Ecology and physiology of parasites

A SYMPOSIUM

Increasing wisdom and ingenuity are required if we are to master our environment and cope with the myriad of organisms that affect our existence. Not the least of these organisms are the parasites and pathogens which can be found in all animals. The ecological implications of parasitism are obvious, and the interrelationships among different organisms within the same host are fascinating, but more knowledge and understanding are needed.

The symposium was held to stimulate discussion of the significance of ecological problems presented by parasites and to develop means of attacking some of these problems. The diversity of parasitism from protozoa to arthropods was emphasized and the speakers and topics were selected to interest those in various biological disciplines and professions. Organized by the Department of Parasitology in the School of Hygiene of the University of Toronto, and held at Toronto in February 1970, the symposium was an unqualified success. The enthusiastic interest, indicated by the attendance of over three hundred people from seven countries, and numerous requests for copies of the proceedings led to the publication in this volume of the twelve papers presented at the symposium.

The opening remarks of the leader of the discussion which followed each paper have been included and a complete bibliography is provided for each topic. The contributors are leading specialists in their fields; their papers present the results of the most recent research and assemble and review the scattered literature on each topic. The text is illustrated throughout with diagrams and photographs.

Parasitism and associated phenomena are excellent examples of problems requiring the interdisciplinary approach taken by the symposium. The results of such an approach are useful in a wide variety of disciplines: microbiology, invertebrate zoology, entomology, and tropical medicine, as well as parasitology.

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Ecology and physiology of parasites

A symposium held at University of Toronto 19 and 20 February 1970

EDITED BY A. M. FALLIS

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Preface

The role of the infinitely small in nature is infinitely great.

Pasteur as quoted by René Dubos in *The Unseen World*, Rockefeller Press, 1962

Increasing wisdom and ingenuity are required if we are to master our environment and cope with the myriad of organisms that affect our existence, not the least of which are the parasites and pathogens which occur in all types of animals. This is not to suggest that every parasite causes disease. On the contrary, as Theobald Smith pointed out fifty years ago, parasitism is a normal phenomenon and the interplay between host and parasite is continuous. The ecological implications of such a situation are obvious, but knowledge is scanty. The interrelationships among different organisms within the same host are fascinating although not well understood. Indeed, only recently the eminent parasitologist W. P. Rogers of Australia remarked, "there is no satisfactory explanation, in physiological terms, for the parasitic habits of any animal parasite." Parasitism and associated phenomena suggest, moreover, excellent examples of problems requiring interdisciplinary research and provide model systems which can be used to furnish exciting information. The possibilities for such uses increase as knowledge of the physiology and ecology of the parasites expands.

A group of colleagues speculated on the significance of, and means of attacking, some of these ecological problems. "Why not," said one, "assemble a group of experts to discuss their diverse researches?" "Let us select topics and speakers," said another, "to emphasize the diversity of parasitism from protozoa to arthropods and interest those in various biological disciplines and professions." This seemed a commendable plan since frequently biologists, because of an understandable interest in life in water and on land, overlook the third type of environment, namely that within other animals. The enthusiasm of colleagues in the Department of Parasitology, and others with whom the idea was discussed, set in motion plans for a symposium. Their realization was made possible by the generous financial

support of the President's Fund, the School of Graduate Studies, the Medical Research Council, and the Wellcome Trust. Publication of the proceedings was facilitated by grants from the Wellcome Trust and the Canadian National Sportsmen's Show.

The venture, as indicated by the papers that follow and an attendance of almost three hundred from seven countries, was an unqualified success. The planning committee is grateful for the encouragement and support of Dr A. J. Rhodes, Director, School of Hygiene, and Dr D. A. Chant, Chairman, Department of Zoology. We are especially indebted to Miss Ourom of the editorial staff of the University of Toronto Press. Her meticulous care and editorial skills are largely responsible for the assembly of the papers in this volume. Special gratitude is owing to Mrs N. Doughty and Mrs M. Staszak for their execution of the seemingly endless secretarial tasks and their conscientious attention to detail in planning the symposium.

Finally the authors deserve our thanks for submitting illustrations and manuscripts requiring minimal editorial changes. We are grateful also to those who led the discussion for their pertinent comments. Possibly some who read the papers will be inspired to investigate problems that remain. The range is enormous. Answers to some, as in the past, will lead to improved health of man and his animals and could change the course of history. Solutions of others will provide knowledge for later application and satisfaction and encouragement to all who probe the unknown in search of it.

A.M.F.
Toronto, March 1970

Ecology and physiology of parasites

A SYMPOSIUM

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The development and ecology of coccidia and related intracellular parasites

DATUS M. HAMMOND

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In recent years, the use of the electron microscope and *in vitro* cultivation have resulted in important advances in our knowledge of the development and ecology of intracellular parasites, as well as of their interrelationships with the host. In this paper, I shall be concerned chiefly with the genera *Eimeria* and *Isospora*, and other related Sporozoa, especially *Toxoplasma*, *Besnoitia*, *Sarcocystis*, and *Plasmodium*. The stages beginning with the oocyst or sporozoites and ending with the first-generation merozoite will be emphasized.

EXCYSTATION

In *Eimeria* species of ruminants in which excystation has been recently studied, two kinds of stimuli, acting in sequence, appear to be necessary to bring about excystation (Jackson 1962; Nyberg and Hammond 1964). The first stimulus, consisting of exposure of the oocysts to carbon dioxide, occurs normally in the rumen. Oocysts altered as a result of response to this stimulus will usually undergo excystation when exposed to the second stimulus, trypsin and bile (Lotze and Leek 1960) or bile salts (Hibbert, Hammond, and Simmons 1969).

The carbon dioxide apparently stimulates the activation or the production of an enzyme, or an enzyme rate-limiting step, which causes a change in the permeability of the micropyle (Hibbert and Hammond 1968). Recently, evidence for the occurrence of such an enzyme or enzyme system was observed in an experiment in which *E. bovis* oocysts were incubated in supernatant fluid previously used for excystation of oocysts and then treated with a trypsin–bile salt mixture (Hibbert and Hammond, in preparation). Some excystation of the oocysts occurred and this took place also when trypsin inhibitor was added to the supernatant. Oocysts incubated in heated supernatant before treatment in trypsin and bile

salt did not excyst. Thus, the presence in the fluid of a heat-labile agent which affects excystation is suggested by these results.

The oocysts of *Eimeria* species from chickens are broken mechanically in passing through the gizzard, and the sporozoites escape from the sporocysts when these are exposed to bile and trypsin in the small intestine (Doran and Farr 1962). Oocysts which remain intact apparently do not undergo excystation, although Lotze and Leek (1968, 1969) found that some such oocysts of *E. tenella* contained active sporozoites when recovered from the small intestine, large intestine, or faeces one to three hours after inoculation. Some of these oocysts had altered walls, possibly as a result of exposure to enzymes or carbon dioxide in the upper digestive tract.

E. tenella oocysts responded to exposure to carbon dioxide in a manner similar to that of ruminant coccidia (Nyberg, Bauer, and Knapp 1968). Ninety per cent of the oocysts contained active sporozoites after 18 hours of exposure to carbon dioxide and 8 hours of incubation in trypsin and bile. However, we found that little or no complete excystation, i.e., escape of sporozoites from the oocyst as well as the sporocyst, occurred in oocysts of *E. acervulina* and *E. necatrix* from chickens, or in two species of *Eimeria* from ground squirrels, after the oocysts had been exposed to carbon dioxide and then trypsin and bile under the usual conditions (Hibbert and Hammond 1968). Moderate levels of excystation occurred in all four of these species when the percentage of carbon dioxide was increased from 50 to 90, and in the two species from chickens when the duration of exposure to carbon dioxide was extended from 10 to 30 hours or longer. Thus, it appears that oocysts of *Eimeria* species of ruminants differ from those of chickens and certain rodents in that, in the former, sporozoites evidently escape from the intact oocyst more readily. These species may differ little, if at all, in the conditions under which sporozoites escape from the sporocysts, however.

