circulating antibodies may gain access to the invasive stages (Rose and Long, 1969; Rose, 1978).

Davis et al. (1978) have suggested that the involvement of locally produced antibodies of the IgA class in immunity to coccidia is of strategic importance as a first line of defense against invasion of the mucosal surfaces. They demonstrated that cecal contents obtained from chickens infected with E. tenella, containing mainly IgA antibodies, had anti-schizont and sporozoite neutralizing activity. According to Rose (1978) secretory immunoglobulin may participate in local immunity to Eimerian infection, and may well be active when no circulating antibodies can be detected.

ii. Cell-mediated immunity

Cell-mediated immunity is considered to be the major factor in the development of host immunity to intracellular parasites (Rose, 1978; Mesfin and Bellamy, 1979).

Neonatal thymectomy and bursectomy have been used in attempts to answer the question of whether immunity to coccidial infection is humoral or cell-mediated (Pierce and Long, 1965; Rose and Long, 1970, Liburd and Mahrt, 1971; Rose, 1973, 1974b). It was found that neonatal thymectomy had either no influence on the acquisition of immunity or only a slight degree of difference was noted in infections between thymectomized and intact animals (Rose, 1973; Mesfin and Bellamy, 1979). According to Rose (1974b), complete surgical thymectomy in chickens is difficult to ensure because of the multilobular nature of this organ and may account for the varying results obtained. Mesfin and Bellamy (1979) showed that nude mice (athymic) discharged more oocysts on primary infection and they never developed immunity to E. falciformis var

pragensis upon reinfections. On the other hand, normal litter mates and Swiss white mice developed immunity to the parasite after the first or second infection.

It is well known that the immune response to some coccidial infections does not interfere with the penetration of epithelial cells by the parasite, but it does act against invasive stages as they develop within host cells (Horton-Smith et al, 1963a; Mesfin and Bellamy, 1979).

Cell-mediated immunity to coccidial infection has been demonstrated with transferred immune lymphocytes, transfer factor, macrophage migration inhibition test, delayed hypersensitivity reaction and in vitro lymphocyte blastogenesis (Klesius, Kramer, Burger and Malley, 1975; Klesius, Kramer and Frandsen 1976; Klesius, Kristensen, Elston and Williamson, 1977; Liburd and Mahrt, 1971; Liburd, Pabst and Armstrong, 1972; Morita, Tsutsumi and Soekawa, 1973; Rose, 1977; Klesius, Elston, Chambers and Fudenberg, 1979; Klesius and Kristensen, 1977; Giambrone and Klesius, 1980; Giambrone, Klesius and Edgar, 1980).

Macrophage migration inhibition tests, which are essentially an in vitro test of cell-mediated immunity, have been demonstrated in spleens of chickens immunized with E. tenella (Morita et al, 1973), and peritoneal exudate cells of chickens immunized with E. tenella and E. maxima (Rose, 1974). Rose (1977) studied the correlation between the delayed skin hypersensitivity reaction and the macrophage migration inhibition (MMI) test in the presence of E. tenella oocyst antigen. Although a few positive MMI tests were observed, the results were highly variable and did not correlate with the wattle delayed hypersensitivity reaction. In addition to chickens, delayed hypersensitivity reaction to

coccidial antigens has been demonstrated in rabbits infected with E. stiedae (Klesius et al., 1976) and in calves infected with E. bovis (Klesius et al., 1977). The most suitable antigen which was used for skin testing of delayed hypersensitivity reaction experiments was prepared from oocysts (Klesius et al., 1976, 1977; Rose, 1977; Giambrone and Klesius, 1980; Giambrone et al., 1980).

Klesius et al. (1977) demonstrated that some calves immunized with E. bovis gave a positive delayed hypersensitivity reaction to E. stiedae oocyst antigen and this cross-reaction was more evident when lymphocyte blastogenesis assays were used. Cross-reactivity was also demonstrated in delayed hypersensitivity reactions using oocyst antigens from E. tenella, E. maxima, and E. necatrix, indicating some antigen sharing among species of chicken coccidia (Giambrone et al., 1980). It was also reported that chickens immunized with their respective species of coccidia demonstrated a delayed hypersensitivity reaction to oocyst antigen prepared from E. bovis (Giambrone et al., 1980). Giambrone and his coworkers found that chickens vaccinated with a commercial vaccine (Coccivac, containing viable oocysts from eight chicken Eimeria species) were able to mount a measurable cell-mediated immune response (delayed hypersensitivity) and were resistant to challenge with E. necatrix and E. tenella. They concluded that delayed hypersensitivity reaction to oocyst antigen can predict resistance to coccidiosis.

