently participated in isolat­
ing the immature merozoite from the cytoplasm of the schizont, along
with infoldings of the surface membrane of the schizont. Merozoites
formed in this manner were long and slender.

Spheroidal schizonts formed by trophozoites usually had a single
surface membrane with inner membrane present only in various small
areas about the periphery of the cell. Conoids were found beneath the
surface membrane and adjacent to the nucleus. In later stages, de­
veloping merozoites grew outward from the surface of the schizont, and
infoldings of the limiting membrane of the schizont occurred at the
margins of each. Such merozoites appeared short and stubby.
Eimeria alabamensis Christensen, 1941, from cattle, is one of three species of Eimeria known to develop in the natural host entirely within the host cell nucleus. It is considered to be pathogenic only when large numbers of oocysts are given to host animals (Davis, Boughton, and Bowman, 1955). Relatively small mature schizonts occur as early as 3 days after inoculation, and the prepatent period averages 8.6 days (Davis, Bowman, and Boughton, 1957). Ruminant species of Eimeria studied in cell culture thus far have relatively large first-generation schizonts which require 10 days or more to mature (Fayer and Hammond, 1967; Clark and Hammond, 1969; Kelley and Hammond, 1970). The relatively rapid rate of development and the occurrence of small, intranuclear schizonts indicated that E. alabamensis would provide favorable material for an in vitro study.

The objectives of this study were:

1. To study the developmental characteristics of the endogenous stages of E. alabamensis in cell culture.

2. To determine the fine structural aspects of development of E. alabamensis in cell culture and the effect of the parasite upon the host cell.

3. To relate the results of this study to those obtained with other species of Eimeria.
REVIEW OF LITERATURE

Growth of Eimeria in Cell Cultures

Several of the intracellular species of sporozoa have been successfully cultivated during the last 25 years. Excellent reviews of this work have been published recently. Pipkin and Jensen (1958) and Trager and Krassner (1967) have discussed cultivation of parasitic protozoa with special emphasis on the malaria parasites and Toxoplasma. Taylor and Baker (1968) have also reviewed the cultivation of the avian and mammalian species of Plasmodium. Pipkin (1960) reviewed the literature concerning the parasitic protozoa other than Plasmodium which have been grown in vitro. Literature on the cultivation of Besnoitia, Sarcocystis, and Theileria has recently been reviewed by Fayer (1968).

Avian species of Eimeria

Although an attempt was made to cultivate schizonts of Gastrocytis gilruthi as early as 1925 (Triffitt, 1925), successful cultivation of an Eimeria species was not reported until 1965. Patton (1965) was able to observe development of the endogenous stages of Eimeria tenella, a cecal parasite of chickens, through the first asexual generation in monolayer cultures of avian and mammalian cells. Mature merozoites developed in secondary and established cultures of bovine kidney cells, as well as secondary cultures of fibroblast-like cells of embryonic
tissues from Japanese quail. Schizonts were demonstrated in fibroblasts from 9-day chick embryos and L cells.

Strout et al. (1965) were able to demonstrate infection by sporozoites of *Eimeria acervulina* in HeLa, human amnion, chick kidney, chick fibroblast, and mouse fibroblast cell cultures. Development proceeded to the trophozoite stage with evidence of some schizogony.

In that same year, Long (1965) was able to demonstrate the entire life cycle of *Eimeria tenella* after inoculation of sporozoites into the chorioallantoic membranes of 10-day chick embryos. Histological sections showed the presence of schizonts 4-7 days after inoculation and free merozoites at 6-7 days post-inoculation. Schizonts, believed to be those of the second-generation, were observed on days 8 and 9. Throughout days 7-11, gametocytes and oocysts were found; later, oocysts were noticed free in the allantoic fluid. Intravenously injected sporozoites did not initiate infections in these embryos.

Long (1966) later compared the development of various species of chicken coccidia after inoculation of the respective sporozoites into the chorioallantoic membrane of the chick embryo. The endogenous life cycles of *Eimeria mivati*, *E. brunetti*, and *E. tenella* were completed in the chick embryo, and after sporulation of the resulting oocysts these oocysts were capable of initiating normal infections in chicks. *Eimeria necatrix* developed to nearly mature merozoites, whereas *E. acervulina* and *E. maxima* did not develop. Long (1966) also attempted, unsuccessfully, to infect the chorioallantoic membranes of chick and turkey
embryos with the rabbit coccidium, *Eimeria stiedae*. *Eimeria tenella* sporozoites did not develop in turkey and quail embryos.

Doran and Vetterling (1967b) used secondary cultures of bovine kidney cells to demonstrate the completion of one asexual generation and possibly a part of another by *Eimeria meleagrimitis*, a turkey coccidium. Development did not take place in primary cultures of bovine and porcine and secondary porcine cells.

Doran and Vetterling (1967a) reported the cultivation of two species of poultry coccidia in mammalian cell cultures. Mature schizonts of *Eimeria meleagrimitis* were demonstrated in secondary bovine embryonic cells, but no development was noted in ovine, porcine, or human cells. *Eimeria necatrix* developed to mature schizonts in bovine kidney cells, and immature schizonts were found in porcine kidney cells. No development occurred in ovine or human kidney cell cultures.

Bedrnik (1967b) used second-generation merozoites of *Eimeria tenella*, obtained from the ceca of infected chicks, to initiate infections in monolayer cultures of HeLa and chick embryo fibroblasts. Heavy infections followed these inoculations, and development of third-generation merozoites was noted in both cell types. These third-generation merozoites did not escape from the host cells.

In further studies, Bedrnik (1967a) noted that development of sexual stages and a few oocysts resulted from inoculation of second-generation merozoites into monolayers of chick embryo cells. Sexual
stages were confined to islands of epithelial cells and were not found in adjacent fibroblast cells.

Doran and Vetterling (1968) reported that *Eimeria meleagritmitis* developed better in cell line cultures of embryonic bovine kidney than embryonic turkey intestine. All cultures were incubated at 40.6 and 43 C alternating at intervals of 12 hours. Schizonts in embryonic bovine kidney cells were larger and had more merozoites which ultimately escaped and penetrated new host cells. This was not evident in cultures of turkey intestine cells. These authors reported greater survival of parasites up to 96 hours, more numerous trophozoites and larger schizonts with greater numbers of merozoites than in their previous investigation with this parasite.

Strout and Ouellette (1969) reported the first successful attempts to obtain sexual stages of a species of *Eimeria* in monolayer cell culture using sporozoites as inoculum. Sporozoites of *Eimeria tenella*, inoculated into primary cultures of embryonic chick kidney, developed into mature gametes within 160 hours.

Strout, Ouellette, and Gangi (1969) demonstrated that inoculation of 500,000 sporozoites of *Eimeria tenella*, followed by a washing 1 hour later, allowed the optimum asexual development of this parasite in cultures of chick embryo kidney cells. Similar results were obtained with an inoculum size of 100,000 sporozoites without subsequent washing or medium change. Mature schizonts were most numerous at 96 hours after inoculation.
Matsuoka, Callender, and Shumard (1969) reported that optimum development of the first asexual generation of *Eimeria tenella* in embryonic bovine trachea cell lines resulted from inoculation of 100,000 sporozoites per ml without subsequent washing. Mature schizonts were most numerous at 72 hours after inoculation.

Bedrnik (1969) compared the capacity for invasion and development of sporozoites and merozoites of *Eimeria tenella* inoculated into cultures of 10-13-day chick embryo cells. Merozoites of the second-generation were found to penetrate host cells as quickly as, but in smaller numbers, than sporozoites. Nevertheless, merozoites developed further and in larger numbers than sporozoites; further advanced asexual generations and sexual stages were found. An unknown growth factor, introduced into the cultures with the inoculum of merozoites harvested from infected chick ceca, was assumed to be responsible for asexual development.

Shibalova (1969) demonstrated complete development of sporozoites of *Eimeria tenella* and *E. brunetti* after inoculation of these into the allantoic cavity of 7-12-day chick embryos. Gametocytes and oocysts were found in the allantoic fluid and chorioallantois 5-10 days after sporozoite inoculation and 2-7 days after merozoite administration. Oocysts were capable of initiating infection in the normal host after sporulation. Sporozoites of *Eimeria tenella* developed through mature first-generation schizonts as early as 96-120 hours in cultures of chicken fibroblasts, embryonic chick kidney cells, human kidney cells, guinea pig and monkey kidney cells, as well as HeLa cells.
Shibalova and Korolev (1969) reported development of *Eimeria tenella* sporozoites in chick fibroblast cultures incubated at 40-41 °C. Mature first-generation schizonts were observed after 96-150 hours in culture. Further development did not occur.

Long (1969) was successful in obtaining completion of the life cycle of *Eimeria tenella* in cultured chorioallantoic membranes (CAM) previously infected with sporozoites. Various types of schizonts were found, and these were correlated with the stage of the infection at the time the CAM cultures were begun. Gametocytes and infective oocysts were found only in those cultures of CAM cells initiated from embryos infected 4-6 days earlier. The most numerous stages of development were found in dense areas of epithelial-like cells.

Fayer (1969) observed the changes in the retractile bodies of *Eimeria tenella*, *E. adenoeides*, and *E. meleagrimitis* sporozoites in cultures of bovine kidney cells. In those sporozoites with anterior and posterior retractile bodies, the anterior body moved posteriad and ultimately merged with the posterior retractile body. Numerous finger-like projections were found at the periphery of the retractile bodies.

Doran (1970a) demonstrated for the first time in monolayer cell cultures the development of sporozoites to infective oocysts. *Eimeria tenella* sporozoites inoculated into cell cultures of embryonic chick kidney and nonembryonic chick kidney developed into gametocytes and oocysts only in areas of cell aggregates. The sexual stages developed earlier and in larger numbers in nonembryonic chick kidney cells.
Oocysts were found as early as 7 days. These were later sporulated and fed to a previously uninfected chicken, and an infection yielding 15,000,000 normal oocysts resulted.

Doran and Vetterming (1969b) demonstrated that the length of time during which oocysts of *Eimeria meleagrimitis* were stored, although not affecting excystation to any great extent, had a pronounced effect upon the development of sporozoites in cell cultures of embryonic bovine kidney. In oocysts stored 53-60 weeks, sporozoites escaped but these disintegrated upon emerging from the sporocyst or died shortly thereafter.

Doran (1970b) used inocula of 100,000; 250,000; 500,000; and 1,000,000 sporozoites of *Eimeria adenoeides* to determine the effect of debris on the development of this parasite in cultures of embryonic bovine kidney cells. Five hours after inoculation, numbers of intracellular forms were directly proportional to inoculum size; however, fewer parasites were found in cultures inoculated with uncleaned suspensions of sporozoites. By 48 hours, numbers of schizonts in cultures inoculated with cleaned sporozoite suspensions were proportional to inoculum size while cultures with uncleaned suspensions showed an increase in the number of schizonts not proportional to inoculum size. Between 5 and 48 hours in culture, there was a greater loss of intracellular parasites in cultures inoculated with uncleaned sporozoites than in those inoculated with cleaned sporozoites.

Bedrmik (1970a) in his search for a stimulating growth factor concluded that neither the temperature (37 or 40 °C) nor the medium in which
merozoites were suspended influenced the capacity for development. Calf and horse sera worked equally well. The best development occurred in cells derived from the intestine and liver of 17–19-day chick embryos. Second-generation merozoites developed best when inoculated into cultures with cells from the inflamed cecum of the infected chicken. An unknown factor, stimulating development of the parasite, is apparently present in these cells.

Bednik (1970b) demonstrated that the third-generation merozoites, developed in cell cultures inoculated with second-generation merozoites, could be transferred by subculturing a fresh tissue. This technique resulted in the development of fourth-generation merozoites and sexual stages. More second-generation merozoites, obtained from infected ceca, developed when collected by a trypsin digestion technique than when obtained by a phosphate buffered saline wash.

**Ruminant species of Eimeria**

The first report of the development of a mammalian species of *Eimeria* in cell cultures was that of Fayer and Hammond (1966). Sporozoites of *Eimeria bovis*, inoculated into monolayer cultures of embryonic spleen, completed one asexual generation. Bovine embryonic kidney supported development to the immature schizont stage.

Hammond and Fayer (1968) and Fayer and Hammond (1967) described the active penetration of host cells by *Eimeria bovis* sporozoites in monolayer cultures of embryonic bovine cells. First-generation
schizonts developed in bovine kidney, spleen, and thymus cell line cultures. Development in primary cultures of bovine intestine and testicle cells was limited to multinucleate and binucleate schizonts, respectively. Cell-free media was found capable of maintaining motile sporozoites for at least 21 hours. Sporozoites were inoculated into established cell line cultures of bovine kidney (Madin-Darby, 1958), human intestine (int 407), mouse fibroblast (L cells), as well as bovine tracheal cell line cultures. Mature first-generation schizonts were demonstrated in all cell types but the L cells. In the latter, only a few sporozoites developed into trophozoites. Mature schizonts were most numerous, and development was found to be optimum in the bovine trachea cells.