Oocysts of different species are known to excyst at different rates, and in certain species of *Eimeria* from poultry (Farr and Doran 1962) and from ruminants (Hibbert and Hammond 1968) the rate of excystation is correlated with the distance which must be travelled in the digestive tract to reach the usual site of development. In these instances, species developing in the anterior portion or middle of the small intestine excyst faster than species developing in the posterior small intestine or large intestine.

The most rapid rate of excystation of sporozoites from sporocysts in any species studied in our laboratory occurs in *E. utahensis* from the kangaroo rat, *Dipodomys ordii*. This species of *Eimeria* has sporocysts with relatively large substiedal bodies. These are plug-like structures lying immediately under the Stieda body, which is a thickened area of the sporocyst wall at one end of the sporocyst. During excystation, the Stieda body appears to swell and then dissolve or disintegrate. The substiedal body is pushed outward, slowly at first, and then explosively, leaving a relatively large opening through which the sporozoites immediately escape. Excystation in *E. utahensis* occurs within 15 seconds to 2 minutes after exposure of sporocysts to a trypsin–bile salt mixture (Hammond, Ernst, and Chobotar 1970). Such excystation in *E. bovis* usually requires 35 to 40 minutes. The substiedal body appears to play an important role in excystation in this species and in *E. larimerensis* and *E. callospermophili* from the Uinta ground squirrel. In the latter two species, bile or bile salts were observed to stimulate the motility of free sporozoites as well as sporozoites within free sporocysts, but excystation did not occur unless trypsin was present (Roberts, Speer, and Hammond 1970).

Little is known about the ability of coccidia to survive as oocysts, free sporozoites, or intracellular stages within hosts such as ground squirrels over the hibernation season. In a recent study (Anderson and Hammond 1969) 20 captive Uinta ground squirrels were inoculated with one of three species of *Eimeria* 1 to 3 days before they became torpid as a result of their being placed in a chamber kept at 7° c. Oocysts, evidently from the inoculum, were discharged for 8

to 239 (mean, 182) days during the brief periods of activity which occurred in the torpid animals at intervals of 4 to 10 days. When the squirrels awakened after removal from the cold chamber in April, 16 of the 20 became infected, the intervals between awakening and the first discharge of oocysts being similar in the majority to the normal prepatent period. These findings indicate that the oocysts and/or early developmental stages survived in the torpid subjects from September through April, and suggest that such survival may occur in hibernating squirrels.

PENETRATION OF HOST CELLS

Invasion of host cells by living sporozoites has been observed with the light microscope in *E. bovis* (Fayer and Hammond 1967), *E. auburnensis* (Clark and Hammond 1969), *E. ninakohlyakimovae* (Kelley and Hammond 1970a), and *E. callospermophili* and *E. bilamellata* (Speer, Hammond, and Anderson 1970). In each of these observations, the sporozoite underwent a gliding movement immediately before penetrating the host cell, and began entering the host cell with its anterior end first, with no evident cessation or marked deceleration of the gliding movement. Usually, only a few seconds were required for invasion of the host cell. In *E. bovis*, when observations were made without a heating stage, sporozoites penetrated host cells more slowly, and sometimes stopped in the process of penetration.

In *E. ninakohlyakimovae* (Kelley and Hammond 1970a) the anterior portion of the sporozoite narrowed to about one-third of the normal body width as it entered the host cell (Fig. 6), the remainder of the body being somewhat wider than usual at this time. The narrowed portion of the sporozoite evidently passed through a relatively small opening in the cell membrane of the host cell. Such an opening, however, can be observed only with the use of the electron microscope. In a similar study of the development of *E. callospermophili* in cultured cells, a sporozoite-shaped schizont (see below) was seen in the process of penetrating a host cell whose surface membrane was interrupted at the site of entrance (Roberts, Hammond, and Speer 1970). This finding indicates that sporozoite-shaped schizonts and sporozoites of *Eimeria* enter host cells through a discontinuity in the cell membrane. Because it develops so rapidly, this opening is probably made chiefly by mechanical means.

In a later stage of penetration of a host cell by a sporozoite of *E. ninakohlyakimovae*, the anterior portion of the sporozoite which was within the host cell expanded, but the part of the body passing through the opening in the cell membrane remained constricted (Fig. 7). When the sporozoite was about halfway within the host cell, a minute, sharply pointed protrusion appeared at the anterior end (Fig. 8). This protrusion may represent the conoid, which possibly plays a role in penetration. McLaren and Paget (1968) proposed that the conoid apparatus of *E. tenella* is extrudable and retractable or includes an extrudable and retractable element. Ryley (1969) found that extruded conoids occurred in negatively stained or shadowed sporozoites of *E. tenella*. In a scanning electron microscope study, Vetterling and Madden (1969) observed protruding conoids at the anterior ends of excysted sporozoites (which they termed "activated") and suggested that the conoid apparatus is used to penetrate cells. In electron microscope studies of the sporozoites of four *Eimeria* species, we have observed specimens of free, presumably "activated," sporozoites with conoids in the retracted position as well as in the protruded position (Roberts and Hammond 1970). This indicates that the location of the conoid in excysted sporozoites is more labile than implied by Vetterling and Madden.

The paired or club-shaped organelles (also called rhoptries) may play a role in penetration, as suggested by McLaren and Paget (1968) for *E. tenella*, but in electron microscope studies of intracellular sporozoites of *E. bovis* in cell cultures no appreciable alteration in the appearance of these structures as compared with those of ex-

tracellular sporozoites was found (Sheffield and Hammond 1968). In *Besnoitia jellisoni*, organisms rapidly enter host cells in cell cultures (Fayer *et al.* 1969). Sometimes a stylet-like tip was observed at the anterior end of pentrating organisms; the body underwent constriction while passing through the host cell membrane. Jadin and Creemers (1968), however, observed injury in the cell membrane and adjacent cytoplasm in a red cell apparently in an early stage of invasion by *Toxoplasma*, and suggested that a proteolytic substance escaping from the conoid region of the *Toxoplasma* was digesting a portion of the erythrocyte.

Recently, it has been found in an electron microscope study that merozoites of *Plasmodium berghei yoeli* and of *P. gallinaceum* begin entering erythrocytes by contacting these with the conoid region (Ladda, Aikawa, and Sprinz 1969). The proper orientation of the *Plasmodium* merozoite towards the host cell was considered to be a passive event, occurring randomly, whereas the sporozoite of *Eimeria* species is moving anteriorly at the time penetration is begun, so that its proper orientation towards the host cell is an active event. A depression in the surface of the red cell occurs at the point of contact with the *Plasmodium* merozoite; the depression deepens to form a cavity as the merozoite enters the cell. When the merozoite is entirely within the cavity, the cell membrane of the host cell pinches together at the surface. The paired organelles decrease in size and density during the early stages of penetration, indicating that they may contain substances that assist in penetration. The host cell membrane remains intact throughout the invasion process, and becomes invaginated to form the lining of the vacuole surrounding the parasite. The manner of penetration of host cells by *Plasmodium* species might be expected to be different from that by *Eimeria* species because of differences in the organelles of the anterior region of the body, particularly the conoid.

