Life Cycles Involving Sexual and Asexual Generations of *Eimeria* in Gallinaceous Birds

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Although a textbook-like presentation of the life cycles of all eimerian parasites of gallinaceous birds is out of question, the reviewer follows the system of textbook descriptions, in order to add in right place all the new informations emerging from recent studies as well as some hypothetic conclusions that might initiate new approaches to one or another problem. Keeping this in mind, I tried to emphasise all details which seemed to be new, but, naturally, could not entirely avoid references to some long established characteristics of coccidian development.

Life cycles of the eimerian parasites of birds in the order Galliformes differ only in minor details. There is consistently an asexual and a sexual stage or, according to another school of thought, an endogenous and exogenous stage of development in the life cycle of all *Eimeriae*. A single specific eimerian parasite (oocyst), settled in the susceptible avian host, gives rise to several hundreds of thousands of oocysts which, once discharged into the outside world, disseminate and infect further hosts to maintain the species.

Since the developmental stages of Eimeriae differ in both morphological and biological properties, these features are regarded specific characteristics for species differentiation.

Albeit the details of exogenic development are well known with most eimerian parasites, information on the endogenic cycle is often scanty. In fact, studies of endogenic cycle have been centred primarily on the coccidia of gallinaceous birds important from economical point of view, and little has been published in this context on the coccidian parasites of other host species.

The strict host specificity and organ specificity, often even with distinct site preference are species characteristics of Eimeriae. Certain species (Eimeria mitis, E.mivati, E.mayurai, E.duodenalis, E.grenieri, etc.) settle chiefly in the duodenum, others (E.maxima, E.meleagridis, etc.) in posterior segments of the intestine and others again prefer the coecum (E.tenella, E.adenoeides, etc.) or even the rectum and cloacal mucosa (E.brunetti). The life cycle of E.nectarix is particular. The schizogyony of this coccidium proceeds in the small intestines of chickens, the telomerozoites released by the schizonts pass down the coecum and gametes develop within the caecal epithelial cells.

A further common feature of Eimeriae is that they develop intra-cellularly; only some motile developmental stages occur temporarily outside the host cell. Such motile stages are the excysted sporozoites, first, second, etc. generations of merozoites as well as the microgametes, which search the macrogametes by active movement after release from the microgametocyte.

Up to recently, it was almost dogmatically accepted that Eimeriae are epithelial parasites, however, light microscopic and, particularly, electronmicroscopic studies have increasingly revealed the presence of endogenous stages also in cells of mesenchymal origin. It remains to be clarified whether these cells
represent a normal location, at least partly, of the developmental stages, or just an effort of the host organism toward phagocytic destruction of the invading parasite.

The intracellular location in relation to the nucleus is also typical of the eimerian parasites of Galliformes. The parasites may occur basally, adjacent to the nucleus or between the lumen and the nucleus. A species character is also the number of schizogonic generations succeeding each other until the start of the last - sexual - stage of endogenous development.

1. Endogenic development. Schizogony. Cyst walls of most oocysts which entered the alimentary canal are burst under the influence of the host's body temperature, action of the digestive juices, primarily pancreatic juice or presumably also by the mechanical effect of the peristalsis. LOTZE and LEEK (1969) state that part of the oocysts do not disintegrate and are eliminated with the stool. The sporocysts liberated by excystation release the sporozoites through the micropyle. Sometimes sporozoite excystation from the sporocyst takes place within the oocyst. According to DORAN and FARR (1962), the E. acervulina oocysts pass the crop without rupture, but start to release sporocysts in the gizzard, very likely owing to the latter's mechanical effect. Sporozoites liberate in the duodenum and jejunum.

The excystation of oocysts can be observed also in vitro. At appropriate temperature and under the influence of pancreatic juice and perhaps also other digestive juices, excystation takes a rapid course from a few minutes to a few hours. NYBERG, BAUER and KNAPP (1968) reported a stimulatory action of CO\textsubscript{2} on in vitro excystation.

On the basis of in vivo experiments ZUCKER (1964) pointed out the important role of dietary calcium content in the promotion of excystation by the pancreatic juice.
The sporozoites force themselves through the narrow micropyle with visible effort and they may sometimes fail.

The sporozoites of E. tenella are elongate cylindrical bodies, tapering toward one end. They have a distinct apical ring (RYLEY, 1969, and others) from which 24 fibrils extend posteriorly beneath the bilamellar membrane. At the apical end there is also a conoid consisting of spirally wound tubes which very likely plays some role during penetration into the host cell. The nucleus of the sporozoite is centrally located and there is a larger homogenous paranuclear body behind it. This structure also occurs in the sporocysts of other Eimeriae often with an additional smaller prenuclear body. In light micrographs, these structures can be visualized by various staining methods but, naturally, their ultrastructure remains to be identified electronmicroscopically.