Fayer and Hammond (1969) described the morphological changes occurring in the sporozoites of *Eimeria bovis* during the first 24 hours after inoculation into cell cultures. The gradual disappearance of the anterior refractile body along with changes in shape of the posterior refractile body was reported. The posterior refractile body was retained during development of the schizonts, and it was suggested that this organelle may be used in some way in the intracellular existence of the parasite.

Hammond, Fayer, and Miner (1969) were unsuccessful in their attempts to obtain further development of *Eimeria bovis* first-generation merozoites, obtained from cell cultures and infected calves and inoculated into cultures of 11 cell types. One calf, given merozoites from cell
cultures, was found to have gametocytes 4 days after the merozoites were introduced into a ligated cecum.

The development of *Eimeria auburnensis* sporozoites through the first-generation schizonts was reported by Clark and Hammond (1969). In cultures of embryonic bovine trachea and spleen cell lines and Madin-Darby (MDBK) and established embryonic human intestine cell lines, inoculated sporozoites and sporozoite-shaped schizonts were observed to undergo lateral outpocketings during transformation into trophozoites or schizonts. This species was the first to be described as having a sporozoite-shaped schizont in the host animal (Chobotar, Hammond, and Miner, 1969). This was also observed in cell cultures. Mature schizonts, somewhat smaller than those found in the calf, were seen as early as 9 days after inoculation.

Development of an intranuclear parasite of cattle, *Eimeria alabamensis*, was demonstrated by Sampson and Hammond (1969) in secondary cultures of embryonic bovine trachea and synovial cells, MDBK, Syrian hamster (BHK-21), embryonic bovine trachea (BETr), and Int-407. Approximately 1-4 per cent of intracellular parasites were found in the nucleus of the host cell after 2 days in culture. Schizonts appeared to be of two types: a larger type usually found at 2 days in embryonic bovine trachea cells had short stubby merozoites, and a smaller type observed at 3 days in MDBK cells had long slender merozoites. Some merozoites penetrated new host cells, and trophozoites of the second-generation were found at 4 days.
The development of sporozoites of the ovine coccidium, *Eimeria ninakohlyakimovae*, in various types of ovine and bovine cells was reported by Kelley and Hammond (1970). The sporozoites actively penetrated host cells. Trophozoites, formed by lateral outpocketing or widening of the sporozoite, were seen as early as 3 days after inoculation in culture. The maximum numbers of mature schizonts, which had thousands of merozoites, represented about 0.4-4.3 per cent of the maximum number of intracellular sporozoites which were observed earlier. Merozoites were formed by radial budding from the blastophores. Merozoites were not seen penetrating new host cells.

Speer and Hammond (1970a) briefly reported on the development of *Eimeria ellipsoidalis* in various cell types. Crescent bodies were seen associated with some stages. Spheroidal schizonts with 18-42 nuclei were found as early as 5 days after inoculation of sporozoites. Migration of nuclei to the periphery of the schizont and an elevation of the surface membrane were associated with merozoite formation. Mature first-generation schizonts were slightly larger but contained fewer merozoites than those reported from calves. An inoculum size of 300,000 per tube was found to be deleterious to development.

**Rodent species of Eimeria**

Recently, the development in cell cultures of several species of *Eimeria* from the Uinta ground squirrel has been studied. Speer and Hammond (1969) and Speer, Hammond, and Anderson (1970) reported on
the development of *Eimeria callospermophili* and *E. bilamellata* in cell cultures of bovine, ovine, and ground squirrel origin. Mechanically released intracellular sporozoite-shaped schizonts were observed to penetrate new host cells after vigorous movement in the medium. Merozoites, formed by peripheral budding, were seen at 15 hours after inoculation of *E. callospermophili* sporozoites and at 4 days in *E. bilamellata*. Immature schizonts of the second generation were observed 44 hours after inoculation of sporozoites of *E. callospermophili* into primary cells of minced whole embryos of ground squirrels.

Speer and Hammond (1969), in a cinemicrographic study, recorded the characteristic movements of sporozoites in cell cultures and their penetration of and development within the host cells. Merozoites were observed escaping from host cells, gliding in circular pathways and penetrating new host cells.

Hammond, Speer, and Roberts (1970) reported the occurrence of refractile bodies in first-generation merozoites of *Eimeria callospermophili* and *E. bilamellata* in cell cultures. Sporozoites in cell cultures developed into sporozoite-shaped schizonts and ultimately became spheroidal schizonts. Within the spheroidal schizont, the refractile bodies were smaller and more numerous than in earlier stages. As merozoites budded radially, an anterior refractile body and later a posterior one were incorporated into each.

A detailed account of nuclear division and refractile body changes in developing *Eimeria callospermophili* sporozoites and schizonts was
given by Speer and Hammond (1970b). As nuclear division commenced, 8-10 hours after inoculation of sporozoites, the nucleolus and nucleus became enlarged and assumed first an ellipsoid shape and then a dumbbell shape before separating into daughter nucleoli and nuclei, respectively. During the early period of intracellular existence, the anterior refractile body decreased in size as small granules formed at its periphery, and finally it disappeared. Later, the posterior body decreased in size and subdivision into several smaller bodies took place about the time of transformation into the spheroidal schizont.

That motility of schizonts can be brought about by a bile-salt stimulus was demonstrated by Speer, Hammond, and Kelley (1970). Mature schizonts and free merozoites of *Eimeria callospermophili*, *E. larimerensis*, *E. bilamellata*, as well as *E. nieschulzi* and *E. ninakohlyakimovae* in cell line cultures of embryonic bovine intestine, kidney, and liver were exposed to bile or a bile salt, or one of these in combination with trypsin. The merozoites, within mature schizonts of all of these species but *E. ninakohlyakimovae*, were stimulated to move; the merozoites escaped and some actively penetrated new host cells. The free merozoites of all species had increased motility in preparations with bile or a bile salt.

Hammond (1969) recently reviewed the findings of studies concerned with the cultivation of three ruminant coccidia. All three, *Eimeria bovis* and *E. auburnensis* from cattle and *E. ninakohlyakimovae* from sheep, have sporozoites which exhibit flexing, gliding, pivoting,
and probing movements before penetration of the host cell. In all of these species, trophozoites and schizonts are associated with a crescent body of unknown function located within the parasitophorous vacuole. As schizonts of *E. auburnensis* mature, the single layer of nuclei, lying on the periphery, forms numerous cylindrical invaginations toward the interior of the schizont. Such invaginations are narrower in diameter in *E. bovis* and *E. ninakohlyakimovae* schizonts and give rise to blasto­phores. Formation of merozoites is associated with, and possibly induced by, an intranuclear fiber apparatus.

Studies of cultivation of *Eimeria* species were reviewed by Speer and Hammond (1970a). Sporozoites of various species of *Eimeria* from various host animals were observed to follow one of two routes of development in monolayer cultures. *Eimeria bovis*, *E. auburnensis*, and *E. alabamensis* from cattle; *E. ninakohlyakimovae* from sheep; and *E. nieschulzi* from the rat all developed through typical trophozoites to mature schizonts. *Eimeria callospermophilii* and *E. bilamellata* from the ground squirrel and *E. auburnensis* and *E. alabamensis* from cattle, at least occasionally, developed sporozoite-shaped schizonts instead of trophozoites. In some species, the sporozoite-shaped schizonts were observed leaving the host cells and penetrating new host cells. The most rapid development occurred in *Eimeria callospermophilii* and *E. larimerensis*; these required only 15 hours and 24 hours, respectively, to reach the mature schizont stage. Schizonts of *E. bovis*, *E. auburnensis*, and *E. ninakohlyakimovae* were usually large and had
thousands of merozoites, whereas the remaining species studied had 4-65. Merozoites with refractile bodies were seen in *E. alabamensis*, *E. callospermophili*, *E. bilamellata*, and *E. larimerensis*. Partial development of the second-generation schizonts was observed in *Eimeria alabamensis*, *E. larimerensis*, and *E. callospermophili*.

**Fine Structural Aspects of Development in Tissue Culture**

A study of *Eimeria bovis* sporozoites in cell cultures was briefly reported by Sheffield, Fayer, and Hammond (1968). Such structures as a limiting membrane associated with a second inner membrane, sub-pellicular microtubules, microneme, conoid, numerous paired organelles, a nucleus with a prominent nucleolus, Golgi apparatus, refractile bodies, micropore, mitochondria with sac-like cristae, and osmiophilic spherical structures were present. These structures are typical of extracellular sporozoites also, as reported by Roberts and Hammond (1970).

Scholtyseck and Stout (1968) reported on the fine structure of *Eimeria tenella* sporozoites, including the motile and the intracellular forms. They observed that in trophozoites and young schizonts the micropores were enlarged and were ingesting particulate matter to form food vacuoles. The material ingested included pieces of host cell cytoplasm which were found in the surrounding parasitophorous vacuole. After 96 hours in culture, parasites possessed many vacuoles containing osmiophilic granules believed to be derived from host cell cytoplasm.
Scholtyszek (1969) described the interrelationship between *Eimeria tenella* from chickens and the infected host cell in vivo and in vitro. A parasitophorous vacuole was observed around the parasite; this vacuole was later limited by a membrane of host cell origin. The host evidently attempts to digest the parasite, but this attempt is usually unsuccessful. Micropores observed in the sporozoites were thought to function in ingesting the host cell cytoplasm. Numerous vesicles assumed to be food vacuoles were distributed in the sporozoite cytoplasm.

Roberts et al. (1969) reported the absence of labelled thymidine in the nuclei of *Eimeria callospermophili* stages grown in cell cultures of bovine intestine, in contrast to a definite uptake by the nuclei of host cells. This suggests that *E. callospermophili* in cultures does not utilize free thymidine during development through the first-generation of merozoites.

Roberts et al. (1970) described the fine structure of the stages of schizogony of *Eimeria callospermophili* in cell cultures and in tissues of ground squirrels. Changes in the refractile bodies and mitochondrial swelling were observed in sporozoite-shaped schizonts. An intranuclear fiber apparatus with associated polar cones was seen; anlagen of merozoites, including inner membrane; conoid; and subpellicular microtubules, were closely associated with these nuclear structures. In the later stages of its development, each merozoite had two refractile bodies. A Golgi apparatus and nucleus were incorporated into each merozoite.

Roberts, Hammond, and Speer (1970) observed several fine
structural features in the sporozoites of *Eimeria callospermophilii* which had not been described previously. Two anterior polar rings, a posterior pore, a posterior annulus, small amylopectin granules surrounding the refractile bodies, and a fibrillar organelle were described. These features were noted in both intracellular and extracellular sporozoites. The membrane lining the parasitophorous vacuole appeared to be synthesized *in situ* after penetration of the host cell by the sporozoite.
MATERIALS AND METHODS

Trypsin-dispersed cell suspensions were prepared as reported previously (Fayer and Hammond, 1967). Cells were suspended in Eagle's minimum essential medium (MEM), containing 15 per cent fetal calf serum with 50 units of penicillin G and 50 μg/dihydrostreptomycin per ml. Concentrated cell suspensions, seeded into Leighton tubes containing 11 x 40 mm coverslips, were incubated at 37 C; cells were normally confluent within 3-4 days.

The terminology used herein is that of the Tissue Culture Association (Federoff, 1967). Bovine embryonic thyroid (BETY), kidney (BEK), and spleen (BES) were used as primary cultures only. Bovine embryonic intestine (BEInt) was used as primary cultures and also as cell line cultures after being cultured in 32-ounce Brockway prescription bottles. Bovine embryonic synovial cells (BESy) were used only as cell line cultures. The established lines of Madin-Darby bovine kidney (MDBK), embryonic human intestine (Int 407), as well as the cell line of embryonic bovine trachea (EBTr) were obtained from the American Type Culture Collection cell repository in Rockville, Maryland. The Syrian hamster kidney established cell line (BHK-21) was obtained from Dr. Rex S. Spendlove, Department of Bacteriology, Utah State University. The established lines and cell lines were treated as described earlier (Fayer and Hammond, 1967).
Oocysts of *Eimeria alabamensis* were obtained from fecal samples collected from experimentally infected calves. The samples were mixed with water, strained through a series of sieves, concentrated by sedimentation, and suspended in 2.5 per cent potassium dichromate solution. Sporulation of the oocysts was induced by constant aeration of the solution for 2 weeks at room temperature (18-24°C) (Ernst, Cooper, and Frydendall, 1970). The sporulated oocysts were then stored at 4°C until used. Oocyst suspensions did not contain any species other than *E. alabamensis* and were less than 6 months old at the time of use. The oocysts were cleaned and sterilized according to the methods of Fayer and Hammond (1967) and Hammond, Chobotar, and Ernst (1968). The oocysts were then ground for 3-5 minutes with a teflon-coated tissue grinder, and the sporocysts were washed by repeated centrifugation to eliminate any toxic materials which may have been released by rupturing the oocysts. The cleaned pellet of sporocysts and oocyst walls was resuspended in a solution of 0.25 per cent trypsin (1:300, Nutritional Biochemicals) and 1 per cent sodium taurocholate in Saline A, at pH 7.5, and placed in a 37°C water bath for approximately 30-40 minutes. The resulting freed sporozoites were washed once with serum-free MEM, counted with a hemacytometer, and seeded into Leighton tubes at approximately 250,000 to 300,000 per 1.5 ml of medium. After 4-6 hours of incubation at 37°C, the medium was replaced with fresh MEM containing 3 per cent fetal calf serum.