The origin of the membrane lining the parasitophorous vacuole surrounding the intracellular sporozoites of *Eimeria* species is unknown. McLaren (1969) reported that no limiting membrane other than that of the parasite could be observed around merozoites of *E. tenella* after these had penetrated host cells and assumed an oval shape. Scholtyseck (1969) observed that sporozoites of *E. tenella* which had just invaded cultured cells were located in a vacuole having an incomplete membrane. These findings indicate that the limiting membrane of the parasitophorous vacuole is not derived directly from the surface membrane of the host cell.

DEVELOPMENT OF SPOROZOITES, TROPHOZOITES, AND EARLY SCHIZONTS

During the period immediately after penetration of a host cell by the sporozoite, the refractile bodies, which have also been called eosinophilic globules and paranuclear bodies, undergo marked changes. In *E. bovis* sporozoites during the first 24 hours in cultured cells, the anterior refractile body frequently appeared to move posteriorly. It usually became smaller as this occurred, and finally disappeared (Fayer and Hammond 1969). In a cinemicrographic study, Fayer (1969) found that in intracellular sporozoites of *E. tenella*, *E. adenoeides*, and *E. meleagrimitis* which had an anterior and a posterior refractile body, the anterior body moved posteriorly and merged with the posterior body. Finger-like projections which appeared along the anterior margin of the posterior refractile body later became detached, and were observed in the sporozoite cytoplasm. We have recently found that the anterior refractile body of *E. callospermophili* undergoes a marked decrease in size during the sporozoite's first 10 hours within the host cell and usually can no longer be seen after this time (Speer and Hammond 1970). A succession of small granules, which later become randomly distributed, is formed at the surface of the refractile body. The unusually large posterior refractile body also decreases in size during this time, and later forms several smaller spherical bodies. In a cinemicrographic study of the development of

this species (Speer and Hammond 1969), refractile bodies underwent frequent changes in shape, size, and location. In addition, the merging of the posterior refractile body with a somewhat smaller refractile body was observed. These changes in the refractile bodies are evidently associated with the use of their substance in the development of the parasite.

In *E. bovis*, sporozoites developing in cell cultures transform into trophozoites during a period which extends from 3 days until 5 to 8 days after inoculation (Fayer and Hammond 1967). The nucleus changes from vesicular to compact and approximately doubles in size. The nucleolus becomes greatly enlarged and the posterior refractile body changes from an ellipsoidal to a spheroidal shape. While these changes are being completed, the parasite is transformed from the elongate slender form characteristic of the sporozoite to the rounded form of the trophozoite. Similar nuclear changes were reported to be associated with this transformation in *E. meleagrimitis* (Doran and Vetterling 1968). *E. ninakohlyakimovae* sporozoites undergo a similar transformation to trophozoites but it occurs earlier than in *E. bovis* (Kelley and Hammond 1970a). The change in shape associated with the transformation takes the form of a gradual widening or a lateral outpocketing of the body, usually in the posterior portion.

In *E. auburnensis*, *E. callospermophili*, and *E. bilamellata*, the early development follows a different course. The trophozoite stage is usually omitted, although some trophozoites of *E. auburnensis* were seen in cell cultures; the parasite undergoes considerable growth and completes several nuclear divisions while retaining the elongate form characteristic of the sporozoite. This stage, which we call the sporozoite-shaped schizont, later transforms into a spheroidal schizont by a rapid formation of a lateral outpocketing or by a gradual increase in width of the entire body. This omission of the trophozoite stage is not an abnormality associated with growth in cell cultures because similar development was seen in *E. auburnensis* (Chobotar, Hammond, and Miner 1969) and in *E. callospermophili* (Roberts, Hammond, Anderson, and Speer 1970) in the host animal.

The sporozoite-shaped schizont of *E. callospermophili* retains the locomotor ability of the sporozoite, as demonstrated by the motility of specimens freed from their host cells by scraping off the monolayer from the coverglass (Speer, Hammond, and Anderson 1970). Such specimens flexed, glided, and entered host cells. A slender protuberance, probably representing the conoid apparatus, was observed with phase-contrast microscopy at the anterior end of the schizont during penetration. In an electron microscope study, sporozoite-shaped schizonts of *E. callospermophili* were found to have all of the cytoplasmic organelles, including pellicular structures, present in the sporozoite (Roberts, Hammond, Anderson, and Speer 1970). However, the anterior refractile body had disappeared or was represented by small granules. Dedifferentiation of the conoid apparatus, micronemes, and pellicular structures occurred only later, in the spheroidal schizont stage (Fig. 5), after the anlagen of merozoites had appeared. This course of development appears to differ considerably from that of merozoites of *Plasmodium* species, which undergo dedifferentiation of the specialized structures of the pellicle and anterior portion of the body while transforming into the trophozoite stage, so that the trophozoite and early schizont are relatively simple in morphology (Ladda 1969). Such a dedifferentiation accompanies transformation of the sporozoites of *E. ninakohlyakimovae* into trophozoites (Kelley and Hammond 1970b).

NUCLEAR DIVISION

Division of the nucleus of the sporozoite of *E. callospermophili* has been observed in living specimens in cell cultures (Speer and Hammond 1970) and also with the electron microscope (Roberts and Hammond, in preparation). In living specimens, the first nuclear division of the sporozoite usually occurred 8 to 10 hours after

inoculation of sporozoites, and required about one hour for completion. A prominent nucleolus was present throughout the division process. In an early stage of division, the nucleus and nucleolus became elongate, with the latter appearing first spindle-shaped (Fig. 9) and then rod-shaped (Fig. 10). In several specimens, the nucleolus and nucleus increased appreciably in length during a period of 3 to 4 minutes, and then assumed a dumb-bell shape. The daughter nucleoli separated and the nuclear membrane became infolded in the area between them (Fig. 11). Thus, the nuclear membrane apparently remains intact during division. However, Canning and Anwar (1969) reported that at the beginning of zygotic meiosis in *E. tenella* the nucleolus and nuclear membrane disappeared and a spindle was formed.

In electron micrographs, a nucleolus could not be seen in sporozoites immediately after they had entered cells, but a prominent nucleolus was present in the enlarged nucleus of sporozoites which apparently had been within the host cell for 6 to 8 hours. A pair of centrioles was seen adjacent to the nucleus in some specimens. Dividing nuclei were greatly elongated, with a narrow middle region, having the Golgi complex in a depression at one side and a rod-shaped nucleolus (Fig. 1). In some specimens, a centriole was seen at one pole, and microtubules were present at the lateral margin of the nucleolus. This suggests the possible occurrence of an arrangement of spindle fibres similar to that reported for trypanosomes by Rudzinska and Vickerman (1968). In a stage in which division was nearly complete, the daughter nucleoli were visible and the nuclear membrane had an infolding which incompletely separated them (Fig. 2). Clumps of granules, possibly representing chromatin, appeared to have a random distribution in the nucleoplasm.

In microgametocytes of *E. auburnensis*, nuclei in an early stage of division were observed to have an intranuclear spindle apparatus, with fibres radiating from opposite poles of the nucleus (Hammond, Scholtyseck, and Chobotar 1969). In the later stages of division, during the separation of the two daughter nuclei, the inner membrane of the nucleus became infolded before the outer one. According to Ladda (1969), nuclear division in *Plasmodium* species occurs by a form of endomitosis in which chromosomal replication occurs independently, and attachment of chromosomes to nuclear membranes may serve for segregation of chromosomes into daughter nuclei. The nuclear membrane remains intact; the nucleus elongates and divides into two equal parts. Elements interpreted as microtubules and chromosomes are seen before and independent of nuclear division, although chromosomes have not yet been adequately demonstrated. Much work remains to be done before an understanding of nuclear division in this group of parasites can be obtained.

