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Electron microscopic study on sporocysts and sporozoites of parental strains and precocious lines of rabbit coccidia *Eimeria intestinalis*, *E. media* and *E. magna*

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Abstract The fine structure of sporocysts and sporozoites of parent strains and precocious lines of rabbit coccidia *Eimeria intestinalis*, *E. magna* and *E. media* was studied. The parent strains and precocious lines differ only in the shape and size of refractile bodies (RB). In the sporocysts of precocious lines of *E. magna* and *E. media*, one extremely large RB was seen, either inside one of the sporozoites, or free in the sporocyst. In the oocysts of the precocious strain of *E. intestinalis*, two sporocysts resembled those of the precocious lines of *E. magna* and *E. media*, whereas the other two sporocysts did not harbour any RB. Sporozoites of all the precocious lines contained no, or very small, RB after in vitro excystation.

Introduction

Precocious lines were derived from three species of rabbit coccidia: *Eimeria intestinalis* (Licois et al. 1989, 1990), *E. magna* (Licois et al. 1995) and *E. media* (Licois et al. 1994) in the Rabbit Pathology Unit, INRA, Tours, France. These strains are characterized by a shortened prepatent period and the terminal merogonies are absent from the life cycle of *E. magna* (Pakandl et al. 1996a) and *E. media* (Pakandl et al. 1996b), whereas the third of four asexual generations is lacking in *E. intestinalis* (Licois et al. 1989, 1992). Oocysts of these lines exhibit an interesting peculiarity. In parent strains, the morphology is as usual, with a single refractile body (RB) in each sporozoite. In contrast, there is only one extremely large RB in every sporocyst in the precocious lines of *E. magna* and *E. media* (Licois et al. 1994, 1995). In the precocious line of *E. intestinalis*, two of the four sporocysts have this appearance, i.e. with a single large RB, and the two remaining sporocysts lack any RB (Licois et al. 1989, 1990). To explain the unusual morphology shown by the precocious lines, the fine structure of sporocysts and of in vitro excysted sporozoites was studied.

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Materials and methods

The parasite strains

Pure strains of *Eimeria intestinalis*, *E. media* and *E. magna*, and precocious lines derived from these strains have already been characterized (Licois et al. 1990, 1994, 1995).

Preparation of sporocysts

After treatment with sodium hypochlorite (20%) for 2 h for *E. intestinalis* and overnight for *E. media* and *E. magna* (Coudert et al. 1995), the oocyst walls were mechanically broken in a homogenizer to obtain sporocysts. Direct fixation and embedding of sporocysts did not give satisfactory results, probably due to the intact sporocyst wall being impermeable to fixatives (glutaraldehyde, paraformaldehyde and osmium tetroxide). Therefore we treated the sporocysts with excystation medium (0.33% bile salts and 0.13% trypsin in PBS) for 30 s at 39 °C. The sporozoites did not leave the sporocysts during such a short time. Then, the sporocysts were fixed with 2.5% glutaraldehyde in cacodylate buffer, postfixed in 1% osmium tetroxide, and processed by routine method for transmission electron microscopy (TEM).