Four experiments were conducted for each cell type mentioned.
above except for primary cells which were tested three times each. Sufficient tubes were used in each experiment so that, at each of the examinations, one tube was used for phase-contrast observation, one for fixation and staining, and one as a control. Observations on living material were made with double coverslip preparations and phase-contrast microscopy 4 hours after inoculation and then at 24-hour intervals for 4 days. Monolayers were fixed in Schaudinn's fluid and stained with iron hematoxylin, PAS-AO, or Gomori's modified trichrome stain. Measurements were made with an ocular micrometer at a magnification of 1000X; specimens measured in fixed preparations were stained with iron hematoxylin. Because few intranuclear stages were seen, these were not measured.

**Electron Microscopy Techniques**

Sporozoites obtained by *in vitro* excystation techniques were inoculated into 8-ounce Brockway prescription bottles containing monolayers of MDBK cells. At intervals of 5 minutes, 6, 24, 48, 60, and 72 hours after inoculation, the cells were removed from the flasks with trypsin-versene, prewarmed to 37°C in a water bath, placed in centrifuge tubes, and pelleted by centrifugation at 1000 rpm for 5 minutes. After aspiration of the digestion medium, the pellet was fixed by one of two methods:

1. Karnovsky's fixative in cacodylate buffer, pH 7.4 for 4 hours at ambient temperature, rinsed overnight in cacodylate buffer, pH 7.4 at 8°C and post-fixed in 2.5 per cent osmium tetroxide in cacodylate buffer,
pH 7.4 for 1 1/2 hours at 8 C and washed with cacodylate buffer; and
(2) 2.5 per cent glutaraldehyde and 2.5 per cent osmium tetroxide to­
gether in phosphate buffer. pH 7.4 for 1 1/2 hours at 8 C and washed
with phosphate buffer.

In preparing free sporozoites for study, freshly excysted spor­
zoites were removed from the excystation medium fixed with either of
the above fixatives, washed in the appropriate buffer transferred to a
small cellulose nitrate tube (No. 305528, Beckman instruments), and
centrifuged. Excess fluid was aspirated off and an amount of Tissue Tac
equal in volume to that of the pellet was thoroughly mixed with the
pellet. A small drop of 25 per cent glutaraldehyde was then added to
the suspension, mixed thoroughly, and the resulting suspension was
immediately centrifuged at 2000 rpm for 5 minutes. During centrifuga­
tion, the suspension underwent gelation, resulting in a compact pellet
of sporozoites for dehydration and sectioning. The cellulose tube con­
taining the pellet of sporozoites was transferred to a petri plate contain­
ing 35 per cent ethanol, and the bottom of the tube was removed. The
pellets of freshly excysted sporozoites and of infected cells were
minced in 35 per cent ethanol. Pieces of material approximately 2-3 mm³
in size were then passed through a graded series of alcohol at 8 C. After
three 5-minute washes in absolute ethanol, the material was quickly
passed through two changes of propylene oxide at ambient temperature
for 10 minutes each. Embedding was done by the method of Sporn,
Wanko, and Dingman (1962) with Luft's Epon mixture, composed of
six parts of solution A and four parts of solution B. Polymerization was carried out by placing the specimens in BLM capsules and incubating at 37, 45, and 60°C for 12 hours each.

In later experiments, Dow Epon was used in place of Luft's and the embedding procedure was that of Lockwood (1964), with mixture number 1. Polymerization was effected by an incubation at 60°C for 12 hours.

After sufficient cooling, usually 24 hours, the blocks were properly trimmed to approximately 0.5-1.0 mm on a side. Sectioning was done with a Sorvall MT-2 ultramicrotome equipped with a diamond knife. Sections 600 Å in thickness were cut. Immediately before lifting the sections onto the bar or carbon-coated grids (200 mesh), a camel's hair brush, previously dipped into chloroform, was held over the sections in the boat to smooth out any wrinkles and assist in orienting the ribbons.

Some tissue was prestained overnight in a solution of 1 per cent uranyl acetate and 1 per cent phosphotungstic acid in 70 per cent ethanol at 8°C. Other tissue was not prestained before embedding. Sections of this material were stained with saturated uranyl acetate (Watson, 1958) for 5 minutes at 60°C and then with lead citrate (Reynolds, 1963) for 5 minutes at ambient temperature. Sections cut from prestained material were stained only with the lead citrate.

Sections were studied and micrographs were taken with a Zeiss EM 9A electron microscope.
RESULTS

Degree of Development in Various Cell Types

Unless otherwise stated, all measurements are given in microns with ranges listed in parentheses.

Numerous intracellular sporozoites were seen in all cell types tried (Table 1). These usually occurred in the cytoplasm of the host cell; development was observed only occasionally within the nucleus. Trophozoites and binucleate schizonts occurred in large numbers in all kinds of cells used except for INT 407 in which relatively few were seen. Mature schizonts were observed 2 and 3 days after inoculation in all cell types except for INT 407 and BHK in which they were observed only 3 days after inoculation. More mature schizonts were observed in BES and MDBK cells than in the others used. The maximum number of immature schizonts in these two kinds of cells was 3.3 and 1.6 per cent, respectively, of the maximum number of intracellular sporozoites earlier observed in these. The maximum number of mature schizonts in these two cell types was approximately 40 and 100 per cent, respectively, of the maximum number of immature schizonts. The maximum number of mature schizonts was 1.3 and 1.7 per cent, respectively, of the maximum number of intracellular sporozoites earlier seen in the two cell types. In BHK cells, the number of immature schizonts observed represented a relatively large proportion (10.5 per cent) of the intracellular
Table 1. Average numbers of *Eimeria alabamensis* endogenous stages found daily after inoculation into cultures

<table>
<thead>
<tr>
<th>Cell type&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Stage&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Number of experiments</th>
<th>Number of parasites found&lt;sup&gt;c&lt;/sup&gt;</th>
<th>% development&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Days after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>EbTr</td>
<td>SF</td>
<td>4</td>
<td>1278</td>
<td>521</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>TR</td>
<td>29</td>
<td>759</td>
<td>169</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>4</td>
<td>182</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>0</td>
<td>24</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>BESy</td>
<td>SP</td>
<td>4</td>
<td>595</td>
<td>345</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>TR</td>
<td>3</td>
<td>176</td>
<td>174</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>1</td>
<td>113</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>0</td>
<td>23</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>MDek</td>
<td>SF</td>
<td>4</td>
<td>992</td>
<td>218</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>TR</td>
<td>0</td>
<td>283</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>2</td>
<td>50</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>0</td>
<td>4</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0</td>
<td>4</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>BHK</td>
<td>SF</td>
<td>4</td>
<td>352</td>
<td>131</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>TR</td>
<td>7</td>
<td>111</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>0</td>
<td>23</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>0</td>
<td>0</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>int 407</td>
<td>SP</td>
<td>3</td>
<td>341</td>
<td>282</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>TR</td>
<td>0</td>
<td>7</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>0</td>
<td>10</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>BEint</td>
<td>SP</td>
<td>3</td>
<td>857</td>
<td>610</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>TR</td>
<td>8</td>
<td>651</td>
<td>350</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>2</td>
<td>146</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>0</td>
<td>11</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>Cell type&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Stage&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Number of experiments</th>
<th>Number of parasites found&lt;sup&gt;c&lt;/sup&gt;</th>
<th>% development&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Days after inoculation</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>BETy</td>
<td>SP</td>
<td>3</td>
<td>1015</td>
<td>216</td>
</tr>
<tr>
<td></td>
<td>TR</td>
<td>3</td>
<td>674</td>
<td>167</td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>1</td>
<td>170</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>IS</td>
<td>0</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>BES</td>
<td>SP</td>
<td>3</td>
<td>1154</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>TR</td>
<td>42</td>
<td>272</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>9</td>
<td>135</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>IS</td>
<td>0</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>BEK</td>
<td>SP</td>
<td>3</td>
<td>889</td>
<td>282</td>
</tr>
<tr>
<td></td>
<td>TR</td>
<td>21</td>
<td>242</td>
<td>261</td>
</tr>
<tr>
<td></td>
<td>BS</td>
<td>26</td>
<td>117</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>IS</td>
<td>1</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Abbreviations: EBTr, embryonic bovine trachea; BESy, bovine embryonic synovium; MDBK, Madin-Darby bovine kidney; BHK, Syrian hamster kidney; Int 407, human intestine; BEInt, bovine embryonic intestine; BETy, bovine embryonic thyroid; BES, bovine embryonic spleen; BEK, bovine embryonic kidney.

<sup>b</sup>Abbreviations: SP, sporozoite; TR, trophozoite; BS, binucleate schizont; IS, immature schizont; MS, mature schizont.

<sup>c</sup>Numbers represent stages found in a strip approximately 0.4 mm by 40 mm along the coverslip.

<sup>d</sup>Per cent development (to mature schizonts) =
\[
\frac{\text{maximum number of mature schizonts}}{\text{maximum number of intracellular sporozoites}} \times 100
\]
sporozoites seen in this kind of cell. In BETy cells, relatively large numbers of intracellular sporozoites were seen and the number of mature schizonts, occurring 2 days after inoculation, was relatively high, but no mature schizonts were seen 3 days after inoculation. More intracellular sporozoites were found in EBTr cells than in any of the others, but fewer mature schizonts occurred in these cells than in BES and MDBK. Thus, the latter two cell types appeared to be most favorable for development of *E. alabamensis*.

**Sporozoites**

During the first 24 hours in culture, extracellular sporozoites underwent slow movements, including flexing of the anterior one-third of the body. At times, the anterior end of the body was extended, forming a stylet-like protuberance and then this was retracted. With their tapered anterior ends, sporozoites made intermittent contact with the monolayer cells. Rarely, after a period of such contact, the sporozoite became attached at the anterior end and underwent a pivoting movement. Usually, pivoting occurred with the posterior end attached to the substrate. Twenty living, extracellular sporozoites, observed 24 hours after inoculation, measured 7.8 (6-8) in length by 2.5 (2-4) in width just anterior to the posterior refractile body. Twenty living intracellular sporozoites measured at the same time were 5.5 (4-6) by 2.9 (2-4). Extracellular sporozoites were observed during the second and third days in cultures, but they were immotile and were therefore presumed to
be dead. Penetration of the host cells by sporozoites were observed during the first 24 hours in culture. This required 20-30 minutes in the several instances seen. However, many intracellular sporozoites were found 5 minutes after inoculation. Sporozoites were constricted to approximately three-fourths of the body width during the penetration process. Penetration of the host cell nucleus, observed only once, 24 hours after inoculation, required about 10 minutes, and the sporozoite was constricted to about one-half of the body width as it passed through the nuclear membrane (Figure 23). After entrance into the nucleus, the sporozoite remained adjacent to the nuclear membrane (Figure 24).

After penetration, the sporozoite moved through the cytoplasm, leaving a clear pathway which was visible for several minutes (Figure 1). By 24 hours after inoculation, the majority of intracellular sporozoites were located adjacent to the nucleus of the host cell. After this location was reached, the sporozoite became shorter and wider, and its anterior end assumed a more or less blunt shape (Figures 2 and 3). Some sporocysts were seen within host cells. Sporozoites in the process of leaving the host cell were not observed.

In EBTr, MDBK, BETy, and BES cultures, host cells, observed 24 hours after inoculation, harbored as many as eight sporozoites; in the remaining cell types, no more than three parasites were seen in one cell. In multiply-infected cells, intracellular parasites were confined to one area of the cell cytoplasm or commonly to the immediate vicinity of the
host cell nucleus (Figure 3). In cells with intranuclear parasites, as
many as two sporozoites or four merozoites were rarely seen.

In all cell types, the maximum number of intracellular sporozoites
was found 24 hours after inoculation (Table 1). Thereafter, the numbers
decreased; this decrease was most rapid in cultures of EBTr, MDBK,
BEK, and BETy cells. Less than 1 per cent of all intracellular sporozoites
were found in the host cell nucleus. Intranuclear sporozoites and
trophozoites were first seen 48 hours after inoculation (Figure 25); these
were similar in appearance to those in the host cell cytoplasm.

Intracellular sporozoites, observed 6 hours after inoculation, had a
large spherical, vesicular nucleus with a distinct, eccentric nucleolus.
The nucleolus stained lightly or not at all with iron hematoxylin. By
24 hours post-inoculation, the majority of intracellular sporozoites each
had an elongate nucleus; the nucleolus was larger and more centrally lo-
cated than in specimens examined earlier. After staining with PAS-AO,
intracellular sporozoites had bright orange refractile bodies, brilliant red
(PAS-positive) ellipsoidal granules surrounded the nucleus.