LATE DEVELOPMENT OF SCHIZONTS AND FORMATION OF MEROZOITES

In the unusually large first-generation schizonts of *E. bovis*, *E. auburnensis*, and *E. ninakohlyakimovae*, which have a maximum diameter of 200 to 300 μm when mature, the nuclei often become arranged near the surface as development proceeds (Hammond, Ernst, and Miner 1966; Chobotar, Hammond, and Miner 1969; Wacha and Hammond 1970). Invaginations or infoldings of the peripheral layer of nuclei then occur. Frequently, these later form spheroidal, ellipsoidal, or lobulated masses having a single layer of nuclei at the periphery. These masses, which have been termed blastophores, are probably comparable to the pseudocytomeres of the schizonts of certain *Plasmodium* species (Garnham 1951; Garnham, Bird, Baker, and Killick-Kendrick 1969). The blastophores have a surface membrane consisting of a single unit membrane derived from that of the sporozoites.

The first indications of merozoite formation in *E. bovis* are thickenings of the surface membrane of the blastophore in the areas overlying nuclei (Sheffield and Hammond 1967). The thickened area of the cell membrane then becomes elevated

into a conical protuberance and an inner membrane becomes separated from the surface membrane. As the merozoite forms, the inner membrane extends posteriorly, and the conoid and subpellicular microtubules, as well as the spheroidal anlagen of the club-shaped organelles, appear. A nucleus and other organelles are incorporated into the forming merozoite and it finally becomes separated from the residual body. A similar process of merozoite development has been observed in *E. nieschulzi* by Colley (1968), in *E. tenella* by McLaren (1969) and Sénaud and Černá (1968, 1969), in *E. magna* and *E. pragensis* by the latter authors (1968, 1969), and in an *Isospora* species by Schmidt, Johnston, and Stehbens (1967).

Scholtyseck (1965) has described a different kind of merozoite formation in *E. perforans* and *E. stiedae*. In these, the merozoites were formed internally, and were separated from each other and from the remaining cytoplasm of the schizont by spaces developing in the endoplasmic reticulum. In *E. callospermophili*, the early development of the merozoites in schizonts, presumably of the second generation, resembled that of *E. bovis*, except that blastophores were not formed (Scholtyseck, Hammond, and Todd 1968). A highly developed endoplasmic reticulum played some part in the separation of the individual schizonts.

Recently, we have found that the merozoites in first-generation schizonts of *E. callospermophili* begin to form internally, instead of at the surface (Roberts, Hammond, Anderson, and Speer 1970). The early stages of formation occur in sporozoite-shaped schizonts having from four to six nuclei. The anlagen of merozoites are observed in association with nuclei having microtubular spindle apparatuses, which are located near the outer margin rather than the centre of the nucleus. Often one or two centrioles are seen adjacent to each pole of the spindle apparatus (Fig. 3), near which the anlage of the merozoite later appears (Fig. 4). The anlage consists of the inner membrane of the anterior portion of the merozoite and the immature conoid. No precursor of these structures could be seen. The subpellicular microtubules then appear, arranged in pairs. Somewhat later, the spheroidal anlagen of the club-shaped organelles and the forming refractile body are seen. A Golgi complex is located immediately to one side of the pole of the spindle apparatus, and associated flattened cisternae are present at the base of the forming merozoite. Possibly the Golgi apparatus participates in the formation of certain of the organelles of the merozoite. At a later stage of development, the nucleus divides and each daughter is incorporated into a merozoite (Fig. 5). As the merozoites develop, the sporozoite-shaped schizont transforms into a spheroidal schizont. The inner pellicular membrane and anterior end organelles gradually undergo dedifferentiation. The immature merozoites are usually oriented so that they point obliquely towards the surface of the schizont. As the merozoites become larger, they assume a more peripheral position, reach the surface of the schizont, and then grow out as radial protuberances (Fig. 5). In this process, an outer surface membrane is evidently acquired by the merozoite from that of the schizont. The later stages of merozoite formation resemble those of *E. bovis*, with the merozoites being attached at their posterior ends to a residual body. Two refractile bodies are present in each merozoite (Hammond, Speer, and Roberts 1970).

Internal formation of merozoites such as occurs in *E. callospermophili* has not been reported in any other coccidian. However, the occurrence of a *centrocône* similar to the pole of the spindle apparatus in *E. callospermophili* and a centriole associated with merozoite formation was reported in *E. pragensis*, *E. magna*, and *E. tenella* by Sénaud and Černá (1968, 1969). These authors pointed out the resemblance of the centrocône to similar structures in endodyogeny of *Toxoplasma gondii* (Sénaud 1967; Sheffield and Melton 1968). Merozoite formation in *E. callospermophili* resembles endodyogeny in *T. gondii* in that each merozoite begins to form internally, in association with a nucleus in an early stage of division.

The nuclear divisions associated with merozoite formation appear to differ from those occurring during the early development of the schizont with respect to the location of the spindle apparatus; this is peripheral in the former and central, if observed at all, in the latter. Centrioles, sometimes appearing in pairs, occur adjacent to each pole of the peripheral spindle apparatus. Nuclear divisions similar to that associated with merozoite formation have been reported to occur in association with microgamete formation in *E. auburnensis* (Hammond, Scholtyseck, and Chobotar 1969) and in *E. intestinalis* and *E. magna* (Snigirevskaya 1969). In the latter two species, a mitotic spindle, with a peripheral location, was seen in dividing nuclei of microgametocytes, and a centriole was located adjacent to each of the poles. In *E. auburnensis*, pairs of centrioles were sometimes seen in association with the poles of the peripherally located spindle apparatus. Thus, it appears that a similar pattern of nuclear division is associated in *Eimeria* species with the development of two different kinds of daughter individuals, merozoites and microgametes.

The poles of the spindle apparatus may play a role in inducing the formation of the merozoites and microgametes. Such an association has been suggested for the formation of sporozoites in *Plasmodium gallinaceum* (Terzakis, Sprinz, and Ward 1967). Peripheral nuclear fibres associated with sporozoite formation were reported in *P. berghei* by Vandenberg and Rhodin (1967). Bradbury and Trager (1968) suggested that substances diffusing through nuclear pores from an intranuclear spindle apparatus, or specialized areas of the nuclear membrane itself, may play a role in organizing axonemes during microgametogenesis of *Haemoproteus columbae*. The centrioles observed in the vicinity of the poles of the spindle apparatus in the *Eimeria* species may play an intermediate role in inducing the formation of merozoites and microgametes. Kinetosomes were observed in the microgametocytes of *P. gallinaceum* and *P. cathemerium* by Aikawa, Huff, and Sprinz (1969). Since these were seen

ABBREVIATIONS, ALL FIGURES: ARB, anterior refractile body; C, conoid anlage; CE, centriole; CO, club-shaped organelle anlage; G, Golgi complex; IF, intravacuolar folds of the vacuolar membrane; IM, inner pellicular membrane of merozoite anlage; M, mitochondrion; MN, microneme; N, nucleus, NU, nucleolus; PRB, posterior refractile body; PV, parasitophorous vacuole; RB, developing anterior refractile body of merozoite; SA, spindle apparatus.

