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Distribution of *Eimeria tenella* Schizonts in the Chorioallantoic Membrane (CAM) of Chicken Embryos

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Abstract

The development and distribution of *Eimeria tenella* second-generation schizonts within the chorioallantoic membrane (CAM) of the chicken embryo was investigated following the inoculation of sporozoites into the allantoic cavity. In the CAMs spread on glass slides, 68% of the developing schizonts were found in colonies with an average of 27 schizonts per colony. Many schizonts (29.2%) were detected adjacent to blood vessels, and 80.2% of all the schizonts were located within 40 μm of blood vessels. In cross section, the schizonts were detected in the connective tissue between the inner allantoic limb and the blood vessels of the CAM. Schizonts were found only within cells of the mesodermal layer of the CAM, not within cells of the allantoic limb or endothelial cells of the blood vessels. The process of lesion formation and hemorrhage in the CAM caused by the developing second-generation schizonts was described.

Key words: chicken embryo, chorioallantoic membrane, *Eimeria tenella*, schizont

Introduction

Eimeria tenella was first cultivated in chicken embryos by Long (1965). Since then, the development of second-generation schizonts in the chorioallantoic membrane (CAM), following inoculation of sporozoites into the allantoic cavity, has been observed repeatedly (Ishii and Onaga, 1971, Itagaki *et al.*, 1972, Long, 1965, 1970, 1973, Nakai *et al.*, 1982). The second-generation schizont of *E. tenella*, which usually causes hemorrhages in chicken embryos, is the most pathogenic developmental stage of the parasite. However, since only limited information is available concerning tissue parasitization and mechanisms of pathogenicity, we thought it important to further investigate the development of second-generation schizonts in the CAM.

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Materials and Methods

Organisms: *E. tenella* K-2 strain was used (Nakai and Ogimoto, 1983).

Cultivation in chicken embryos: The parasites were cultivated by the methods described previously in detail (Nakai *et al.*, 1982). Briefly, however, they are as follows: excysted sporozoites were obtained from washed and sterilized oocysts. Approximately $1\text{-}5\times10^4$ sporozoites were inoculated into the allantoic cavity of 10-day-old white leghorn chicken embryos, after which the infected embryos were incubated at 41°C for up to 5 days.

Schizont development: On the 4th or 5th day of infection, the CAMs were washed in saline, spread on glass slides, air-dried, fixed in absolute methanol and stained with Giemsa stain. When parasites were detected, the number and location of the second-generation schizonts were recorded, and microphotographs were taken. Some pieces of fresh CAM were fixed in Bouin's fluid, embedded in paraffin, sectioned, stained with hematoxylin and eosin (HE), and examined microscopically.