Preparation of sporozoites

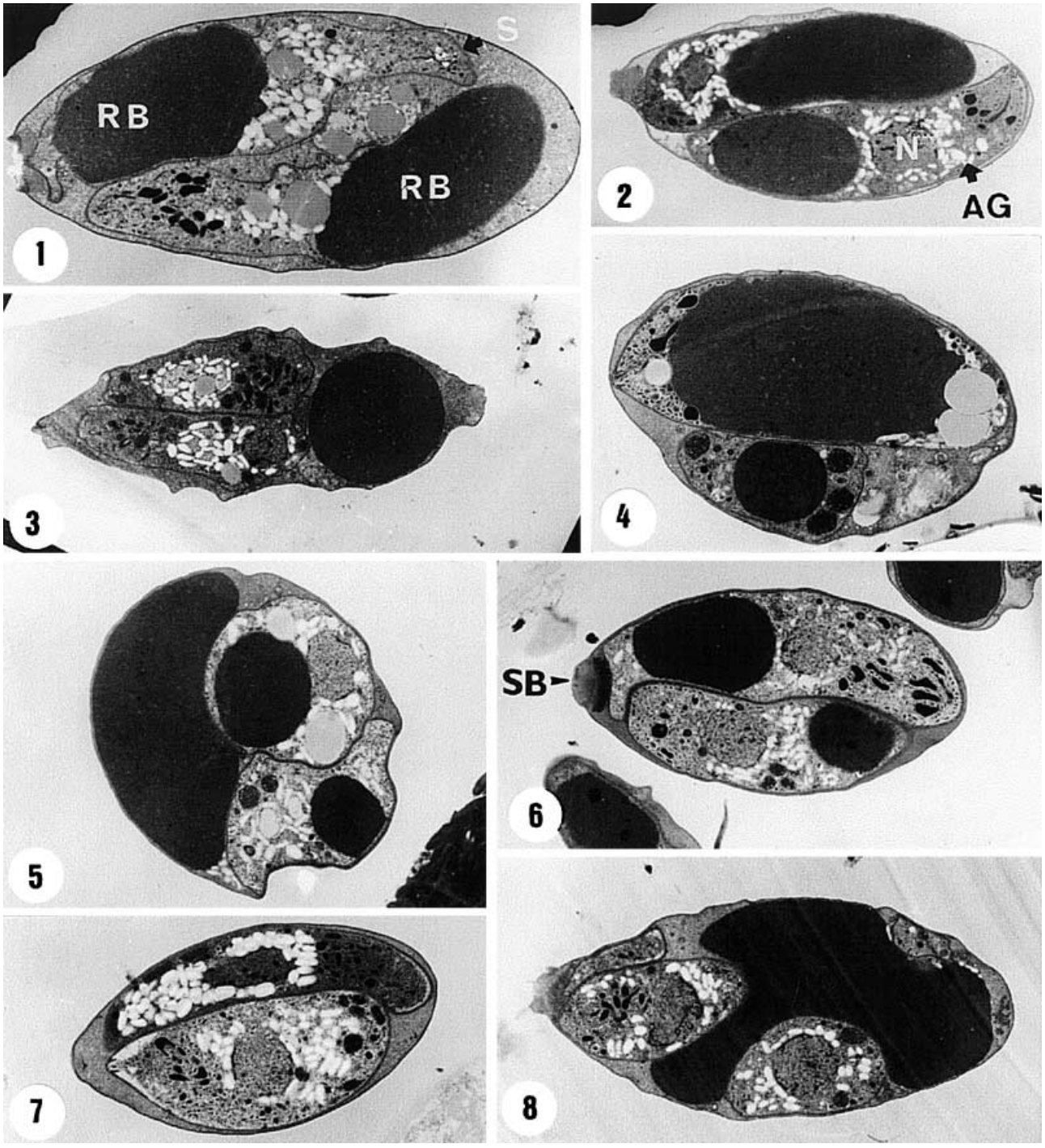
After mechanical destruction of oocysts, the sporocysts were treated with routinely used excystation medium (Ceré et al. 1995). Liberated sporozoites were purified in a column packed with cellulose and were processed for TEM in a similar way to the sporocysts.

Results

Sporocysts

Eimeria magna and E. media

Parent strains. A typical picture was observed in the parent strains of both species. The sporocysts contained two sporozoites oriented "head to tail" (Figs. 1, 2). Both sporozoites had large RB in the posterior part and, depending on the plane of section, smaller anterior RB were seen in some of them. In addition to the RB, the sporozoites contained a nucleus, apical complex, tri-layered pellicle and mitochondria. Numerous amylopectin granules and large round inclusions were seen in the cytoplasm. A Stieda body, observed in longitudinal sections, resembled a plug closing a hole in the sporocyst wall. The Stieda body consisted of two parts: an upper portion, homogenous and uniformly electron-dense, and



a lower part, formed by a layer of a more electron-dense substance, about 0.4 μm thick. A homogenous matrix filled the sporocysts. Because of the influence of the excystation medium, we could see some sporocysts in a more advanced phase of excystation, in which the Stieda body and matrix disappeared.

Precocious lines. Generally, the sporocysts of precocious lines of both *Eimeria magna* and *E. media* differed from those of their parent strain only in the size, shape and placement of RB. All the sporocysts of the precocious strains of *E. magna* and *E. media* were of one type and three pictures were seen:

(1) Sporozoites did not contain any RB, but one large RB was observed inside the sporocyst (Fig. 3). In this case, the RB was free in the sporocyst "matrix" and it was not surrounded by any membrane.
(2) One of the sporozoites had an extremely large RB, whereas the second had none, or had only a RB smaller than those present in sporozoites of the parent strain (Fig. 4).
(3) One or both sporozoites contained small RB and, as in the first case, a free RB was present in the sporocyst (Fig. 5).

E. intestinalis

The sporocysts of the parent strain of *E. intestinalis* (Fig. 6) were basically similar to those of *E. magna* and *E. media*.

The sporocysts of the precocious line of *E. intestinalis* were of two types. In the first, neither sporozoites nor sporocyst contained any RB (Fig. 7). The second type, however, resembled the sporocysts of the precocious lines of *E. magna* and *E. media*. RB were inside the sporozoites and/or free in the sporocysts (Fig. 8).

Sporozoites after in vitro excystation

Sporozoites of all three parental strains had ultrastructural features typical for the genus *Eimeria*. They harboured at least one RB (Fig. 9). In contrast, sporozoites of the precocious lines usually had no RB; and if any RB was present, it was relatively small (Fig. 10).