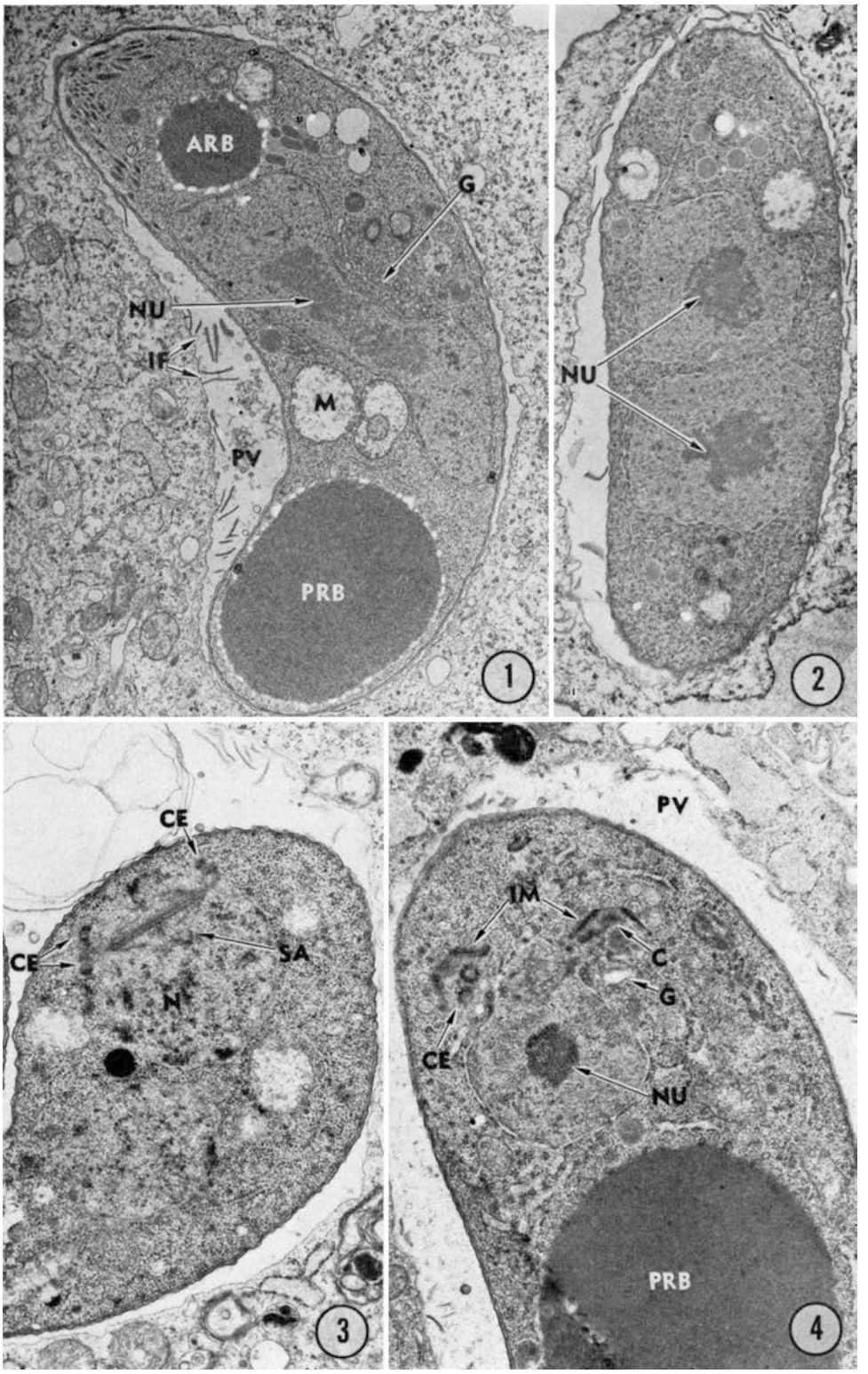
FIGURES 1–4. Electron micrographs of *Eimeria callospermophili* sporozoites and schizonts grown in embryonic bovine intestinal cells, fixed according to the method of Karnovsky (1965), embedded in Epon, sectioned with a diamond knife and Sorvall ultramicrotome, and stained with uranyl acetate and lead citrate, unless otherwise stated. Prepared by William L. Roberts with a Zeiss 9A electron microscope.

1 Sporozoite with dividing nucleus. Note elongate shape of nucleus and nucleolus, and intact nuclear membrane. From culture fixed 12 hours after inoculation. ×11,000

2 Portion of sporozoite with nucleus almost completely divided. Note infolding of nuclear membrane between the two nucleoli. From culture fixed 14 hours after inoculation. ×11,000

3 Portion of sporozoite-shaped schizont, showing nucleus with spindle apparatus near its outer margin and three longitudinally sectioned centrioles (two adjacent to one pole and one adjacent to the other). From culture fixed 16 hours after inoculation. ×15,000

4 Portion of sporozoite-shaped schizont, showing anlagen of two merozoites, each adjacent to a pole of a spindle apparatus. Note centrioles in anlage at left and Golgi complex at base of anlage at right. From culture fixed 16 hours after inoculation; fixative, 2.5 per cent glutaraldehyde and 2.5 per cent osmium tetroxide together. ×15,000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in proximity to darkened areas in the nucleus, it was suggested that the nucleus may play some role in their formation. Structures resembling centrioles were seen in young oocysts of *P. berghei yoeli* and microtubules thought to be spindle fibrils were observed at the periphery of the nucleus in this stage and in ookinetes of this species by Garnham *et al.* (1969).

MOTILITY AND FURTHER DEVELOPMENT OF MEROZOITES

Usually, mature merozoites show little or no motility while retained within the parasitophorous vacuole of the host cell in which they developed. However, moving merozoites were seen within a schizont of *E. auburnensis* in a cell culture (Clark and Hammond 1969). Occasionally, gliding movements and motion of the anterior ends of merozoites in a lateral direction were seen within schizonts of *E. callospermophili.* Merozoites of this species (Speer, Hammond, and Anderson 1970) and of *E. alabamensis* (Sampson, Hammond, and Ernst 1971) were observed leaving the parasitophorous vacuole by their own motility. In the latter, merozoites often left in twos. In an electron micrograph showing a merozoite of *E. callospermophili* in the process of escaping, the body of the merozoite was constricted at the point of exit, in a manner similar to that of sporozoites while entering cells (Roberts, Hammond, Anderson, and Speer 1970). In *E. ninakohlyakimovae*, merozoites which had penetrated new host cells were markedly constricted as they left these cells (Kelley and Hammond 1970a). These findings suggest that merozoites enter and leave cells in a similar manner to sporozoites.

Recently, we have found that merozoites in mature schizonts, as well as free merozoites, of *E. callospermophili* and other species in cell cultures become motile when stimulated by bile or

FIGURES 5, 9–11. *E. callospermophili*, continued.
FIGURES 6–8. *E. ninakohlyakimovae.*

5 Electron micrograph of spheroidal schizont, showing outgrowth of two merozoites. Note that inner pellicular membrane of schizont has almost completely dedifferentiated by this stage. From a ligated intestinal segment of a ground squirrel fixed 16 hours after introduction of sporozoites; prepared as for Figure 4. ×12,000

6–8 Photomicrographs by G. L. Kelley of a living specimen, phase-contrast microscopy, in process of penetrating host cell, with constriction of sporozoite at point of passage through cell membrane (arrow). In each figure, anterior end of sporozoite is oriented towards top of page. From embryonic lamb thymus cell culture, two days after inoculation. ×1,500

6 Sporozoite shortly after beginning penetration of cell, with constricted anterior portion of body.

7 Anterior fourth of sporozoite within cell; this portion has become wider, so that constriction at point of entrance is evident.