Freshly excysted sporozoites had only one refractile body which
was located posteriorly. In intracellular sporozoites, observed 1 day
after inoculation, several small spheroidal granules were becoming sepa-
rated from the refractile body (Figures 3 and 4). In sporozoites, ex-
amined 2 days after inoculation, small granules of refractile material,
preumably derived from the posterior refractile body, were found at the
anterior end; they had coalesced in some specimens into one or more
refractile bodies (Figure 5). At 48 hours after inoculation, some of the extracellular sporozoites had more than one refractile body, suggesting the possibility that these sporozoites had entered and left cells.

**Trophozoites**

Trophozoites were first seen 24 hours after inoculation; they were most numerous at 48 hours in all cell types except for Int 407, but smaller numbers were seen at 72 and 96 hours. More trophozoites were observed in EBTr, BETy, and BEInt cells than in the others. Twenty trophozoites, fixed at 2 days after inoculation in EBTr cells, measured 4.8 (3-5) by 3.8 (2-4.5). These usually had a single, spheroidal refractile body measuring approximately 1.5-2 in diameter. In trophozoites, the nucleus was relatively large and the nucleolus prominent (Figure 6).

**Sporozoite-shaped schizonts**

Nuclear division sometimes occurred in parasites retaining the sporozoite shape. Of 100 binucleate specimens in cultures of MDBK cells, examined at 48 hours, 68 were spheroidal (Figure 7) and 32 were sporozoite-shaped (Figure 17). Ten sporozoite-shaped specimens, fixed at this time, measured 6 (5-6) by 3.3 (2.6-3.5). Each of these schizonts had one or two large refractile bodies and several small refractile granules (Figure 17). Transformation to the spheroidal shape was found first at 24 hours after inoculation. An outpocketing occurred in the anterior region of the body, and this later became extended posteriorly
along one side (Figure 17). Sporozoite-shaped schizonts with 2–3 nuclei were most numerous at 48 hours.

**Spheroidal schizonts**

Binucleate spheroidal schizonts were first seen at 24 hours in all cell types except for Int 407 and BHK; they were most numerous in BEK cells. Ten such schizonts, fixed at 48 hours in EBTr cultures, measured 5 (4-5) by 4.5 (3.5-4.7). Schizonts with four or more nuclei were found at 24 hours in BEK cultures; in all other cell types they were not seen until 48 hours after inoculation. Ten fixed schizonts, each with four nuclei, measured 8.0 (7.5-8.5) by 7.6 (7.5-7.7) at 3 days after inoculation in cultures of MDBK cells. Intranuclear schizonts with four nuclei were rarely seen and resembled those occurring in the cytoplasm. Four- or five-nucleate schizonts usually had one large refractile body located peripherally (Figure 8); rarely, several small refractile bodies were found in addition to or in place of the large one.

**Merozoite formation**

Formation of merozoites apparently occurred at the periphery of the schizont. As early as 24 hours after inoculation, schizonts were found with small elevations at the surface (Figure 18). These became more pronounced and then elongated to form merozoites. Each immature merozoite had a refractile body and a nucleus; however, some merozoites had two nuclei (Figure 22). In some specimens, a dumbbell-shaped nucleus, apparently in the process of division, was observed.
Extracellular binucleate merozoites were not seen.

Six schizonts in which merozoites were forming, found in MDBK cultures and fixed 2 and 3 days after inoculation, measured 14.2 (11.5-18.5) by 10.2 (8.5-11.5). The majority (39 of 50) appeared elongate (Figure 20); the remaining ones were spheroidal (Figure 19). Each schizont had 6-14 (mean, 12) merozoites. These escaped from host cells 2 and 3 days after inoculation in all cultures except for BESy, Int 407, and BHK in which this was seen only 3 days after inoculation.

Ten living, free merozoites, observed 2 days after inoculation, were 5.2 (4.9-6.0) by 3.1 (1.6-4.3); each had a centrally located nucleus, two refractile bodies, and a tapered anterior end (Figure 21). The refractile bodies stained red with Gomori's modified trichrome, orange with PAS-AO, and grey to black with iron hematoxylin. Extracellular merozoites flexed the anterior one-third of the body. Rarely, merozoites were observed entering new host cells within 1 hour after their escape. The merozoite underwent constriction as it passed through the limiting membrane of the host cell; after this was completed, the anterior end appeared swollen. Penetration required only a few seconds; the merozoite usually did not move further within the cell after entering. Partially rounded intracellular merozoites were seen 4 days after inoculation.

Distinctly smaller schizonts were also observed; these were more numerous than the larger ones. Mature specimens were found in all cell types 3 days after inoculation. Twenty living specimens of such mature schizonts in cultures of MDBK and EBTr cells, measured at 3 days, were
9.7 (5.5-13) by 6 (5-7.5). These schizonts were observed to form 6-10 merozoites apparently by radial budding (Figure 10). At 48 hours, nuclei and refractile bodies were arranged at the periphery of the schizont (Figure 9). Immature merozoites were attached to a central residuum which had nuclei (Figures 11 and 12). As merozoites became larger, a refractile body (Figure 13) could be seen in the anterior region of each. In some mature schizonts, the merozoites had a parallel arrangement (Figure 14). The anterior ends of the merozoites moved laterally within the parasitophorous vacuole, apparently causing enlargement of the vacuole. Thereafter, merozoites escaped in pairs. Often, the host cell cytoplasm appeared granulated at this time. The merozoites apparently underwent no constriction as they escaped from the host cell. Often, a period of 10-20 minutes elapsed before other merozoites escaped. Frequently, 1-4 merozoites remained in the parasitophorous vacuole after the others had escaped. Free merozoites were long and slender with bluntly rounded ends (Figure 15). Six live merozoites, observed at 3 days in MDBK cells, measured 7.7 (7-8) by 2. The refractile bodies, which were about 2 in diameter, stained as did those described above. The movements of these merozoites and their entrance into new host cells were similar to those of the small merozoites. Intracellular parasites were rarely seen after 4 days post-inoculation, and those found were degenerate.

Schizonts with forming merozoites filled most of the space within the enlarged nucleus; immature merozoites appeared spheroidal (Figure 27).
and mature ones elongate in fixed material. Often the immature spheroi-dai forms appeared to be partially isolated from one another by septa-like structures connected with the membrane lining the parasitophorous vacuole (Figure 27). Binucleate merozoites were seen within host cell nuclei. As merozoites escaped, the nuclear membrane of the host cell disintegrated. At 4 days after inoculation of MDBK cells, three host cell nuclei were found to have been invaded by one or more merozoites (Figure 28) which in two cases had transformed into second-generation trophozoites.

Host cell changes

No alteration in host cells was seen until 2 days after inoculation at which time a distinct indentation of the host cell nuclear envelope adjacent to the parasite was noticeable. A parasitophorous vacuole was observed in association with intracytoplasmic and intranuclear parasites. During development of the parasites within the host cell cytoplasm, the parasitophorous vacuole remained narrow and the host cell nucleus became enlarged (Figures 5, 8, 18, 20, 23, 26, and 28). With intranuclear infections, the host cell nucleoplasm underwent an apparent decrease in volume as the parasite grew; nuclear enlargement was greater than in cells with intracytoplasmic infections. Infrequently, cells harboring parasites became binucleate (Figure 7). Blebs at the surface of the host cell in the vicinity of the intracellular parasite were seen only rarely. Clear spaces in the monolayer were visible around some infected cells. The host cell cytoplasm appeared highly granular 3 days after inoculation (Figure 8). Host cells usually disintegrated as merozoites escaped.
Figures

Figures 1-53 are photomicrographs and electron micrographs of developmental stages of *Eimeria alabamensis* in cultured cells. Figures 1-21 and 23-24 are of living specimens photographed with phase-contrast microscopy; Figures 22 and 25-28 are of specimens fixed in Schaudinn's and stained with iron hematoxylin; Figures 29-53 are electron micrographs from specimens in MDBK cells fixed with the Karnovsky method, except for Figures 31, 33, 41, and 52 which were fixed with glutaraldehyde-osmium tetroxide. The type of cell culture and interval between inoculation and taking of photographs or fixation are given in parentheses.

**Abbreviations all figures: light microscopy.** AR, anterior refractile body; DM, developing merozoites; DN, dividing nucleus; E, elevation representing an early stage of merozoite formation; HN, host cell nucleus; M, merozoite; N, nucleus of parasite; NU, nucleolus of parasite; PR, posterior refractile body; R, refractile body; RB, residual body; RG, refractile granule; S, sporozoite; T, trophozoite.

**Abbreviations all figures: electron microscopy.** A, annulus; AR, anterior refractile body; CO, conoid; C, centriole; ER, endoplasmic reticulum; FV, food vacuole; G, Golgi complex; GA, Golgi adjunct; HC, host cell cytoplasm; HN, host cell nucleus; IM, inner membrane complex; L, lipid; MI, mitochondria; MB, myelin-like body; MP, micropore; MN, micronemes; N, nucleus of parasite; NE, nuclear envelope of parasite; NU, nucleolus of parasite; OM, outer membrane; P, pellicle;
FC, polar cone; PG, polysaccharide granules; PR, posterior refractile body; PV, parasitophorous vacuole; RA, rhoptries anlage; RB, refractile body; RH, rhoptries; RG, refractile granule; SM, subpellicular microtubules.
Figure 1. Sporozoite which has just entered host cell. Note clear pathway in cell cytoplasm (BEInt, 6 hours). X1000

Figure 2. Intracellular sporozoite (BEInt, 24 hours). X1280

Figure 3. Four sporozoites around the host cell nucleus (MDBK, 4 hours). X1000

Figure 4. Intracellular sporozoite with refractile granules forming at the surface of refractile body (MDBK, 48 hours). X1000

Figure 5. Sporozoite with anterior and posterior refractile bodies (MDBK, 48 hours). X1000

Figure 6. Trophozoite with enlarged nucleus and nucleolus (BEInt, 48 hours). X1000

Figure 7. Schizont with two nuclei in binucleate host cells (BEInt, 48 hours). X1000

Figure 8. Schizont with five visible nuclei and large refractile body (MDBK, 48 hours). X1000

Figure 9. Schizont of small type in early stage of merozoite formation (BEInt, 48 hours). X1000

Figure 10. Schizont of small type in intermediate stage of merozoite formation (MDBK, 48 hours). X1000

Figure 11. Schizont of small type, with immature merozoites, each having a nucleus and a refractile body (MDBK, 72 hours). X1000

Figure 12. Schizont of small type with merozoites attached to a multinucleate central residual body (MDBK, 72 hours). X1000

Figure 13. Schizont of small type, with nearly mature merozoites, each with a prominent anterior refractile body (MDBK, 72 hours). X1000

Figure 14. Mature schizont of small type with merozoites arranged in parallel in a degenerating host cell (MDBK, 72 hours). X1000

Figure 15. Free merozoite of long type (MDBK, 72 hours). X1000

Figure 16. Sporozoite-shaped schizont with cytoplasmic outpocketings (arrows) (BEInt, 24 hours). X1000
Figure 17. Binucleate sporozoite-shaped schizont with one nucleus in lateral outpocketing (arrow) (BEInt, 24 hours). X1000

Figure 18. Intermediate schizont of large type in early stage of merozoite formation (MDBK, 48 hours). X1000

Figure 19. Schizont of large type with nearly mature merozoites (BEInt, 48 hours). X1000

Figure 20. Schizont of large type with developing merozoites attached to a central residual body (MDBK, 48 hours). X1000

Figure 21. Free merozoite of short type with anterior and posterior refractile bodies (BEInt, 48 hours). X1000

Figure 22. Large schizont with nearly mature merozoites, some of which have two nuclei (arrows) or a dividing nucleus (MDBK, 72 hours). X1000

Figure 23. Sporozoite entering a host cell nucleus. Note constriction of sporozoite body at site of entrance (MDBK, 24 hours). X1280

Figure 24. Intranuclear sporozoite (MDBK, 48 hours). X1000

Figure 25. Intranuclear trophozoite (MDBK, 48 hours). X1000

Figure 26. Intranuclear multinucleate schizont (MDBK, 72 hours). X1000

Figure 27. Intranuclear schizont with immature merozoites (MDBK, 72 hours). X1000

Figure 28. Four second-generation trophozoites within a host cell nucleus (MDBK, 96 hours). X1000
Fine Structural Observations

Extracellular sporozoites

Extracellular sporozoites fixed 10 minutes after excystation did not differ from the intracellular sporozoites found in material fixed 5 minutes after inoculation, except as noted below.

Intracellular sporozoites

The stubby, slightly curved sporozoites fixed 5 minutes (Figure 29), 6 hours, and 24 hours (Figure 31) after inoculation of MDBK cultures had a limiting pellicle (Figures 31 and 32). This structure was composed of an outer membrane approximately 70-125 Å in thickness which consisted of two osmiophilic layers separated by an osmiophobic layer, thus resembling a unit membrane (Robertson, 1959). The inner, more dense layer of the pellicle terminated at the anterior, osmiophilic polar ring (Figure 32). In some sections, the inner membrane, which was 125-200 Å in thickness, appeared to consist of two closely apposed unit membranes (Figure 35). This inner structure appeared as a five part "sandwich" with a central, thick, dense layer separated from the thinner osmiophilic layers on each side by an osmiophobic layer. Beneath the inner dense layer of the pellicle, 22 subpellicular microtubules about 215-250 Å in diameter were evenly distributed about the periphery of the cell. These appeared to be attached to the polar ring at the anterior end and extended posteriorly as far as the anterior margin of the posterior
refractile body. They were not seen in sections at the level of the posterior refractile body.