Excystation of the sporozoites and their penetration into the intestinal epithelial cells introduces schizogony, the first asexual stage of endogenous development. Up to now it was generally accepted that sporozoites invaded directly the epithelial cells where they rounded up and developed to schizonts each within a pale parasitophorous vacuole. VAN DOORNINCK and BECKER (1957) showed that the sporozoites of E. necatrix first entered the lamina propria via transport by macrophages and passed only in a second step into the lining epithelium of the LIEBERKÜHN crypts. Sometimes the sporozoites are phagocytized by the macrophages. The macrophage-transport of E. tenella sporozoites was described by PATILLO (1957), CHALLEY and BURNS (1959) and others. The occurrence of sporozoites in fibrocytes and macrophages was noted also by us and these findings have been confirmed by SCHOLTYSECK (1953), and SCHOLTYSECK et al. (1969).

According to TYZZER's (1929) studies, the sporozoites of E. tenella may settle not only in the coecum, but also in the mucous epithelium of the intestinal segments adjacent anteriorly and
posteriorly to the caecal orifices. My experiments on coecectomized chickens (ined.) have verified that the above intestinal segments and the coecal mucosa served not only as settlements for E. tenella sporozoites, but the coccidium completed its full endogenous development in these sites. Depending on the severity of the infection, inflammatory reactions with petechiae developed not only in the ileum close to the caecal orifices and on the mucosa of the caecal stump, but also in the rectum. The inflammation involved no life danger even when the infestation was massive. This was, however, merely an implication as all experimental birds were killed for pathological and histological examination on the 7th-9th days after infection. Perhaps deaths from tenella coccidiosis may have followed later on.

Roughly consistent conclusions were deduced by LEATHEM (1969) from a similar, but more reliable experimental system. In coecectomized chickens, LEATHEM found the schizogonic and gametogonic stages of E. tenella in the final segment of the small intestine close to the caecal orifices as well as in the large intestine. He did not mention the rectal occurrence of these stages.

In my further experiments with E. necatrix the telomerozoites released by the schizonts did not find their site of preference, the caecum, in the coecectomized chickens and, therefore, only a few of them developed to gametes in the caecal stump and in the adjacent area, but never settled in the rectum unlike the corresponding stages of E. tenella. These findings clearly indicated that both E. tenella and E. necatrix maintained organ specificity even under abnormal conditions.

Organ specificity of this kind is not infrequent among eimerian parasites of the birds belonging to the order Galliformes. The pheasant coccidium E. colchici (Norton, 1967) behaves similarly to E. necatrix, as it undergoes schizogony in the small intestine, whereas the final process of schizogony and gametogony as well, proceeds in the caecum.
Studies by HORTON-SMITH and LONG (1965) threw a new light on the question of host specificity, but the new interpretation deduced from their findings does not contradict the previous ones. They noted that the sporozoites of *E. necatrix* and *E. brunetti*, which normally settled in the small intestine, could also settle in the caecum in case they were injected directly into it. The sporozoites which penetrated the caecal epithelial cells started endogenic development as if they had had settled in the small intestine. The oocysts passed with the faeces were used with success for experimental infection of susceptible chickens.

The parasitophorous vacuole formed by the cytoplasm around the penetrated parasite separates it from the host cell. Vacuole formation seems to be a result of the protective mechanism of the host cell, but it provides at the same time also certain protection for the developing parasite.

The rounded-off sporozoites (trophozoites) give rise to merozoites by paramitosis (SCHOLTYSECK, 1953). The released merozoites invade further host cells. The number of merozoites may vary from 2-3 to several hundreds. Second generation schizonts of the chicken coccidia *E. tenella*, *E. necatrix* and *E. brunetti* from each at least 100 merozoites. The latter stages represent a certain transition to giant schizonts - globidia - in which, several thousands (100 000) of merozoites may develop. Demonstration of schizonts of the above type in scrapings is of diagnostic value.

The endogenous development of the Eimeriae of gallinaceous birds includes very likely more than one schizogonic generation. While TYZZER (1929) identified a single schizogonic generation of *E. acervulina*, WARREN and BALL (1967) found that there were at least three, and VETTERLING and DORAN (1966) described four of them. Four generations of schizonts were found also in the cycle of *E. mivati* (LONG and HORTON-SMITH, 1968). In most of the better known species, 3 schizogonic generations are usual.
The single schizogonic stage of *E. maxima*, as described by BHATIA and PANDE (1968), seems to be an exception.