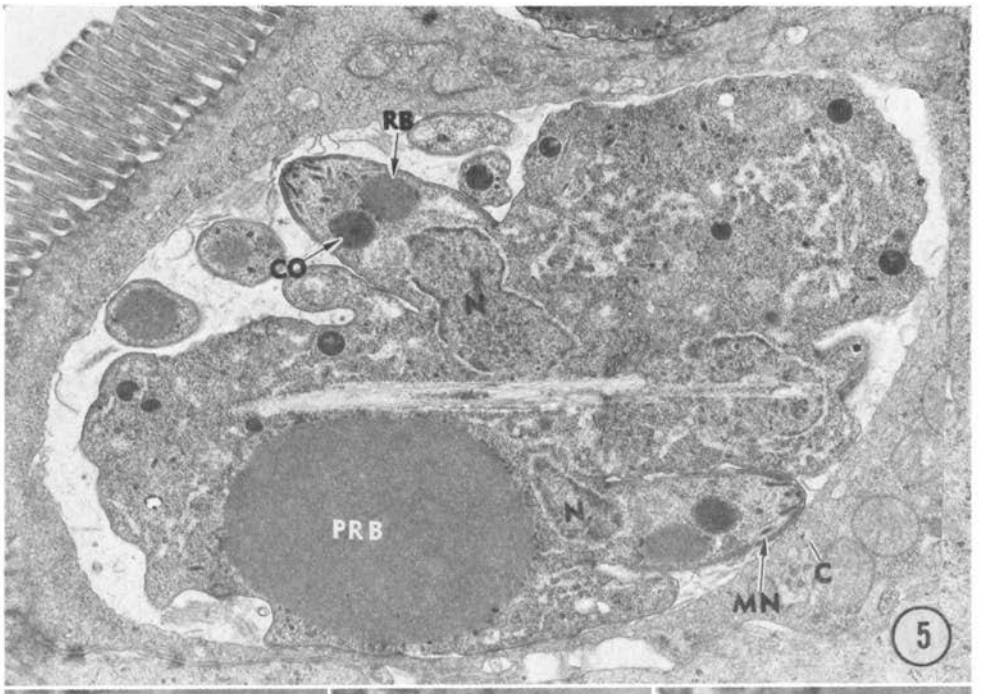
8 Penetration about half completed; posterior refractile body is included in the constricted portion of the body; note proturberance near anterior end of body.

9–11 Photomicrographs by C. A. Speer of intracellular sporozoites undergoing nuclear division, phase contrast microscopy. ×1,600

9 Sporozoite with relatively wide sporozoite body, elongated nucleus, and spindle-shaped nucleolus (arrow). From culture of third-passage embryonic bovine kidney cells, 8 hours after inoculation.

10 Sporozoite with nucleus in stage comparable to that of Figure 1, having rod-shaped nucleolus (arrow) lying in the narrow middle region of the elongated nucleus. From culture of third-passage embryonic bovine kidney cells, 8½ hours after inoculation.

11 Sporozoite with nucleus in stage comparable to that of Figure 2, showing invagination of nuclear membrane (arrow) between the two nucleoli. From culture of fourth-passage embryonic bovine intestinal cells, 9 hours after inoculation.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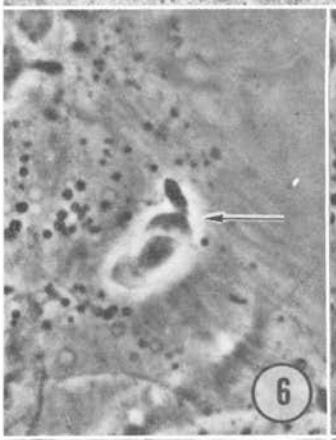
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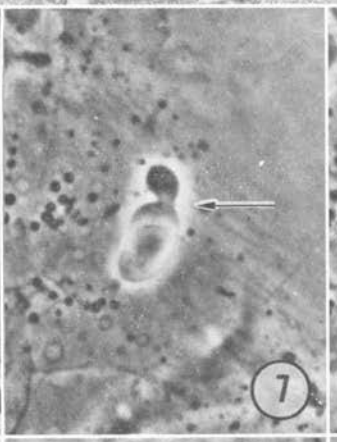
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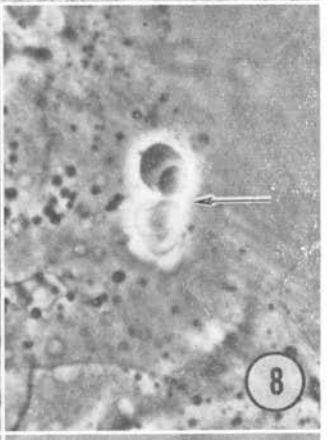
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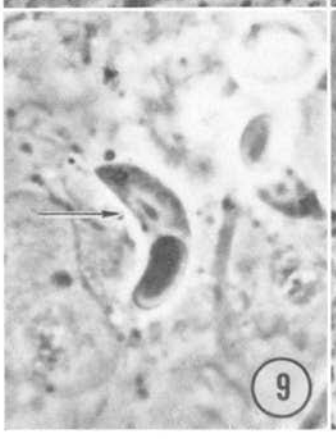
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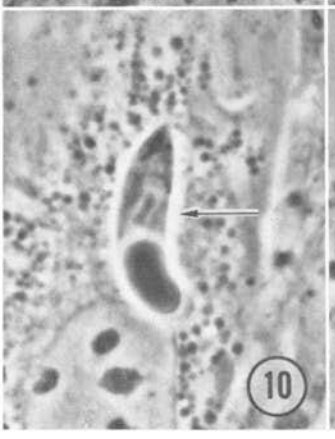
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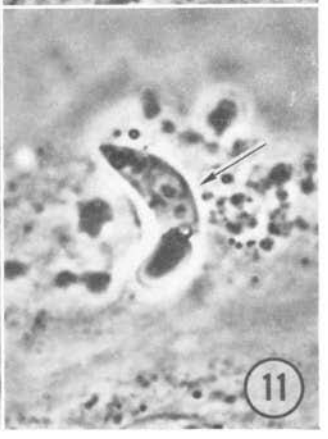
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bile salts (Speer, Hammond, and Kelley 1970). The merozoites within mature schizonts usually undergo a marked increase in motility when bile or a bile salt is added to the coverslip preparation, and soon begin leaving the host cell. Motility of free merozoites, previously released by rupture of the host cells, is observed with much greater frequency than normal in preparations to which bile or bile salts have been added. The stimulation of motility in merozoites in this way has also been observed in *E. bilamellata* and *E. larimerensis*, also from the Uinta ground squirrel, as well as *E. nieschulzi* from the rat and *E. ninakohlyakimovae* from the sheep. If this is found to hold true more widely in the coccidia, it may represent an important ecological aspect of the host-parasite relationship.