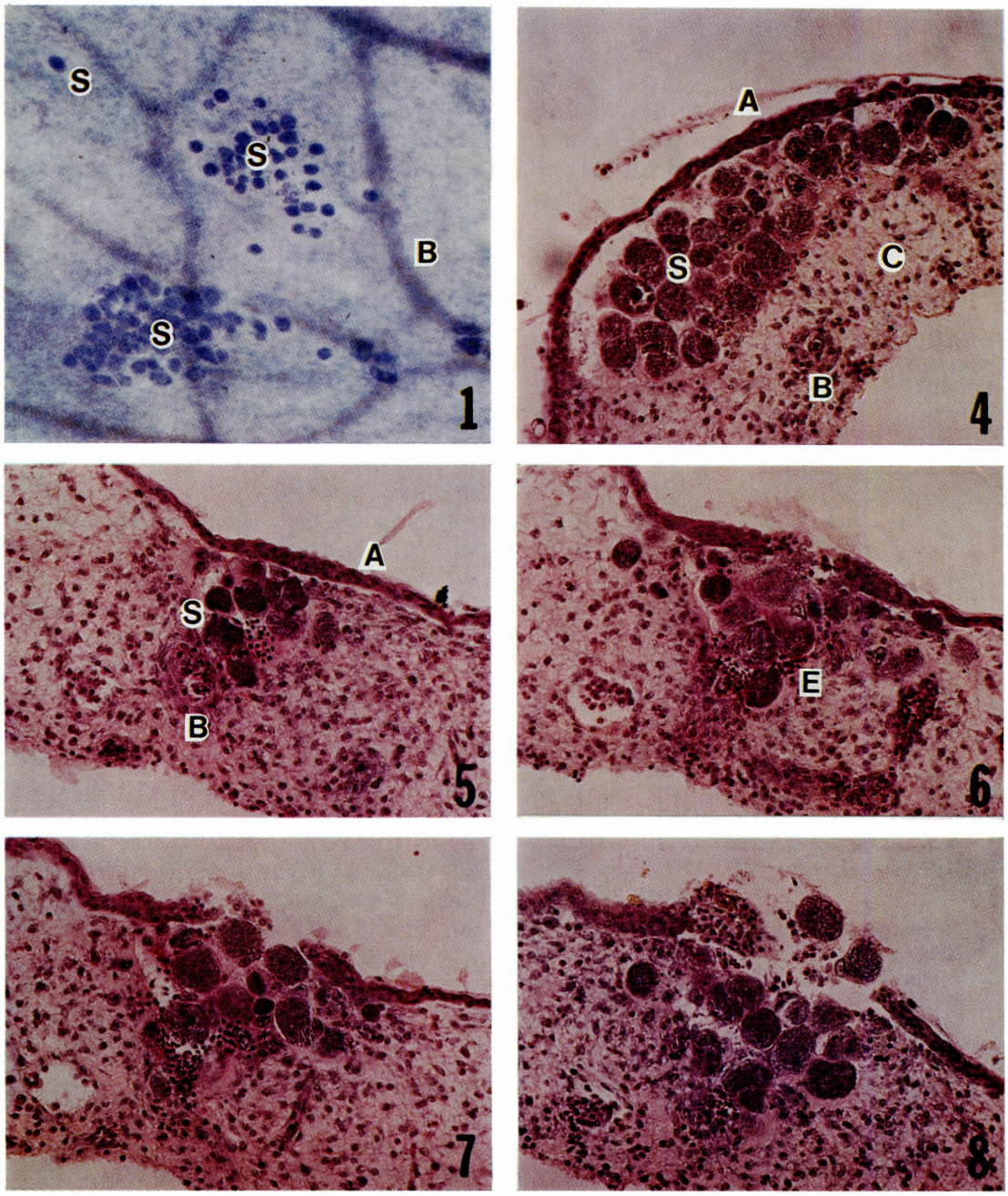

Fig. 1 and 4—8. **1.** Whole mount of infected CAM. Giemsa stain. ×100. **4—8.** Cross sections of infected CAMs. HE. ×185. Pathological changes in blood vessels and CAMs related to the development of second-generation schizonts. **A,** inner allantoic limb; **B,** blood vessel; **C,** connective tissue; **E,** erythrocytes; **S,** second-generation schizonts.

Results

Second-generation schizonts were found singly and in colonies within the CAM (Fig. 1). The total number of schizonts within colonies greatly exceeded the number of solitary parasites, 898 to 418 (Table 1). Most of the colonies contained less than 30 schizonts, but one large colony containing 82 schizonts was encountered. The average number of schizonts per colony was 27 (Fig. 2).

Table 1. Colony formation by second-generation schizonts.

Number of schizonts	Solitary development	Within colonies
1316 (100%)	418 (31.8%)	898 (68.2%)

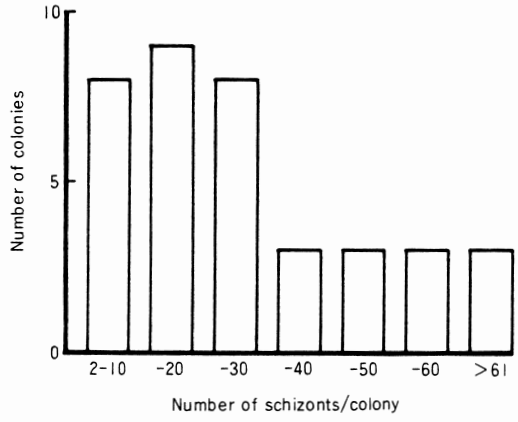

Fig. 2. Number of schizonts per colony. The average was 26.6 ± 2.3 (M ± SE; n = 1316). The largest colony consisted of 82 schizonts.