The sporozoites of *E. tenella* finally invade the caecal epithelial cells (MCLAREN and PAGE, 1968; MCLAREN, 1969). For a certain time they are intimately connected with the host cell's cytoplasm, as their limiting membranes contact directly. The rounded-off trophozoites are already enclosed in a vacuole. The schizonts are surrounded by a unit membrane. In young schizonts the endoplasmic reticulum increases and several nuclei appear scattered in the schizont body. The merozoites separate by evagination of the nuclear protrusions. In light micrographs, the conoid appears as a pale eosinophilic detail at the tapering end of the developing merozoites. Mature merozoites remain for a given time attached to the schizont by one end. They seem to be held by a ring-like structure. In the pale cytoplasmic sectors of juvenile merozoites, thready structures (mitochondria?) surrounded by a double membrane are seen by electron microscopy. Immature merozoites are characterized also by a round vacuole, surrounded by an electron dense membrane. This vacuole disappears by the time of maturation. After the maturation of the merozoites, only a small portion of cytoplasm is retained in the schizont as a residual body. The latter contains linear mitochondria, lipid inclusions and elements of endoplasmic reticulum.

The parasitophorous vacuole is lined with a double limiting membrane. The vacuole increases parallelly with the growth of the parasite so that in the gametogonic stage it is hardly recognized as such. The xenon (parasite + host cell complex) tends to separate from the organism and in this phase it should be regarded a pseudocyst.

According to TYZER, the *E. tenella* xenons, particularly from the second generation on, sink deeply under the epithelial layer owing to the pressure of the epithelial cell row. This seems to account for the subepithelial location of xenons in most histological preparations. GILL and RAY (1957) found that the sporo-
zoites pierce between the epithelium passing downward to the lamina propria, until finally they are phagocytized by the macrophages.

Gametogony follows upon schizogony, but they may run synchronically for a while. This accounts for the circumstance that the birds excrete oocysts for several days (patent period) after gametogony, too, though an intercurrent infection could be excluded.

During gametogony, the telomerozoites may differentiate either into a single female individual (macrogamete) or into several motile flagellated males (microgametes).

Microgamete development of several eimerian species (microgametogony) was studied also electronmicroscopically. In the young microgametocyte, the nucleus divides several times in succession and the new nuclei thus formed arrange peripherally. The chromatin-rich nuclei elongate, assume a parallel order along the periphery and with a little cytoplasm added, they develop to microgametes.

The greater part of the microgamete's body consists of the nucleus. The perforatory, which plays a role in fertilization is localized anteriorly to the nucleus. The perforatory of *E. tenella* microgametes consists of 3 kinetosomes from which arise 3 posteriorly extending flagella. According to JURAJDOVA (1969), also the *E. acervulina* microgametes are triflagellated and there is reason to suppose that this applies also to other *Eimeriae*.

The microgametes insert the appropriate end of the perforatory into macrogametes very likely prior to the development of the wall.

The development of the female stages (macrogametogony) has been disclosed by light- and electronmicroscopic studies (McLAREN, 1969; SCHOLTYSECK, 1962; SCHOLTYSECK and WEISENFELD, 1956,
etc.). With the growth of the macrogametocyte, its nucleus and the enclosed nucleolus tend to become more and more conspicuous. Later on, granules appear in the cytoplasm which play a role in forming a wall around the fertilized macrogamete (zygote). First the so-called wall-forming or membranaceous bodies can be identified already in very early macrogametes, whereas the dark bodies (polysaccharide material) develop only later. They may be denoted according to SCHOLTYSECK's proposal as wall-forming bodies I and II. SCHOLTYSECK, HAMMOND and ERNST (1966) believe that these bodies develop inside a large bladder of endoplasmic reticulum, but McLAREN (1969) failed to verify this concept. McLAREN stated that the earliest wall-forming bodies were spirals freely located in the cytoplasm. The larger wall-forming bodies are membrane-bound and sit in a vacuole.

In a more advanced stage of gametogony, dark bodies appear in increasing numbers in the macrogamete's cytoplasm. They are very likely the electronmicroscopic counterparts of the structures known light microscopically as plastic granules (REICH, 1913; CHEISSIN, 1958, etc.). According to PATTILLO and BECKER (1955), they consist of mucoprotein, mucopolysaccharide or glycoprotein.

As the E. tenella macrogamete tends to become more and more ovoid, the dark bodies tend to arrange peripherally, until they merge to form the middle layer of the cyst wall. The outer layer of the wall is derived from the zygote's outer limiting membrane, while the inner layer seems to be constituted by the wall-forming bodies. Indeed, these bodies disappear as soon as the inner layer is completed.

The duration of endogenous development that is, the time elapsing between the ingestion and shedding of oocysts, varies with the species and is, therefore, a species characteristic. The endogenic phase of the cycle has been called the prepatent period - the correctness of this term may be disputed - and the phase beginning with the appearance of oocysts in the faeces the patent period. With the Eimeriae of gallinaceous birds the
prepatent period may last from 84 hours (E. praecox) to 7 days (E. tenella, E. necatrix, etc.).