In the species of *Eimeria* from ruminants and rodents in which *in vitro* cultivation has been attempted in our laboratory, the merozoites apparently have much less capacity to develop than do the sporozoites. In all of these species, sporozoites have developed to mature first-generation schizonts, but so far we have been unsuccessful in obtaining development of merozoites to mature second-generation schizonts in cell cultures. This is also true of results obtained when merozoites of *E. bovis* collected from experimentally infected calves were used (Hammond, Fayer, and Miner 1969). Except for a single instance of a binucleate second-generation schizont of *E. bovis* (Hammond and Fayer 1968), further development of merozoites has been observed only in *E. callospermophili*. Small numbers of multinucleate second-generation schizonts of this species, some of which were in the early stages of merozoite formation, were seen (Speer, Hammond, and Anderson 1970). These results differ from those of Bedrnik (1969), who found that merozoites of *E. tenella* obtained from chickens developed, in general, in tissue culture much better than did sporozoites. He noted, however, that merozoites obtained from chickens on the sixth day of infection grew best, indicating that later generations of merozoites may have better *in vitro* growth potential than those of the first generation. Bedrnik (1967) reported the development of gametocytes and oocysts of *E. tenella* from second-generation merozoites, and Strout and Ouellette (1969) obtained mature microgametocytes and macrogametes of this species in cell cultures inoculated with sporozoites.

HOST-PARASITE RELATIONSHIPS

Each intracellular stage of *Eimeria* species lies in a vacuole which has been termed the *parasitophore Vakuole* (Scholtyseck and Piekarski 1965) or "parasitophorous vacuole" (Hammond, Scholtyseck, and Miner 1967) and "periparasitic vacuole" (Stehbens 1966). The membrane of the host cell lining this vacuole is usually smooth, but in some species it has numerous fine folds or villus-like structures protruding into the vacuole. In *E. auburnensis* macrogametes, these intravacuolar folds evidently disintegrate, forming particulate material, which may be taken into the parasite (Hammond, Scholtyseck, and Chobotar 1967). Similar, but somewhat smaller folds or villus-like structures are found along the membrane lining the parasitophorous vacuole of schizonts of *E. callospermophili* (Fig. 1), *E. nieschulzi* (Colley 1968), and *E. miyairii* (Andreassen and Behnke 1968). In *Adelina tribolii*, the vacuolar membrane protrudes into the vacuole, forming vesicles and membranous structures, which may assist in the nutrition of the parasite (Žižka 1969). Similar vesicles and membranes were found in the vacuoles of *Lankesterella hylae* (Stehbens 1966) and in *Myriosporides amphiglenae* (Henneré 1967). In the former, this material was evidently taken into the parasite by engulfment or surface activity of the pellicle. Stereocilia, at least some of which are outgrowths of the vacuolar membrane, were observed in the vacuoles surrounding *T. gondii* organisms (Sheffield and Melton 1968).

The vacuolar membrane surrounding the large first-generation schizont of *E. bovis* has numerous blebs, formed apparently in the process of pinching off to form free vesicles, which evidently

disintegrate in the vacuole (Sheffield and Hammond 1966). This observation suggests the transfer of material from the host cell into the vacuole, from which it is probably taken in for use by the parasite. Andreassen and Behnke (1968) observed small vesicles in the cytoplasm of host cells harbouring *E. miyairii* schizonts. These were often near the vacuolar membrane, and sometimes protruded into it. The suggestion was made that these might empty into the vacuole, providing a means of nourishment for the parasite. Other structures occurring at the surface of the vacuolar membrane or at the surface of the parasite have been discussed by Scholtyseck (1968) and by Scholtyseck, Volkmann, and Hammond (1966).

The mechanism of ingestion of nutrients by the parasite is still incompletely known. The occurrence of the invaginations in the surface of the parasite, variously called micropores, micropyles, or cytostomes, is widespread among *Eimeria* species and in *Toxoplasma*, *Sarcocystis*, *Besnoitia*, *Plasmodium*, and other genera. Evidence of ingestion of nutrients through these structures has been obtained in only a few *Eimeria* species. Snigirevskaya and Cheissin (1968) reported the occurrence of active micropores in all of the endogenous stages with the exception of the microgamete in *E. intestinalis*. Scholtyseck (1969) observed micropores with food vacuoles forming at their bases in sporozoites of *E. tenella* within cultured cells. He found that the cytoplasm of the host cell in the vicinity of the parasite undergoes disintegration soon after invasion of the host cell by the parasite, and that this material is included in the parasitophorous vacuole and ingested through the micropores. Sénaud and Černá (1968) reported the occurrence of active micropores in the merozoites of *E. pragensis*. We have seen such micropores also in the intracellular sporozoites of *E. alabamensis* (Sampson and Hammond 1970). In each of these instances, the micropores evidently function as cytostomes. Ingestion of nutrients through a cytostome has also been reported for *Besnoitia jellisoni* (Sheffield 1967) and for *Plasmodium* species of birds and mammals other than rodents (Ladda 1969). A branched cytostome was observed in *Sarcocystis* by Sénaud (1966). In other species, the micropores may be vestigial or may function in some manner as yet unknown. In the microgametocytes of *E. auburnensis*, micropores were seen to occur in clusters of as many as nine (Hammond, Scholtyseck, and Chobotar 1969). Ingestion of nutrient materials may occur by pinocytosis in the macrogametes of *E. auburnensis*, usually in association with v-shaped invaginations at the surface (Hammond, Scholtyseck, and Chobotar 1967).

The factors affecting susceptibility of hosts to coccidia are incompletely understood. It is known, however, that older chickens are more susceptible than young ones to certain *Eimeria* species (Long 1967; Rose 1967), that the breed or strain of chickens also influences susceptibility to *Eimeria* infections (Long 1968), and that strains of parasites differ in pathogenicity (Joyner and Norton 1969).

Changes which have been observed in host cells harbouring stages of *Eimeria* species include increases in the number of mitochondria in cells parasitized by macrogametes of *E. perforans* (Scholtyseck 1963). This indicates a higher than normal rate of metabolism in infected cells. The mitochondria later undergo degeneration. Host cells parasitized by several different *Eimeria* species undergo marked changes, including enlargement of the nucleus, a marked increase in size of the nucleolus, and rearrangement of the chromatin into finer masses than normal. The changes are most pronounced in host cells harbouring large schizonts such as those of *E. bovis*, *E. auburnensis*, and *E. ninakohlyakimovae*; in the two former species the cytoplasm of the host cell also increases markedly in volume. Evidently, substances originating in the parasite stimulate the host to undergo these changes, which may be associated with production by the host cell of materials used in the growth of the parasite. Thus, parasites of this kind are evidently able to cause modifications in the physiology and growth of the host cell. Such

changes presumably result in a more favourable environment for the development of the parasite.

REFERENCES

Aikawa, M., Huff, C. G., and Sprinz, H. 1969. Comparative fine structure study of the gametocytes of avian, reptilian, and mammalian malarial parasites. *J. Ultrastruct. Research* 26:316–31

Anderson, L. C., and Hammond, D. M. 1969. Experimental coccidian infections in captive Uinta ground squirrels, *Spermophilus armatus*, during the hibernating season. *J. Protozool.* 16(Suppl.): 15–16

Andreassen, J., and Behnke, O. 1968. Fine structure of merozoites of a rat coccidian *Eimeria miyairii*, with a comparison of the fine structure of other sporozoa. *J. Parasitol.* 54:150–63

Bedrnik, P. 1967. Development of sexual stages and oocysts from the second generation of *Eimeria tenella* merozoites in tissue cultures. *Folia Parasitol.* 14:364

– 1969. Some results and problems of cultivation of *Eimeria tenella* in tissue cultures. *Acta Vet.* 38:31–5

Bradbury, P. C., and Trager, W. 1968. The fine structure of microgametogenesis in *Haemoproteus columbae* Kruse. *J. Protozool.* 15:700–12

Canning, E. U., and Anwar, M. 1969. Nuclear studies on sporozoan oocysts. *Progr. in Protozool., Proc. III Intern. Congr. Protozool., Leningrad*, p. 23