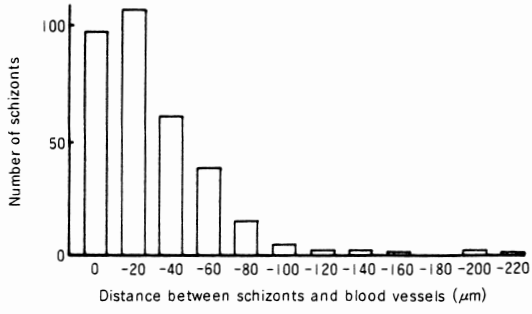

Fig. 3. Distance between schizonts and blood vessels. Of a total of 329 schizonts monitored, 264 (80.2%) were within 40 μm of the nearest blood vessel.

The distance between schizonts and blood vessels was measured (Fig. 3). Of 329 schizonts which were examined at randam, 96 were adjacent to vessels, and 168 were within 40 μm of the nearest blood vessel. Thus, 80.2% of the schizonts were located within 40 μm from blood vessels.

In cross sections of the CAMs, schizonts were observed spreading out in the connective tissue from just under the inner allantoic limb to the midst of the connective tissue layer. No schizonts were observed either in cells of the allantoic limb or in the endothelial cells of blood vessels. Since the blood vessels were in the connective tissue adjacent to the outer chorionic limb, schizonts were distributed between the inner allantoic limb and the vasculature.

The progression of damage to blood vessels and CAMs resulting from development of the second-generation schizonts was observed in serial cross sections (Figs. 5—8). Schizonts parasitizing cells bordering a blood vessel are seen in Fig. 5. With the maturation of the schizonts and the emergence of merozoites, erosion of the blood vessel and hemorrhage into the surrounding connective tissues is visible (Fig. 6). The inner allantoic limb also was destroyed by the active merozoites (Fig. 7), and hemorrhage and migration of merozoites into the allantoic cavity occurred in this grossly damaged area (Fig. 8).

Discussion

Long (1970, 1973) observed macroscopically discrete focal lesions in the CAMs of chicken embryos inoculated with *E.tenella* sporozoites, and reported their association with parasite schizogony. Microscopically, second generation schizonts were located beneath the epithelium of the allantois, and lesions were seen along the walls of blood vessels (Long, 1965, 1970, 1973).

The present paper reveals further details on the distribution of second-generation schizonts in tissues of the CAM. Numerous schizonts

developed in colonies which were seen macroscopically as focal lesions (Table 1 and Figs. 1, 2). The second-generation schizonts in one colony likely come from one first-generation schizonts.

Most schizonts were located next to blood vessels (Table 3). This observation suggests that the parasites preferably parasitize tissues adjacent to blood vessels in order to obtain oxygen and nutrients.

The schizonts were found in cells of the connective tissue adjacent to the inner allantoic limb, but not in cells of the allantoic limb or endothelial cells of blood vessels (Fig. 4). The CAM is formed by fusion of the outer mesodermal layer of the allantois with the mesodermal lining of the chorion in embryonic development. Thus, the CAM is composed of three germ layers, the endodermal layer (1—3 layers of cells of the inner allantoic limb), the mesodermal layer (the connective tissue formed form the allantois and the chorion), and the ectodermal layer (8 or more layers of cells of the outer chorionic limb) (Romanoff, 1960). The present observations revealed that the second-generation schizonts parasitized only the mesodermal layer of the CAM. This is similar to what is seen in chicken cecal infections, where the mesodermal cells of the lamina propria, instead of the endodermal epithelial cells, are parasitized by the second-generation schizonts.

Acknowledgments

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