Lying within the encircling polar ring was a truncate conoid (Figures 30-33, and 40), consisting of spirally arranged fibrillar structures (Figure 30). The conoid, 1350-1500 Å across the top; 1800-2000 Å across the bottom; and 1875-2750 Å in length, was seen in extended and retracted positions. In the extended position, the posterior end of the conoid was located opposite the polar ring, and in the retracted position the anterior end of the conoid was opposite the polar ring (Figures 30-33). Anterior to the conoid were the two annular rings which appeared as dense osmiophilic, triangular structures in transverse sections (Figure 32).

Portions of 2-6 osmiophilic rhoptries (paired or club-shaped organelles) were located within the conoid. Longitudinally oriented rhoptries were observed as far posteriorly as the nucleus (Figures 29, 32, and 33). The anterior portions of the rhoptries, which were circular in transverse section, were smaller in diameter than the portions lying posterior to the conoid. Their density varied in different regions. Numerous micronemes, with a density similar to that of the rhoptries and a round to elongate outline, appeared to follow an irregular pathway in the anterior end of the sporozoite (Figures 29 and 32).

The round to oval nucleus was relatively small in specimens fixed 5 minutes after inoculation of sporozoites (Figure 29). Such a nucleus had prominent particles, having the appearance of ribonucleoprotein, and osmiophilic masses, apparently heterochromatin, irregularly spaced along
the inner side of the distinct nuclear envelope (Figure 29). This had ribosomes attached to its outer surface (Figures 29, 31, and 33). Rarely, rough endoplasmic reticulum was attached to the outer membrane of the nuclear envelope. Nuclear pores were irregularly spaced along the periphery of the nucleus. A distinct nucleolus was not seen in these specimens. As early as 6 hours after inoculation of MDBK cells, however, intracellular sporozoites had several irregularly shaped, osmiophilic masses grouped near the center of the nucleus. Sporozoites, fixed 24 hours after inoculation of cells, had a definite nucleolus and a nucleus about twice as large as that in specimens fixed 5 minutes after inoculation (Figure 32). Closely associated with the nucleus was a large complex of flattened, curved vesicles presumed to be the Golgi complex (Figure 31). In most specimens, this was located in an indentation of the nucleus. In this area, the outer membrane of the nuclear envelope had no ribosomes, and no heterochromatin masses occurred along the inner membrane of the nuclear envelope (Figures 31, 39, and 40). The nuclear envelope was separated from the more proximal row of Golgi lamellae by cytoplasm which lacked ribosomes. A row of spherical membrane-bound vesicles, approximately 350-435 Å in diameter, were linearly arranged and equally spaced from each other in this region (Figure 39). Lamellae located at the apex of the Golgi complex appeared longer, wider, and more curved than those at the base. Associated with the multilayered array of cisternae were small vesicles clustered about the edges of the cisternae and along the outer surfaces of the Golgi
complex. The position of the Golgi complex did not appear to have a specific location in relation to organelles other than the nucleus.

In intracellular sporozoites, fixed 5 minutes after inoculation, numerous ellipsoidal, osmiophilic granules, presumably polysaccharide, were observed between the nucleus and the retractile body (Figure 29). In such specimens, the nucleus was found in the anterior portion of the body (Figure 29). By 24 hours after inoculation, sporozoites had few, if any, polysaccharide granules and the nucleus was centrally located (Figure 31).

Elongate to spheroidal mitochondria, limited by two unit membranes, contained peripherally located tubular cristae. Spheroidal mitochondria measured 0.1-0.3 µ in diameter, and the elongate ones were 0.4-0.9 µ in length by 0.1-0.2 µ in width. These were most often located between the nucleus and the posterior retractile body (Figure 31). Elongate mitochondria frequently occurred along the anterior margin of the posterior retractile body (Figure 31).

The posterior retractile body occupied the posterior one-third of the body. This organelle stained variably with osmium tetroxide. The single retractile body found in each extracellular sporozoite and in each intracellular sporozoite, fixed up to 6 hours after inoculation, appeared more osmiophilic (Figure 29) than the majority of those found in sporozoites fixed at 24 hours after inoculation (Figure 31). Some sporozoites, observed in cultures fixed 24 hours after inoculation, had one or more small bodies attached to the anterior margin of the posterior retractile body or
located between it and the nucleus. These were less intensely stained with osmium tetroxide than the posterior refractile body. By 48 hours after inoculation, sporozoites had 3-5 large lightly stained bodies, grouped in a compact mass at the anterior end of the sporozoite; these bodies were similar in appearance to those earlier observed near the posterior refractile body. Some specimens, fixed 48 hours after inoculation, had both anterior and posterior refractile bodies (Figure 29). These were osmiophilic and similar in density to that of the refractile body of the extracellular sporozoite immediately after excystation.

Ribonucleoprotein particles were evenly distributed in the cytoplasm (Figures 29, 31, and 32). Infrequently, rough endoplasmic reticulum was observed (Figure 31). Slightly osmiophilic bodies, presumably lipid, were observed in specimens fixed 5 minutes to 48 hours after inoculation. These were commonly found lying against the nuclear envelope (Figures 29 and 31). They were not seen in extracellular sporozoites.

In specimens fixed at 6 and 24 hours after inoculation, as many as four micropores were found in the pellicle in a single section. In these, the outer membrane was interrupted, forming a circular depression approximately 800 Å in diameter. The inner membrane adjacent to the opening extended toward the interior of the cell for a distance of about 300-500 Å; it was 140-210 Å in thickness. In active micropores (Figures 35-38), found as early as 6 hours after inoculation of sporozoites and also seen in trophozoites 18 hours later, host cell cytoplasm
occurred in the lumen of the invagination (Figure 35). In some specimens, the invagination was longer and wider than in inactive micropores (Figures 36 and 37), and a small food vacuole could be seen adjacent to its inner end (Figure 37). Such vacuoles had apparently just been formed by pinching off from the micropore. In other specimens, food vacuoles containing structures probably representing host cell cytoplasm were observed near the micropore (Figure 38). Sporozoites were found which had large star-shaped vacuoles containing particulate material resembling host cell cytoplasm. These were located somewhat more deeply in the cell (Figure 40). Micropores were always anterior to the posterior refractile body and usually anterior to the nucleus. Inactive micropores, seen in sporozoites and sporozoite-shaped schizonts from the time of excystation until transformation into trophozoites or spheroidal schizonts, were not expanded into an elongate duct-like structure or associated with a vacuole as previously described.

**Trophozoites**

Trophozoites, which were most numerous at 48 hours after inoculation (Figures 34, 39, and 40), were spheroidal and lacked polysaccharide granules. They had fewer micronemes and smaller rhoptries but more ribosomes and endoplasmic reticulum than did the sporozoites. The mitochondria were slightly larger and some appeared to be in the process of division. Trophozoites had a single refractile body which was ellipsoidal to spheroidal. These appeared smaller than those
found in sporozoites 1 day earlier. Two or three smaller refractile bodies were often distributed within the cytoplasm. The nucleus was larger, and in some specimens appeared to be elongating in preparation for division (Figures 33 and 40). Nucleoli usually appeared to have two components of different densities and structure. A lighter staining and more granular appearing area, comparable to the pars amorpha, appeared singly or in two locations in the nucleolus. A more dense, fibrillar area, possibly the nucleolonema, made up the major portion of the nucleolus (Figures 39 and 40). The nucleolus was larger in 48-hour specimens than in those fixed at 24 hours (Figure 12).

In some cells with an elongate nucleus, a pair of centrioles could be seen at one end of the intact nuclear envelope (Figures 33 and 34). The centrioles were usually in parallel formation. They measured 1375-1500 Å in diameter and 1875-2250 Å in length. Each had nine peripheral microtubules encircling one central microtubule (Figure 34). It could not be determined with certainty whether they were single or double. Two pairs of centrioles, one pair at each pole of a dividing nucleus and lying outside its envelope, were seen in a trophozoite fixed 48 hours after inoculation. The centrioles appeared to lie within a ground cytoplasm devoid of any ribonucleoprotein particles. An intranuclear spindle apparatus, consisting of microtubules, was seen at one side of the nucleus. An interruption in the nuclear envelope at each pole of the intranuclear spindle could not be observed. Two to three microtubules apparently traversed the width of the nucleus and possibly connected the
two poles. Several other microtubules radiated from each pole at an angle of about 45 degrees. These extended one-third to one-half the distance of the nucleus. Only a small area of the nucleus was occupied by the spindle, and chromatin-like material was not found in this region. An intact nucleolus was located in another area of the nucleus. A similar number of trophozoites, undergoing nuclear division at this time, had a spindle apparatus lying across the middle of the nucleus.

The Golgi complex appeared more distinct (Figures 39 and 40) than in sporozoites, and the cisternae associated with the side of the organelle away from the nucleus usually were enlarged. Several specimens had a Golgi complex which appeared to have two identical parts as if in the process of division (Figure 39). Several in trophozoites, two or more rows of membrane-bound vesicles were located between the nuclear envelope and the Golgi complex. Some trophozoites had a membrane-bound, circular mass of cytoplasm free of ribosomes adjacent to the nuclear envelope. This mass was associated with membrane-bound vesicles similar in size to those associated with the Golgi complex. Since a normal Golgi complex was not observed in these specimens, this mass was presumed to be a Golgi complex anlage. Ribosomes were abundant and frequently were attached to one or more whorls of endoplasmic reticulum. The posterior refractile body had granules at its margin apparently formed from the refractile body, and such granules often were found free in the cytoplasm of the cell.

Trophozoites, fixed 48 hours after inoculation, had incomplete
inner membranes (Figure 40). Areas in which the surface had only a single membrane apparently occurred at random around the cell periphery. However, in the region immediately posterior to the polar ring, the inner membrane was always present.

**Multinucleate spheroidal schizonts**

Multinucleate stages found in MDBK cells, fixed 48 hours after inoculation of sporozoites, were of two types. In one type, which was spheroidal in shape, the inner membrane of the pellicle was lacking in most regions (Figure 43). The conoid, polar ring, annular rings, rhoptries, subpellicular microtubules, and micronemes were not observed. The nuclei were smaller than that found in the trophozoite, and each had a distinct nucleolus and masses of heterochromatin in the interior of the nucleus as well as attached to the inner membrane of the envelope (Figure 43). The nuclear envelope had numerous ribosomes attached to its outer surface. Rough endoplasmic reticulum was present. Mitochondria with tubular cristae similar to those found in earlier stages were seen. Free ribosomes were numerous throughout the cytoplasm. In some specimens, food vacuoles were found which contained numerous small membrane-bound vesicles (Figure 43). A distinct Golgi complex was located adjacent to the nuclear envelope. A few specimens had two or more large refractile bodies as well as several small refractile granules. This type of schizont evidently developed from trophozoites.
**Multinucleate sporozoite-shaped schizonts**

The second type of multinucleate schizont resembled the sporozoite in shape and cytological features, and is called the sporozoite-shaped schizont (Figure 44). This stage had 2-4 nuclei and was longer and somewhat wider than the sporozoite stage. The conoid, polar ring, sub-pellicular microtubules, micronemes, and rhoptries were present as well as a complete pellicle. Usually, specimens had one large refractile body at the posterior end similar to that found in the sporozoite as well as several smaller refractile granules. Mitochondria were usually confined to an area adjacent to the posterior refractile body. Each nucleus had a prominent nucleolus and heterochromatin masses similar to those found in earlier stages. A Golgi complex was associated with each nucleus. Lipid bodies were seen.

The sporozoite-shaped schizont later transformed into a spheroidal schizont, and the anterior organelles, the inner membrane, and micro-pores were apparently lost during this process. Specimens undergoing such a transformation were found at 48 hours after inoculation, but these were more common at 72 hours (Figure 45). Some transforming schizonts, fixed at 48 hours, had a dense membrane-bound structure lying within an indentation of the nucleus. Since a normal Golgi complex was not observed in these specimens, this structure was presumed to be a developing Golgi complex or a Golgi adjunct (Figure 45) (Sheffield and Melton, 1968).
Merozoite formation in spheroidal schizonts

One specimen found 48 hours after inoculation of sporozoites had a pair of centrioles located between the nucleus and the limiting membrane of the schizont (Figure 41). Adjacent to the centrioles was an area of dense material similar in density but thicker than the inner membrane of earlier stages. This was presumed to be an indication of inner membrane formation.