Chobotar, B., Hammond, D. M., and Miner, M. L. 1969. Development of first-generation schizonts of *Eimeria auburnensis*. *J. Parasitol.* 55:385–97

Clark, W. N., and Hammond, D. M. 1969. Development of *Eimeria auburnensis* in cell cultures. *J. Protozool.* 16:646–54

Colley, F. C. 1968. Fine structure of schizonts and merozoites of *Eimeria nieschulzi*. *J. Protozool.* 15:374–82

Doran, D. J., and Farr, M. M. 1962. Excystation of the poultry coccidium, *Eimeria acervulina*. *J. Protozool.* 9:154–61

Doran, D. J., and Vetterling, J. M. 1968. Survival and development of *Eimeria meleagrimitis* Tyzzer, 1929, in bovine kidney and turkey intestine cell cultures. *J. Protozool.* 15:796–802

Farr, M. M., and Doran, D. J. 1962. Comparative excystation of 4 species of poultry coccidia. *J. Protozool.* 9:403–7

Fayer, R. 1969. Refractile body changes in sporozoites of poultry coccidia in cell culture. *Proc. Helminthol. Soc. Wash., D.C.* 36:224–31

Fayer, R., and Hammond D. M. 1967. Development of first-generation schizonts of *Eimeria bovis* in cultured bovine cells. *J. Protozool.* 14:764–72

– 1969. Morphological changes in *Eimeria bovis* sporozoites during their first day in cultured mammalian cells. *J. Parasitol.* 55:398–401

Fayer, R., Hammond, D. M., Chobotar, B., and Elsner, Y. Y. 1969. Cultivation of *Besnoitia jellisoni* in bovine cell cultures. *J. Parasitol.* 55: 645–53

Garnham, P. C. C. 1951. The mosquito transmission of *Plasmodium inui* Halberstaedter and Prowazek, and its pre-erythrocytic development in the liver of the rhesus monkey. *Trans. Roy. Soc. Trop. Med. Hyg.* 45:45–52

Garnham, P. C. C., Bird, R. G., Baker, J. R., Desser, S. S., and El-Nahal, H. M. S. 1969. Electron microscope studies on motile stages of malaria parasites. VI. The ookinete of *Plasmodium berghei yoelii* and its transformation into the early oocyst. *Trans. Roy. Soc. Trop. Med. Hyg.* 63:187–94

Garnham, P. C. C., Bird, R. G., Baker, J. R., and Killick-Kendrick, R. 1969. Electron microscope studies on the motile stages of malaria parasites. VII. The fine structure of the merozoites of exoerythrocytic schizonts of *Plasmodium berghei yoelii*. *Trans. Roy. Soc. Trop. Med. Hyg.* 63: 328–32

Hammond, D. M., Ernst, J. V., and Chobotar, B. 1970. Composition and function of the substiedal body in the sporocysts of *Eimeria utahensis*. *J. Parasitol.* 56:618–19

Hammond, D. M., Ernst, J. V., and Miner, M. L. 1966. The development of first-generation schi-

zonts of *Eimeria bovis*. *J. Protozool.* 13:559–64

Hammond, D. M., and Fayer, R. 1968. Cultivation of *Eimeria bovis* in three established cell lines and in bovine tracheal cell line cultures. *J. Parasitol.* 54:559–68

Hammond, D. M., Fayer, R., and Miner, M. L. 1969. Further studies on *in vitro* development of *Eimeria bovis* and attempts to obtain second-generation schizonts. *J. Protozool.* 16:298–302

Hammond, D. M., Scholtyseck, E., and Chobotar, B. 1967. Fine structures associated with nutrition of the intracellular parasite *Eimeria auburnensis*. *J. Protozool.* 14:678–83

– 1969. Fine structural study of the microgametogenesis of *Eimeria auburnensis*. *Z. Parasitenk.* 33:65–84

Hammond, D. M., Scholtyseck, E., and Miner, M. L. 1967. The fine structure of microgametocytes of *Eimeria perforans*, *E. stiedae*, *E. bovis*, and *E. auburnensis*. *J. Parasitol.* 53:235–47

Hammond, D. M., Speer, C. A., and Roberts, W. 1970. Occurrence of refractile bodies in merozoites of *Eimeria* species. *J. Parasitol.* 56:189–91

Henneré, E. 1967. Etude cytologique des premiers stades du développement d'une coccidie: *Myriosporides amphiglenae*. *J. Protozool.* 14:27–39

Hibbert, L. E., and Hammond, D. M. 1968. Effects of temperature on *in vitro* excystation of various *Eimeria* species. *Exptl. Parasitol.* 23:161–70

Hibbert, L. E., Hammond, D. M., and Simmons, J. R. 1969. The effects of pH, buffers, bile and bile acids on excystation of sporozoites of various *Eimeria* species. *J. Protozool.* 16:441–4

Jackson, A. R. B. 1962. Excystation of *Eimeria arloingi* (Marotel, 1905); stimuli from the host sheep. *Nature* 194:847–9

Jadin, J., and Creemers, J. 1968. Ultrastructure et biologie des toxoplasmes. III. Observations de toxoplasmes intraerythrocytaires chez un mammifère. *Acta Trop.* 25:267–70

Joyner, L. P., and Norton, C. C. 1969. A comparison of two laboratory strains of *Eimeria tenella*. *Parasitology* 59:907–13

Karnovsky, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* 27:137A–8A

Kelley, G. L., and Hammond, D. M. 1970a. Development of *Eimeria ninakohlyakimovae* in cell cultures. *J. Protozool.* 17:340–9

– 1970b. The fine structure of the early stages of *Eimeria ninakohlyakimovae* in cell cultures. *J. Protozool.* 17(Suppl.):17

Ladda, R. L. 1969. New insights into the fine structure of rodent malarial parasites. *Military Med.* 134:825–65

Ladda, R. L., Aikawa, M., and Sprinz, H. 1969. Penetration of erythrocytes by merozoites of mammalian and avian malarial parasites. *J. Parasitol.* 55:633–44

Long, P. L. 1967. Studies on *Eimeria praecox* Johnson, 1930, in the chicken. *Parasitology* 57:351–61

– 1968. The effect of breed of chickens on resistance to *Eimeria* infections. *Brit. Poult. Sci.* 9:71–8

Lotze, J. C., and Leek, R. G. 1960. Some factors involved in excystation of the sporozoites of three species of sheep coccidia. *J. Parasitol.* 46(Suppl.):46–7

– 1968. Excystation of the sporozoites of *Eimeria tenella* in apparently unbroken oocysts in the chicken. *J. Protozool.* 15:693–7

– 1969. Observations on *Eimeria tenella* in feces of inoculated chickens. *J. Protozool.* 16:496–8

McLaren, D. J. 1969. Observations on the fine structural changes associated with schizogony and gametogony in *Eimeria tenella*. *Parasitology* 59:563–74

McLaren, D. J., and Paget, G. E. 1968. A fine structural study on the merozoite of *Eimeria tenella* with special reference to the conoid apparatus. *Parasitology* 58:561–71

Nyberg, P. A., Bauer, D. H., and Knapp, S. E. 1968. Carbon dioxide as the initial stimulus for excystation of *Eimeria tenella* oocysts. *J. Protozool.* 15:144–8

Nyberg, P. A., and Hammond, D. M. 1964. Excystation of *Eimeria bovis* and other species of bovine coccidia. *J. Protozool.* 11:474–80

Roberts, W. L., and Hammond, D. M. 1970. Ultrastructural and cytological studies of sporozoites of four *Eimeria* species. *J. Protozool.* 17:76–86