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Fig. 1 Transmission electron micrograph of sporocyst in the parental strain of *Eimeria magna* (×7,000). *AG* amylopectine granules, *N* nucleus, *RB* refractile body, *S* sporozoite, *SB* Stieda body
Fig. 2 Parental strain of *E. media*
Fig. 3 Precocious line of *E. media*. The sporocyst contains a large RB which is outside the sporozoites
Fig. 4 Precocious line of *E. magna* with a huge RB in one sporozoite and a small RB in the other
Fig. 5 Precocious line of *E. magna*. The two sporozoites each contain one small RB and another RB is free in the sporocyst
Fig. 6 Parental strain of *E. intestinalis*
Fig. 7 Precocious line of *E. intestinalis*: First type of sporocyst. Neither sporocysts nor sporozoites contain any RB
Fig. 8 Precocious line of *E. intestinalis*. Second type of sporocyst, harbouring a large free RB

Discussion

Because of the difficulty in preparing oocysts and sporocysts for TEM, there is a paucity of ultrastructural studies of this stage in eimerian parasites. Two methods have been used to overcome the impermeability of the oocyst/sporocyst wall to fixatives, dehydrating reagents and resins. Birch-Andersen et al. (1976) used double sectioning (frozen oocysts in a cryocut and embedded sections in an ultramicrotome) for this purpose. Beesley and Latter (1982) developed a less complicated method to break the oocyst/sporocyst wall. They immersed the oocysts in liquid nitrogen for a short time. We consider our method, described in the present paper, to be the simplest and to give satisfactory results. However, it should be kept on record that the beginning of excystation is effectively observed.

Beesley and Latter (1982) described a Stieda body in *Eimeria tenella*. Unlike our observation, they did not distinguish its two parts by TEM, but noted that the oocysts of *E. tenella* contained a substieda body. To our knowledge, the ultrastructure of oocysts or sporocysts of rabbit coccidia has never been studied.

We have noted differences in the size and shape of the RB. The only method used was TEM and it would be incorrect to give measurements based exclusively on sectioned material. Only one large RB (inside or outside the sporozoite) was seen in sporocysts of the precocious lines of *E. magna* and *E media* and in one of the two types of sporocysts found in *E. intestinalis*, while the other RB were very small or absent. It should be noted that the size of RB depends on the plane of section; but our conclusions are corroborated by light microscopy observations (Licois et al. 1989, 1990, 1994, 1995), since only one very large RB is visible within the sporocysts.

In the parent strains, the RB are equally distributed in the first generation merozoites and small RB are even seen in the merozoites of second generation. The presence of two RB is a typical picture for first generation merozoites of *E. magna* (Pakandl et al. 1996a, b).

The absence of RB within the excysted sporozoites of precocious lines of *E. magna* and *E. media* is consistent with our observations on the first and second generation merozoites of these lines (Pakandl et al. 1996a, b). It can be concluded that the material composing RB is not equally distributed between the sporozoites within one sporocyst in the precocious lines of *E. magna* and *E. media* during oocyst formation. In the precocious line of *E. intestinalis*, there is an unequal amount of this material in the two types of sporocyst.

The sporocysts of precocious lines contain a RB (except one of the two types of sporocysts in the precocious line of *E. intestinalis*), whereas it is usually lacking in excysted sporozoites. Using light microscopy (unpublished results), free RB can be seen outside as well