In a spheroidal schizont found in material fixed 60 hours after sporozoite inoculation, a conoid anlage was observed (Figure 46). This was located beneath the cell membrane near a region of the nucleus which lacked a definite nuclear envelope, possibly the site of a nuclear pore. The inner membrane of the pellicle at this location was thicker and more dense than in other areas of the surface. No other significant changes in the schizont could be observed at this time. In somewhat later stages, immature merozoites were growing outward from the surface of the schizont (Figure 51). Each immature merozoite had a Golgi complex and one or more dense bodies representing refractile bodies and/or rhoptries anlagen. A Golgi adjunct was observed in one immature merozoite. Some Golgi cisternae appeared large at the active center. In some specimens, indications of a polar cone, extending from the nucleus into the forming merozoite, was observed. One or two large refractile bodies remained near the center of the schizont. The limiting membrane of the schizont appeared to be single in regions where merozoites were not developing.
In more advanced stages of merozoite development, the limiting membrane of the schizont appeared to infold around the anterior end of each merozoite (Figure 50). Each immature merozoite had a refractile body, rhoptries, and micronemes. In some specimens, the inner membrane extended inward farther than the outer membrane of the merozoite.

**Merozoite formation in sporozoite-shaped schizonts**

A sporozoite-shaped schizont, which apparently was transforming into a spheroidal form, was found in an MDBK cell in a monolayer fixed 72 hours after inoculation (Figure 47). In this schizont, anlagen of merozoites, each including a conoid, subpellicular microtubules, membrane-bound vesicles, and structures probably representing anlagen of rhoptries and/or micronemes, were observed. Two nuclei were present and microtubules were observed in one of these. The Golgi complex associated with this nucleus was large and had numerous small, membrane-bound vesicles and outpocketings from the perinuclear space. This schizont had a complete pellicle except that the inner membrane was interrupted at several places. At one of these, the inner layer extended into the interior of the schizont; another portion of inner layer was seen in another area of the interior of the schizont. Inward extensions of the inner membrane were also observed in earlier stages (Figure 42). Rarely, sporozoite-shaped schizonts with an intact pellicle and remnants of some of the anterior organelles were seen in MDBK cultures fixed 72 hours after sporozoite inoculation. Such stages had several forming merozoites,
each with a conoid and an associated polar ring, rhoptries, micronemes, and Golgi complex within the interior of the cell (Figure 48). A large refractile body and several smaller ones were present in the schizont.

A schizont, found in a monolayer fixed 60 hours after inoculation, had a forming merozoite with a conoid, rhoptries and/or micronemes, and an anterior refractile body at one side of the schizont. This forming merozoite was parallel with the surface and separated from the remainder of the schizont by two parallel membranes extending from the inner membrane of the pellicle (Figure 49). Another forming merozoite located in a more central position had rhoptries, micronemes, and a Golgi adjunct. Such schizonts had a complete pellicle with only one or two areas where the inner membrane was lacking. As merozoite development continued, the merozoites became separated from the schizont by an infolding of the outer limiting membrane of the latter. The merozoites at this time appeared to be budding from the surface of the schizont (Figure 52). Daughter nuclei, each with an associated Golgi complex and mitochondria, were incorporated into each budding merozoite. In a nearly mature schizont found 72 hours after inoculation of sporozoites, almost completely formed merozoites were present (Figure 53). The longest merozoite of this group measured 8.8 µ in length which falls in the range of the slender merozoites measured 3 days after inoculation of sporozoites in the light microscope study (Sampson and Hammond, 1969).
Merozoites

Mature merozoites were found only in cultures fixed 72 hours after inoculation. Each mature schizont had 3-14 merozoites associated with small membrane-bound vesicles, probably representing residual material. Each merozoite was covered by a pellicle similar to that of the sporozoite, the outer unit membrane of which covered the entire surface of the cell. The inner membranes were interrupted at the micropores, the polar ring, and the posterior pore. Located at the anterior end was a conoid with a spirally arranged fibrillar substructure and two osmiophilic annuli (Figure 53). Two or three dense rhoptries extended from the anterior end of the conoid posteriorly to a position just anterior to the nucleus. Many micronemes were observed in the anterior portion of the cell. The nucleus had dense heterochromatin irregularly spaced along the periphery of its envelope, and a prominent nucleolus. A distinct Golgi complex was found in some specimens adjacent to the outer membrane of the nucleus, and a Golgi adjunct was sometimes present. Mitochondria were distributed throughout the cytoplasm. Large and/or small refractile bodies were present. The cytoplasm had numerous ribosomes, some free and some attached to endoplasmic reticulum. Some specimens had one or two micropores in the anterior portion of the body. Some host cells contained several merozoites, each apparently within its own parasitophorous vacuole. Commonly, such specimens also contained a group of 2-3 merozoites partially surrounded by a
parasitophorous vacuole. This finding might be interpreted as resulting from escape of some of the merozoites from the parasitophorous vacuole into the cytoplasm of the host cell.

**Host cell changes**

In intracellular sporozoites fixed 5 minutes after inoculation, a partial host cell membrane was visible around the parasite. In specimens fixed 6 hours after inoculation, the parasite was in a vacuole completely bound by a membrane of the host cell cytoplasm. In some specimens, this membrane was doubled back and forth within the parasitophorous vacuole. Small vesicles and narrow finger-like projections of the host cell cytoplasm occurred within this vacuole. In several sporozoites and trophozoites, this material was found within the lumen of the micropore of the parasite (Figures 31, 35-38). In many specimens a "myeline-like" body occurred adjacent to the parasite within the parasitophorous vacuole. In the host cell cytoplasm were numerous mitochondria adjacent to the limiting membrane of the parasitophorous vacuole. The cytoplasm of host cells with mature schizonts in cultures fixed 72 hours after inoculation appeared degenerate. The cytoplasm had many vacuoles containing osmiophilic material not seen in host cells in earlier stages of parasite development. Few or no ribosomes were seen in the area adjacent to the limiting membrane of the parasitophorous vacuole, and in some specimens the nucleus appeared to have fewer heterochromatin masses than those in noninfected cells. The nucleolus did not appear to be enlarged in infected cells.
Figure 29. Longitudinal section of sporozoite (5 minutes). Note numerous polysaccharide granules and absence of nucleolus. X17,000

Figure 30. Longitudinal section of anterior end of sporozoite (24 hours). Note annuli (arrows) and spirally arranged fibrillar substructure of conoid (arrow). X57,000

Figure 31. Longitudinal section of sporozoite (24 hours) with enlarged nucleus, nucleolus, and active micropore. X17,000

Figure 32. Longitudinal section of anterior portion of sporozoite (48 hours). Note position of anterior organelles. X22,000

Figure 33. Longitudinal section of trophozoite (48 hours). Note anterior organelles and elongate nucleus with centrioles. X22,000

Figure 34. Transverse section of centrioles (48 hours), each with nine peripheral tubules and one central tubule. X97,300
Figure 35. Longitudinal section of an active micropore of a sporozoite (24 hours). X60,000

Figure 36. Longitudinal section of an active micropore of a sporozoite (24 hours) with enlarged inner portion. X60,000

Figure 37. Longitudinal section of an active micropore of a sporozoite (24 hours) with elongation of inner portion and an adjacent food vacuole. X52,000

Figure 38. Longitudinal section of an enlarged micropore and a food vacuole in a sporozoite (24 hours). X30,000

Figure 39. Transverse section of a trophozoite (48 hours) with apparently dividing Golgi complex and enlarged nucleus. X20,000

Figure 40. Longitudinal section of trophozoite (48 hours) with elongate nucleus and partial disappearance of inner membrane. X22,000

Figure 41. Partial section of trophozoite (48 hours) with centrioles and thickened inner membrane (arrow). X24,000
Figure 42. Oblique section of trophozoite (48 hours) with several refractile bodies and extension of inner membrane into the interior. X17,500.

Figure 43. Section of binucleate schizont (48 hours) with two refractile bodies and a single limiting membrane over most of the surface. X16,000.

Figure 44. Longitudinal section of transforming sporozoite-shaped schizont. Note the presence of two nuclei and anterior organelles. X15,000.

Figure 45. Longitudinal section of transforming sporozoite-shaped schizont (48 hours). Note the presence of two nuclei and partial disappearance of inner membrane and anterior organelles. X16,000.

Figure 46. Section of binucleate schizont (48 hours) with two refractile bodies and extension of inner membrane into the interior. X16,000.
Figure 46. Partial section of schizont (60 hours) with conoid adjacent to thickened inner membrane. X24,000

Figure 47. Longitudinal section through multinucleate schizont (60 hours) showing merozoite anlagen and inner membrane extensions. X20,000

Figure 48. Longitudinal section of sporozoite-shaped schizont (60 hours). Note slender immature merozoites. X17,500

Figure 49. Stage in transformation of sporozoite-shaped schizont to spheroidal schizont (60 hours). Note the two immature merozoites. X16,000
Figure 50. Partial section of schizont (72 hours) with stubby merozoites forming at surface. X20,000

Figure 51. Multinucleate schizont (72 hours) with immature stubby merozoites growing outward at surface. X20,000

Figure 52. Schizont with forming merozoites of slender type (72 hours). X17,250

Figure 53. Longitudinal section of three mature merozoites of slender type (72 hours). X10,000
DISCUSSION

*Eimeria alabamensis* was found to differ somewhat with respect to its growth in cell cultures from the other two bovine species which have been studied in this way. EBTr cultures were reported to be most suitable for the growth of *E. bovis* (Hammond and Fayer, 1968). With *E. auburnensis*, however, more immature schizonts developed in MDBK than in EBTr cells; the number of mature schizonts was about the same in these two cell types (Clark and Hammond, 1969). In the present study, immature schizonts were most numerous in EBTr and BES cells, whereas the largest number of mature schizonts occurred in MDBK and BES cells. BES cells were also favorable for the growth of *E. auburnensis* (Clark and Hammond, 1969) and *E. bovis* (Fayer and Hammond, 1967). The proportion of maximum numbers of immature schizonts to intracellular sporozoites in the two most favorable cell types in *E. bovis*, *E. auburnensis*, *E. ninakohlyakimovae*, and *E. alabamensis* was 60 and 40 per cent, 12 and 6 per cent, 87 and 63 per cent, and 1.6 and 3.3 per cent, respectively. The proportions of maximum numbers of mature schizonts to immature schizonts in the two most favorable cell types for these four species were 8 and 0.4 per cent, 14 and 6 per cent, 7.3 and 0.4 per cent, and 100 and 40 per cent, respectively. Thus, a larger proportion of immature schizonts formed mature schizonts in *E. alabamensis* than in any of the other three species. Probably this was associated with the shorter period of time required for the development of the first-generation
schizonts in E. alabamensis. E. alabamensis resembled E. auburnensis in these aspects of development more closely than it did the other two species.

Several features of the infection with E. alabamensis differed from those reported for E. bovis and E. auburnensis. Sporozoites of E. alabamensis moved more slowly and took considerably longer to penetrate host cells than those of the other two species. During the first 24 hours of the infection, E. alabamensis sporozoites usually became located adjacent to the host cell nucleus, whereas the sporozoites of the former two species were more randomly distributed in the host cell cytoplasm. This is to be expected, since E. alabamensis develops within the host cell nucleus in the normal host. It is of interest that this species usually develops to mature first-generation schizonts in the cytoplasm instead of in the nucleus of host cells in cultures.

In E. alabamensis, the first nuclear division frequently occurred after transformation to the trophozoite stage, but a minority of specimens retained the sporozoite shape until two or three nuclei were present. In E. bovis, nuclear division was observed only in trophozoites, whereas in E. auburnensis the majority of parasites underwent several nuclear divisions before transformation to a spheroidal shape. Lateral cytoplasmic outpocketings during transformation of sporozoites or sporozoite-shaped schizonts to spheroidal forms, as described for E. auburnensis (Clark and Hammond, 1969), were commonly seen in E. alabamensis. Clark and Hammond (1969) observed some such transformations to occur rapidly.
Such cytoplasmic outpocketings have recently been observed in cultures of *E. callospermophili* from the ground squirrel (Speer, Hammond, and Anderson, 1970) and in cultures of *E. ninakohylakimovae* from sheep (Kelley and Hammond, 1970).

The significance of the two sizes of mature schizonts and merozoites in *E. alabamensis* is unknown. Because they occurred simultaneously, both apparently represent first-generation schizonts. Doran and Vetterling (1967b) found two kinds of schizonts in *E. meleagrimitis* grown in BEK cultures; they considered both of these to be of the first generation. They also found two kinds of schizonts in *E. necatrix* in cell cultures.

The formation of merozoites at the surface of the schizont by apparent radial budding in the spheroidal schizonts of *E. alabamensis* is similar to that reported in *E. bovis* (Fayer and Hammond, 1967) and *E. ninakohylakimovae* (Kelley and Hammond, 1970). Roberts et al. (1970) described merozoite formation in *E. callospermophili* as beginning within the interior of the schizont. The inner membrane, along with other organelles of the anterior region of the forming merozoite, first appeared close to a nucleus near the pole of an intranuclear fiber apparatus and associated centrioles. In the later stages of merozoite formation, the immature merozoites appeared at the surface of the schizont and then grew out radially as in *E. bovis* (Sheffield and Hammond, 1967). In the sporozoite-shaped schizonts, the internal formation of merozoite anlagen
appeared to be similar to that described in *E. callospermophili* by Roberts and Hammond (1970).