2. Exogenic development. Sporogony follows upon gametogony. Sporogony, i.e. the sporulation of the oocysts passed with the faeces to the outside world is completed in 1-2, exceptionally in 3 or more days. Since the conditions of experimental observation have been dissimilar, in most cases it is rather supposed than verified that the sporulation temperature were 22-24° C. It would be more correct to state the sporulation time at a standard temperature with an internationally standardized method.

Under natural conditions the sporulation time of oocysts depends largely on external temperature. GLEBEZDIN (1969) reports that in Turkmenia where there are extreme climatic changes, optimal sporulation of chicken coccidia in the soil took place in November, February and March. In these months the soil temperature did not rise above 26-32° C. At higher - 45° C - temperature, the oocysts failed to sporulate and deteriorated. Alternation of temperatures below and above the freezing point seems to be particularly deleterious to the oocysts. Under such conditions most of them deteriorate and only a very small proportion can complete sporulation.

As to the size, the coccidium oocysts of gallinaceous birds correspond to the small or medium large types. Even E. maxima, whose name suggests extraordinary dimensions, may seldom reach 40μ in length. The cross sections of the sphaerical, subsphaerical, ovoid or elliptic oocysts are usually round but some species are exceptions. The oocysts of E. ventriosa are flat and this flattening is quite conspicuous under the microscope, where the oocysts are seen to rotate slowly around their longitudinal axis in the fluid.

Up to recently, the cyst walls of the eimerian parasites of gallinaceous birds were believed to be bilamellar. Newer in-
vestigations have shown the walls of *E.tenella* (McLAREN, 1969, etc.) and *E.megalostomata* oocysts (ORMSBEE, 1939) to be trilamellar. The concept that the oocysts of *Galliformes* had unilamellar walls was apparently erroneous.

On the sphaerical oocysts the micropyle is as a rule invisible. The more or less conspicuous micropyle of the oval and elliptic oocysts may be focussed by light microscope; sometimes the structural change of the cyst wall at a given site is the only indication of the presence of a micropyle.

With all *Eimeriae* of gallinaceous birds, sporogony takes place in the external world. The sporont of the freshly excreted oocyst soon shrinks to a round body. Its division to sporoblasts is preceded by the so-called pyramid stage (*E.maxima, E.meleagridis, E.tetricis*, etc.). In the pyramid stage, the sporont extends 4 tapering processes, from which separate the round sporoblasts. The latter structures elongate and their further differentiation results in 2 sporozoites within each sporoblast. The pyramid stage is short and occurs very likely with the majority of *Eimeria* but may have escaped attention because of its short course.

Sporocysts containing 2 sporozoites have as a rule a micropyle (Stieda body) at one end. The sporozoites arrange parallelly in the sporocysts, with one end broadened and club-like, the other tapering, usually in head to tail position. By the end of sporogony 8 invasive sporozoites develop in each oocysts.

Development of a primary or oocystic residual body is infrequent in the oocysts of the *Eimeriae* of gallinaceous birds. One or two polar bodies are more often seen inside the oocyst. In the overwhelming majority of the cases, the polar bodies arise by the end of sporogony, though they may sometimes be present already in freshly excreted oocysts. The aggregated or scattered granula occurring within the sporocyst outside the sporozoites is called the secondary or sporocystic residual body. The pre-
sence or absence of the residual bodies or their disappearance from the sporulated oocysts at a given time is a reliable characteristic species identification.

Oocyst discharge lasts for a limited time (patent period) which is characteristic of the species. LONG and ROSE (1970) made the interesting statement that the period of excretion of *E. mivati* oocysts can be prolonged by cortisone treatment of the host. Cortisone also effected an increase in the number of excreted oocysts (ROSE, 1970).

PELLÉRDY, L.: Tyúkfélék (Galliformes) *Eimeria*-fajainak fejlődési ciklusa

A szerző a Galliformes rendbe tartozó madarak *Eimeria*-fajainak endogen és exogen fejlődési ciklusára vonatkozó legújabb ismereteit összegezi. Megállapítja, hogy az egyes fejlődési formák morfológiai viszonyainak és biológiai viselkedésének tanulmányozása a faji determináció szempontjából alapvető fontosságot. A gazda-specificitás, az egyes fejlődési alakok morfológiai sajátosságai, szervi és szöveti megtelepedésükben megnyilvánuló szigorú fajlagosság, az intracellularis lokalizáció törvényes ségei ugyanis faji bélyegei, amelyek ismerete nélkülözhetetlen az egyes *Eimeria*-fajok megkülönböztetése tekintetében.

References


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