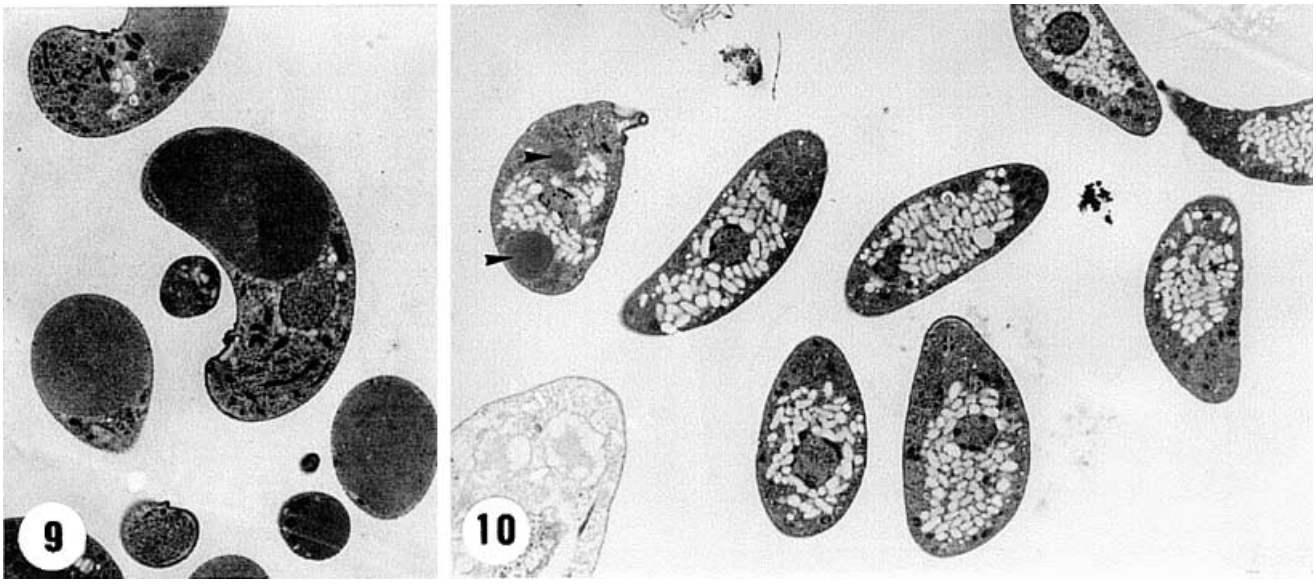

Fig. 9 Sporozoites of the parental strain of *E. intestinalis* (×5,000). At least one RB is present in the sporozoites
Fig. 10 Sporozoites of the precocious line of *E. media*. No RB or, exceptionally, very small RB (*arrowheads*) can be seen in the sporozoites

as inside the sporocysts during excystation. Two different explanations for this phenomenon may be considered:

(1) The RB can easily be lost during excystation. All the sporocysts observed were treated for 30 s with excystation medium and that may explain why the RB were inside as well as outside the sporozoites. However, we do not know how the RB can vacate the sporozoites without fatal damage to the cell.
(2) Sporozoites with extremely large RB, which represent an aberrant form, die during or immediately after excystation. In this case, various configurations observed in the sporocysts (RB outside and/or inside the sporozoites) are not the result of treatment with the excystation medium; such a situation can occur even in non-treated sporocysts.

Nevertheless, it is certain that sporozoites without any RB are able to penetrate into host cells and give rise to meronts. Hence, the exact role of RB in the species which we have studied is not clear.

It should be pointed out that sporocysts, sporozoites, meronts and merozoites of parent strains and precocious lines derived from them differ in their morphology (besides other properties). This very interesting peculiarity has not been recorded in chicken species, in which precocious lines have also been obtained.

References

Beesley JE, Latter VS (1982) The sporulation of *Eimeria tenella* as revealed by a novel preparative method. Z Parasitenkd 67: 255–260

Birch-Andersen A, Ferguson DJP, Pontefract RD (1976) A technique for obtaining thin sections of coccidian oocysts. Acta Path Microbiol Scand Sect B 84: 235–239

Ceré N, Licois D, Humbert JF (1995) Study of the inter- and intraspecific variation of *Eimeria* spp from the rabbit using the random amplified polymorphic DNA. Parasitol Res 81: 324–328

Coudert P, Licois D, Drouet-Viard F (1995) *Eimeria* and *Isospora*. *Eimeria* species of rabbits. In: Eckert J, Braun R, Shirley MW, Coudert P (eds) Biotechnology. Guidelines on techniques in coccidiosis research. Office for Official Publications of the European Communities, Luxembourg, pp 52–73

Licois D, Coudert P, Bahagia S (1989) Some biological characteristics of a precocious line of *Eimeria intestinalis*. In: INRA (ed) Coccidia and intestinal coccidiomorphs. 5th Int Coccidiosis Conf, Tours., INRA, pp 503–508

Licois D, Coudert P, Boivin M, Drouet-Viard F, Provot F (1990): Selection and characterization of a precocious line of *Eimeria intestinalis*, an intestinal rabbit coccidium. Parasitol Res 76: 192–198

Licois D, Coudert P, Bahagia S, Rossi GL (1992) Endogenous development of *Eimeria intestinalis* in rabbits (*Oryctolagus cuniculus*). J Parasitol 78: 1041–1048

Licois D, Coudert P, Drouet-Viard F, Boivin M (1994) *Eimeria media*: selection and characterization of a precocious line. Parasitol Res 80: 48–52

Licois D, Coudert P, Drouet-Viard F, Boivin M (1995) *Eimeria magna*: pathogenicity, immunogenicity and selection of a precocious line. Vet Parasitol 60: 27–35

Pakandl M, Eid Ahmed N, Licois D, Coudert P (1996a) *Eimeria magna* Pérard, 1925: life cycle studies with parental and precocious strains. Vet Parasitol 65: 347–351

Pakandl M, Gaca K, Licois D, Coudert P (1996b) *Eimeria media* Kessel, 1929: comparative study of the endogenous development between precocious and parental strains. Vet Res 27: 465–472