The finding of the apparent binucleate condition of some of the merozoites before they matured and escaped from the host cell was unexpected. This condition might indicate a nuclear division after incorporation of the nucleus into the forming merozoites or an abnormal process whereby two nuclei were incorporated simultaneously. The finding of the dumbbell-shaped nuclei supports the former possibility. Hitchcock (1955) and Lickfeld (1959) observed multinucleate bodies during the schizogony of *Isospora* felis of the cat. These multinucleate forms gave rise to smaller forms also containing more than one nucleus, and these later formed merozoites. More recently, Shah (1969) mentioned seeing multinucleate stages in bodies giving rise to merozoites in his study of *Isospora* in the cat, but their importance was not discussed. Cheissin and Snigirevskaya (1964) and Pellerdy (1953) found multinucleate merozoites in *E. magna* and *E. piriformis*, respectively, but were unable to explain the significance of these findings. Such merozoites were also reported to occur during the life cycle of *E. stiedae* (Pellerdy and Dür, 1970).

The occurrence of refractile bodies of the merozoites of *E. alabamensis* is the first such instance among the bovine species of *Eimeria*, but this has been described in rodent coccidia. Roudabush (1937) reported the presence of two small eosinophilic globules in the first-generation of merozoites of *E. nieschulzi* in the rat. He described these globules as very strikingly similar to those in the sporozoites, but they
appeared different in iron hematoxylin preparations. Recently, Hammond, Speer, and Roberts (1970) found typical refractile bodies in first-generation merozoites of *E. bilamellata* and *E. callospermophili* grown in cell cultures. These bodies appeared similar to those of the sporozoites, whereas the so-called paranuclear bodies reported by Colley (1968) in his electron microscopical observations of merozoites of *E. nieschulzi* were somewhat different.

The effect of *E. alabamensis* on the host cell resembled that of *E. bovis* with respect to the enlargement of the host nucleus. This was more pronounced in cells harboring *E. alabamensis* stages within the host cell nucleus. Enlargement of the nucleolus and a disappearance of the chromatin clumps in the host cell nucleus as in *E. bovis* and *E. ninakohlyakimovae* infections, however, usually did not occur in *E. alabamensis*. The clear spaces around the cells infected with *E. bovis* (Fayer and Hammond, 1967) were observed in *E. alabamensis* infections. The occurrence of multiple host cell nuclei in *E. alabamensis* infections was similar to that observed for *E. auburnensis*, but occurred less frequently.

The development of *E. alabamensis* in cell cultures differed considerably from that in calves as described by Davis, Boughton, and Bowman (1955). Extracellular sporozoites in cultures were similar in size to the range of 5.6 to 9.8 by 1.4 as reported from the calf, but nothing similar to the nonmotile sporozoites measuring 15 in length found in the calf was seen in cultures. Living intracellular sporozoites,
measured after 1 day in culture, were similar to those seen in calves but varied less in size. Fixed trophozoites in cultures at 2 days were slightly larger than the immature schizonts (2.8 to 3.5, average, 3.1, in diameter) observed in calves at 2 days (Davis, Bowman, and Boughton, 1957). Possibly, differences in methods of fixation and staining may have been responsible, at least in part, for this difference in results.

In calves, mature schizonts at 3 days after inoculation had a mean size of 8.4 by 7.4, whereas those seen at 4, 6, and 8 days after inoculation had mean sizes from 11.2 by 8.9 to 12.6 by 9.7. It was suggested that this size difference might have been associated with a difference in stage of development or with the occurrence of more than one generation of schizonts (Davis, Bowman, and Boughton, 1957). The smaller of the two kinds of schizonts observed in cell cultures was intermediate in size between the large and small types reported from calves, whereas the larger schizonts in cell cultures exceeded in size the large schizonts in calves. Mature schizonts were observed 1 day earlier in cultures than in calves, probably because of the time required for excystation and reaching the site of infection in the latter.

Mature schizonts, measured at 4 days after inoculation in the calf, had 16-32 mature merozoites which were 7-9 in length; these merozoites found in the calf and those found 3 days after inoculation in cell cultures were similar in size. Schizonts with more than 14 merozoites were not found in cell cultures. Merozoites found free in the crypts of Lieberkühn on days 6 and 8 measured 3-5 by 1.4 and 4.2 by 1.4, respectively.
The short stubby merozoites found in cultures at 2 and 3 days after inoculation were similar in size to those observed 6 and 8 days after inoculation in calves.

**Fine Structural Observations**

**Sporozoites**

The pellicle covering the surface of *Eimeria alabamensis* sporozoites had an inner "membrane" consisting of two closely applied unit membranes similar to that reported for *Toxoplasma gondii* (Vivier and Petitprez, 1968). These investigators found that the outer membrane was a plasma membrane, whereas the two membranes lying beneath it formed an "inseparable whole of changeable molecular architecture" (Vivier and Petitprez, 1968, p. 329).

The posterior pore found in sporozoites of *E. tenella* (Ryley, 1969) and *E. callospermophili* (Roberts, Hammond, and Speer, 1970) was not found to be present in sporozoites of *E. alabamensis* nor in any other ruminant species of *Eimeria* (Roberts and Hammond, 1970).

The polar ring surrounding the conoid appeared to be formed by a thickening at the anterior termination of the inner membrane; thus, it is similar in derivation to that in *E. auburnensis*, *E. ninakohlyakimovae*, *E. ellipsoidalis*, and *E. bovis* (Roberts and Hammond, 1970); *E. nieschulzi* (Colley, 1967); and *E. tenella* (Ryley, 1969). Colley (1967), Ryley (1969), and Roberts and Hammond (1970) found that the polar ring was apparently attached to the subpellicular microtubules in
E. auburnensis, E. bovis, E. ellipsoidalis, E. ninakohlyakimovae, E. tenella, as well as E. nieschulzi, respectively. Attachment of the subpellicular microtubules to the polar ring was not observed in E. alabamensis nor in E. miyairii by Andreassen and Behnke (1968). Recently, Roberts, Hammond, and Speer (1970) observed two polar rings in E. callospermophili, one attached to the inner membrane and the second to the subpellicular microtubules.

The 22 subpellicular microtubules in E. alabamensis were evenly distributed around the cell. This arrangement was found in E. auburnensis, E. bovis, E. ellipsoidalis, and E. ninakohlyakimovae (Roberts and Hammond, 1970); E. tenella (Ryley, 1969), all of which possess 24 microtubules; and in E. nieschulzi (Colley, 1967) which had 25 microtubules. In E. callospermophili (Roberts, Hammond, and Speer, 1970), however, the 24 microtubules were arranged in loose pairs about the periphery of the cell.

The numerous large polysaccharide granules found in E. alabamensis were similar to those found in E. auburnensis, E. bovis, E. ellipsoidalis, E. ninakohlyakimovae (Roberts and Hammond, 1970) and E. tenella (Ryley, 1969). In E. callospermophili, however, these granules were located only at the peripheral margin of the refractile body (Roberts, Hammond, and Speer, 1970). The rapid disappearance of the polysaccharide granules during the first 24 hours after inoculation of sporozoites into cultures might indicate that the sporozoite of E. alabamensis relies heavily upon its stored carbohydrate for the energy used during the
early period of intracellular existence.

The free sporozoites of *E. tenella* (Ryley, 1969) and the ruminant species described by Roberts and Hammond (1970) all lacked a distinct nucleolus. Roberts and Hammond (1970) reported the presence of a nucleolus in *E. ellipsoidalis* and *E. auburnensis* with the use of the light microscope but failed to find it with the electron microscope. A diffuse nucleolus was reported in the extracellular sporozoites of *E. nieschulzi* (Colley, 1967).

In *E. alabamensis* no nucleolus was seen in extracellular sporozoites or in intracellular sporozoites fixed 5 minutes after inoculation. In specimens fixed 6 hours after inoculation, a nucleolus was seen with the light microscope but not with the electron microscope. In specimens fixed 24 hours and later, a nucleolus was observed in specimens with the electron microscope as well as with the light microscope. In *E. callospermophili*, a nucleolus was observed in intracellular sporozoites in preparations fixed 10 hours or more after inoculation but not in free sporozoites (Roberts, Hammond, and Speer, 1970).

The appearance of the Golgi complex was similar to that found in *E. callospermophili* (Roberts, Hammond, and Speer, 1970) and *Toxoplasma* (Sheffield and Melton, 1968). The close association of this organelle with the nuclear envelope may indicate a possible origin from the latter. Sheffield and Melton (1968) observed outpocketings of the nuclear envelope into the Golgi area, suggesting that Golgi vesicles originate in this manner. The lack of chromatin on the inner side of the
nuclear envelope and the absence of ribosomes in the adjacent cytoplasm might indicate the occurrence of an active membrane formation. Possibly, the membrane-bound vesicles found adjacent to the nuclear envelope are formed by pinching off from the latter, similar to the formation of annulate lamellae in other systems (Kessel, 1968).

The micropore is a structure common to a number of members of the Sporozoa. The occurrence of this organelle in various species has been reviewed by Roberts and Hammond (1970). Snigirevskaya (1968) postulated that *Eimeria intestinalis* has a micropore active in ingestion of nutrients on the basis of its presence in all developmental stages except the microgametes and the similarity of its shape and size at all stages. The sporozoite of *E. tenella* was reported to have an active micropore during its initial existence in tissue cultures (Scholtyseck and Strout, 1968). Ryley (1969) presented evidence in *E. tenella* of a connection between the micropore and a duct-like structure leading into the cell to a vacuole. The appearance of numerous micropores with indications of active vacuole formation and the presence in the cytoplasm of food vacuoles containing particulate matter indicates that *E. alabamensis* feeds by phagotrophy. The presence of host cell cytoplasm within the micropore and evidence that food vacuoles are formed by pinching off substantiates the view that *E. alabamensis* feeds through the micropore on host cell cytoplasm in a manner somewhat similar to that described by Aikawa et al. (1966) and Aikawa, Huff, and Sprinz (1967) in avian and
mammalian Plasmodium species. This is the first report of such activity in Eimeria from ruminants.

One would expect to find lysosomes or similar structures associated with digestion of the food taken into the parasite. Pelomyxa (Roth 1960) and Paramecium (Esteve, 1970) have such organelles. Recently, the presence of acid phosphatase has been demonstrated in food vacuoles in Tetrahymena (Elliot and Clemmons, 1966). Aikawa, Huff, and Sprinz (1967) failed in their attempts to demonstrate this enzyme in the food vacuoles of Plasmodium elongatum. Heller and Scholtyseck (1970) recently found in a cytochemical study with the electron microscope of coccidia that alkaline phosphatase was associated with the organelles involved in food intake (micropore, food vacuoles) and also occurred in the parasitophorous vacuole. Acid phosphatase was found in those vacuoles and vesicles concerned with metabolic functions. ATPase was found in various vesicles and the nuclear membrane.

Eimeria alabamensis had but one refractile body, whereas many other Eimeria species usually have two. Later, after the sporozoites of E. alabamensis had been within cells for some time, they had an anterior refractile body. The group of lightly staining bodies, observed at the anterior end of some specimens, were probably derived from the posterior refractile body (Sampson, Hammond, and Ernst, 1970); these may coalesce to form the anterior refractile body. It has been shown that refractile bodies undergo alterations in shape and position within the sporozoite (Clark and Hammond, 1969; Fayer, 1969; Fayer and Hammond, 1969; Speer and Hammond, 1970b). The changes in the refractile body observed
in the light microscope study of *E. alabamensis* (Sampson and Hammond, 1969), were more pronounced than those seen in the fine structural study. The variation of the staining intensity among the refractile bodies in sporozoites, found at different intervals during the first 2 days of intracellular existence, is interesting. Although a previous cytochemical study (Hammond, Chobotar, and Ernst, 1968) has shown that this organelle exhibits a staining reaction indicating a protein nature, the variation in staining intensity with osmium tetroxide and the coalescing activity might suggest the presence of lipid. Lipids have been observed to fuse in other systems (Foor, 1966). The variability of the staining of such organelles seen in sporozoites may be related to the degree of saturation of lipids therein. The apparent close association of these organelles with mitochondria, which contain the enzymes for the metabolism of triglycerides, may be another indication for the presence of lipid. Further cytochemical and/or biochemical studies will have to be completed before the composition of the refractile body can be resolved.

**Trophozoites**

Trophozoites, fixed 48 hours after inoculation of sporozoites into cultures of MDBK cells, appeared to retain most of the organelles of the sporozoite. The transformation to a spheroidal shape was accompanied by an increase in organelles necessary for active protein synthesis, presumably in preparation for repeated nuclear division and merozoite
formation. The enlargement of the nucleus along with the fibrillar appearance of the chromatin is similar to the changes occurring in activated nuclei reported in the trophozoites of Plasmodium berghei (Ladda, 1969). This is related to the initiation of RNA, DNA, and protein synthesis described in other species of Plasmodium (Polet and Barr, 1968). An increase in the number and size of ribosomes was found to occur in the trophozoite of Toxoplasma (van der Zypen and Piekarski, 1966).

Centrioles were found during schizogony in E. magna, E. tenella (Senaud and Cerna, 1969), E. pragensis (Senaud and Cerna, 1968), E. intestinalis (Snigirevskaya, 1969), and E. bovis (Sheffield and Hammond, 1967) and during microgametogenesis of E. auburnensis (Hammond, Scholtyseck, and Chobotar, 1969) and E. magna (Snigirevskaya, 1968). The centrioles in E. alabamensis appeared similar in substructure to those of E. callospermophili (Roberts et al., 1970). Eimeria magna and E. tenella (Senaud and Cerna, 1969) and E. pragensis (Senaud and Cerna, 1968) were reported to have nine dark masses surrounding a tenth central element. It was not reported whether these structures were single or double.

The associated intranuclear spindle apparatus found in E. alabamensis trophozoites and binucleate schizonts was similar to that reported for schizonts of E. callospermophili (Roberts et al., 1970). However, in this species the spindle had a central location in early
stages and an eccentric location in the later stages in which merozoites were forming. In *E. alabamensis*, both locations were observed in different specimens of trophozoites undergoing nuclear division; in later stages only the eccentric location was seen. The morphology of the apparatus was similar to that reported in eccentrically located spindles in microgametocytes of *E. auburnensis* (Hammond, Scholtyscek, and Chobotar, 1969). Intranuclear spindle fibers have also been reported from various species of *Plasmodium* during the formation of sporozoites (Terzakis, Sprinz, and Ward, 1967) and merozoites (Aikawa, Huff, and Sprinz, 1967). In these studies, centriolar plaques occupied the polar areas. Robinow and Marak (1966) stated that the spindle apparatus found in dividing yeast cell nuclei, because of its variable location, contributes a "form-giving rigidity" to division rather than the force needed for nuclear division.

*Eimeria callospermophili* (Roberts et al., 1970) had a cone-like structure at the poles of the spindle apparatus. The "nuclear polar areas" in *Toxoplasma* (Sheffield and Melton, 1968; Sheffield, 1970), "nuclear poles" in *Besnoitia* (Sheffield, 1966), "polar protuberances" in *E. auburnensis* microgametocytes (Hammond, Scholtyscek, and Chobotar, 1969), "centrocone" in *Toxoplasma* and *Sarcocystis* (Senaud, 1967), *E. pragensis* (Senaud and Cerna, 1968), *E. magna*, and *E. tenella* (Senaud and Cerna, 1969) are similar structures. In *E. alabamensis*, the polar cone appeared to extend from the nucleus into the developing merozoite.
The nuclear envelope remains intact during division in various sporozoan species, including Toxoplasma (Sheffield and Melton, 1968), Plasmodium (Aikawa, Huff, and Sprinz, 1967; Ladda, 1969), and Eimeria (Hammond, Scholtyseck, and Chobotar, 1969; Roberts et al., 1970). This was also found to occur in E. alabamensis.

**Multinucleate schizonts**

Trophozoites are a characteristic stage in the development of many species of Eimeria (Scholtyseck, 1965; Senaud and Cerna, 1968, 1969). The sporozoite-shaped schizont was first described by Chobotar, Hammond, and Miner (1969) in their light microscope study of the development of E. auburnensis in calves. The *in vitro* study of this species by Clark and Hammond (1969) confirmed this observation. More recently, Speer and Hammond (1969) and Speer, Hammond, and Anderson (1970) have found such a stage in the development of Eimeria callospermophili grown in cell cultures and in the host animal. These investigators reported that this stage is capable of locomotion and penetration of host cells. E. alabamensis in cell cultures may have sporozoite-shaped schizonts or trophozoites in their development. In an earlier study on the growth of this parasite in cell cultures (Sampson and Hammond, 1969), motility of sporozoite-shaped schizonts was not observed.

The sporozoite and the sporozoite-shaped schizont usually undergo a dedifferentiation process during transformation to the trophozoite and the spheroidal schizont, respectively (Hammond, 1969). The gradual
breakdown of the inner membrane and the loss of organelles, characteristic of the transforming sporozoite of *E. alabamensis*, is similar to that reported in other *Eimeria* species. Colley (1968) stated that young schizonts of *E. nieschulzi* possessed micronemes but were limited by a single membrane. Recently penetrated merozoites of *E. tenella* retained only remnants of surface fibrils as they transformed into trophozoites (McLaren, 1969). Snigirevskaya (1969) reported that trophozoites in *E. intestinalis* lacked the organelles characteristic of merozoites and stated that these organelles were probably synthesized *de novo* during schizogony. Sporozoites and merozoites of *Plasmodium* spp. rapidly dedifferentiate shortly after penetration (Ladda, 1969). Clark and Hammond (1969); Kelley and Hammond (1970); Sampson, Hammond, and Ernst (1970); Speer and Hammond (1970b) reported that the transformation process occurred by a lateral cytoplasmic expansion along one side of the sporozoite or by a gradual widening of the body.

**Merozoite formation**

*Eimeria perforans* and *E. stiedae* from rabbits form merozoites by a splitting process (Scholtyscek, 1965). Endoplasmic reticulum, distributed throughout the cytoplasm of the schizont, isolates developing merozoites, and later these individuals break away from the mother cell. Nothing was mentioned as to the origin of the organelles. In the species of *Eimeria* which form large schizonts, such as *E. bovis* (Hammond, Ernst, and Miner, 1966), *E. auburnensis* (Chobotar, Hammond, and
Miner, 1969), and *E. ninakohlyakimovae* (Wacha and Hammond, 1970), the nuclei become arranged at the periphery of the schizont. Later, invaginations or infoldings occur whose walls are lined with the nuclei; this results in blastophore formation. Merozoites are formed at the surface of the blastophores or of the invaginations by a kind of radial budding. *E. magna, E. tenella, E. pragensis* (Senaud and Cerna, 1968, 1969), and *E. nieschulzi* (Colley, 1968) have small schizonts similar to those of *E. perforans* and *E. stiedae*. The merozoites, however, bud radially from the schizont surface as in *E. bovis*. Although there was no migration of daughter nuclei to the periphery of second-generation schizonts in *E. tenella* (McLaren, 1969), merozoites were formed by budding.

The ultrastructural events in the formation of merozoites of *E. bovis* are initiated by the synthesis of an inner membrane below the single limiting membrane of the schizont (Sheffield and Hammond, 1967). A site of inner membrane production occurs adjacent to each daughter nucleus. A conoid and other organelles are then formed, and as each developing merozoite protrudes from the schizont an infolding of the surface membrane takes place to further separate the cell. In *E. alabamensis* schizonts, the earliest stages of merozoite formation were not observed. In spheroidal schizonts, which were apparently formed from the trophozoite in *E. alabamensis*, merozoite anlagen develop beneath the pellicle of the schizont and adjacent to the nucleus, thus resembling merozoite formation in *E. bovis* (Sheffield and Hammond, 1967). The further
development of merozoites in this type of schizont results in the budding appearance. The short, stubby merozoites, found 2 and 3 days after inoculation of sporozoites into cell cultures (Sampson and Hammond, 1969), are presumed to be the products of this type of schizont.

The earliest stages of merozoite formation in *E. callospermophili* (Roberts et al., 1970) resembles in some respects the process of endodyogeny in *Toxoplasma* (Senaud, 1967; Sheffield and Melton, 1968), *Besnoitia* (Sheffield, 1966, 1970), and *Sarcocystis* (Senaud, 1967). Recently, Sheffield (1970) has described a kind of schizogony in *Toxoplasma gondii*, the early stages of which are similar to the comparable stages of *E. callospermophili* (Roberts et al., 1970). In the former species, the daughter organisms at first appeared as "two open top dome-shaped inner membranes" situated adjacent to the Golgi complex of a parent nucleus. In *E. callospermophili*, centrioles and polar cones were closely associated with merozoite anlagen, suggesting the possibility that they served as an inducing system for merozoite formation.

In the earliest stage of merozoite formation observed in a recently transformed sporozoite-shaped schizont, each interiorly located anlagen of the merozoites had a conoid, rhoptries, and or microneme anlagen and subpellicular microtubules. This resembles a stage in the internal formation of merozoites as described in *E. callospermophili* by Roberts et al. (1970). In *E. callospermophili*, only about one-third of the body of the merozoite is formed before it protrudes from the schizont surface. The merozoites of *E. alabamensis*, formed in this manner, appear long and
slender rather than short and stubby as were the merozoites from spheroidal schizonts. Thus, the former merozoites apparently correspond with those which were formed by the small, compact schizonts 3 days after inoculation of sporozoites (Sampson, Hammond, and Ernst, 1970).

The daughter organisms in Toxoplasma (Vivier and Petitprez, 1968) and Eimeria (Roberts et al., 1970) apparently acquire the outer membrane of their pellicle from the parent cell. This occurs similarly in E. alabamensis in both types of merozoite formation. In Toxoplasma, however, Vivier and Petitprez (1968) stated that the daughter organisms also acquire their inner membrane complex from the parent organism. This is associated with the extension of the inner membrane complex into the interior of the organism in a fashion similar to that found in E. alabamensis in the present study. Such a phenomenon is not known to occur in other Eimeria species. These internal extensions of the inner membrane in E. alabamensis appear to be related to the isolation of the merozoite anlagen within the parent cell, as found by Vivier and Petitprez (1968) in Toxoplasma.

The presence of a complete pellicle in schizonts of E. alabamensis was apparently associated with the sporozoite-shaped schizont and the spheroidal form into which it transformed. E. callospermophili (Roberts et al., 1970), Toxoplasma (Senaud, 1967; Sheffield and Melton, 1968), and Sarcocystis (Senaud, 1967) are known to retain the complete pellicle during at least the early stages of formation of daughter organisms.
Host cell changes

The intracellular stages of *Eimeria* species are typically each harbored in a "parasitophore vacuole." Scholtyseck and Piekarski (1965) described the "parasitophore vacuole" in their study of the fine structure of merozoites. Later, Hammond, Scholtyseck, and Miner (1967) used the term "parasitophorous vacuole" to describe the space in the host cell within which the parasite was located. Stehbens (1966) used the term "periparasitic vacuole" in his description of the vacuole containing *Lankesterella hylae*.

Vesicles and extensions of the host cell membrane and cytoplasm into the parasitophorous vacuole have been described by a number of workers. Sheffield and Melton (1968) indicated that such structures, which they termed "stereocilia," possibly originate from the membrane lining the parasitophorous vacuole in *Toxoplasma gondii* infections. Porchet-Hennere (1967) reported the presence of membrane formations, often having a myelin-like appearance, in the vicinity of the intracellular stages of *Coelotropha durchoni*. The myelin-like bodies, observed in *Selenidium* (Vivier and Schrevel, 1964), *E. alabamensis*, as well as the structures consisting of tightly wound membranes associated with *Myriosporides* (Hennere, 1967a), are all located in the parasitophorous vacuole. Scholtyseck (1969) indicated that the parasitophorous vacuole may represent a host defense mechanism. Pal (1967) stated that the vigorous migration of mitochondria of the host cell to the border of the parasitophorous vacuole in *Toxoplasma* is evidence of energy drainage.
This condition is found in cells infected with *E. alabamensis* also. In *E. alabamensis*, the occurrence of numerous folds of the membrane, limiting the parasitophorous vacuole adjacent to the accumulation of host cell mitochondria, suggests that the mitochondria supply energy for membrane synthesis.


Untersuchungen an Merozoiten von Eimerien (Eimeria perforans und
E. stiedae) und Toxoplasma gondii. Zun systematischen Stellung

Scholtyseck, E., and R. G. Strout. 1968. Feinstrukturuntersuchungen
über die Nahrungsaufnahme bei Coccidien in gewebekulturen

Merozoiten von Eimeria tenella in Makrophagen. Z. Parasitenk.
32:284-296.

structure of the schizont and merozoite of Isospora sp. (Sporozoa:
Eimeriidae) parasitic in Gehyra variegata (Dumeril and Bibron,

Senaud, J. 1967. Contribution a l'étude des sarcosporidies et toxo­

Senaud, J., and Z. Cerna. 1968. Etude en microscopie electronique des
merozoites et de la merogonie chez Eimeria pragensis (Cerna et
Senaud, 1968), coccidie parasite de l'intestine de la soris (Mus

Senaud, J., and Z. Cerna. 1969. Etude ultrastructurale des merozoites
et de la schizogonie des coccidies (Eimeriina): Eimeria magna
Perard, 1925, de l'intestine des lapins et E. tenella (Railet et

Unpublished PhD dissertation. University of Illinois Library,
Urbana, Illinois.


microscope observations on the sporozoites of Eimeria bovis in
cultured bovine kidney cells. J. Protozool. 15(Suppl.):18.


VITA

J. Robert Sampson

Candidate for the Degree of

Doctor of Philosophy

Dissertation: Growth of *Eimeria alabamensis* from Cattle in *vitro* and the Changes in Fine Structure Associated with Schizogony

Major Field: Zoology

Biographical Information:

Personal Data: Born at Streator, Illinois, April 15, 1940, son of Robert E. and Margaret M. Sampson.

Education: Attended elementary school in Dwight, Illinois; graduated from Dwight Township High School in 1958; received the Bachelor of Science degree in Education from Northern Illinois University in 1964, with a major in biology and minors in mathematics and education; received the Master of Science degree from Northern Illinois University with a major in zoology in 1965.