The essential role of T-lymphocytes in immunity to E. neischulzi reinfection has been demonstrated in nude (athymic) rats by Rose and Hesketh (1979) and Rose, Hesketh, Ogilvie and Festing (1979). Bovine transfer factor prepared from lymph node lymphocytes of calves showing delayed hypersensitivity reaction to E. bovis was able to transfer the

hypersensitivity reaction and partial protection when given to susceptible calves. In addition, rabbits given this bovine transfer factor demonstrated delayed hypersensitivity reaction to the cross-reacting E. stiedae (Klesius and Kristensen, 1977).

GENERAL MATERIALS AND METHODS

Experimental Animals

Male white Leghorns (De Kalb) were obtained as day-old chicks and raised under coccidia-free conditions in isolation. Chicken starter* containing 20% crude protein prepared free of any anticoccidial agents or other drugs was used as feed. Chickens ranging from 6-10 weeks of age were used in all experiments, unless otherwise stated. Birds infected with Eimeria necatrix were kept in ammonia fumigated isolation rooms inside disposable cardboard boxes (20" x 16" x 18"). One to ten chickens were placed in one box, depending on their age, size or experiment. Feed and water were provided ad libitum.

Eimeria necatrix

1. Strain

The strain of E. necatrix used in all the experiments was isolated from a single oocyst infection as described by Remmler & McGregor (1964) and maintained in the Department of Pathology since 1972. The isolate was proven uncontaminated using histological procedures and repeated infection. Sporulated oocyst cultures older than one month were not used in the experiments except for stock culture preparation. Required oocyst numbers were administered directly into the crop using a sterile disposable syringe fitted with a 15 gauge udder infusion canula.

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* Shur Gain, Floradale Feed Mill Ltd., Floradale, Ontario.

2. Stock Culture

Ten to fifteen-week-old chickens were usually used for preparation of stock cultures. Chickens were inoculated with approximately 20,000 sporulated oocysts. At about 9 days post infection the chickens were killed, their ceca were removed and opened longitudinally. All contents and mucosa were scraped using a scalpel blade and suspended in sterile 2.5% potassium dichromate solution. The suspension was placed in a glass dish or Erlenmyer flask, so that the depth of the suspension in the dish or the flask did not exceed 3 cm to allow good oxygenation during mixing. To facilitate sporulation the suspension was either continuously stirred or shaken in a water bath at a temperature of 26°C for up to 2 days.

During the 2nd day samples from the oocyst suspension were examined to determine the stage of sporulation. Under these conditions a high proportion were completely sporulated, as indicated by the formation of the Stieda body of the sporocyst, in about 2 days. Potassium dichromate was then removed by decanting the supernatants after centrifugation for 10 minutes at 650 xg. The oocysts were washed at least three times in distilled water by suspension and centrifugation until no trace of potassium dichromate could be seen. If large pieces of tissue were present, the final preparation was seived to get rid of these. The oocysts were counted, labelled and stored in sterile containers at 4°C until use.

3. Total daily oocyst production

Beginning on day 6 post infection chickens were placed on a one inch wire mesh in disposable cardboard boxes. Feces was collected every

24 hours in stainless steel trays covered with aluminum foil which was placed underneath the wire mesh. Total oocyst production per 24 hours was determined according to the method of Long and Rowell (1958). The collected feces was suspended in a measured volume of tap water and saturated sodium chloride solution added so that the final concentration of sodium chloride was 35%, repreenting a specific gravity of 1.076. According to Long and Rowell (1958), at this specific gravity the oocysts of E. necatrix neither floated nor sank. Total volumes used were totally dependent on the amount of feces collected and on the consistency of the mixture. Volumes ranging from 300 to 1000 ml were used and counts made in the following manner:

1. Feathers and feed were removed from the feces and the feces were placed in a beaker.

2. Feces was first mixed well with the correct volume of water and then the required volume of saturated sodium chloride added.

3. The mixture was homogenized in a Waring blender for 1 minute, transferred to a clean beaker and stirred very well for about 5 minutes to ensure uniform oocyst distribution in the mixture.

4. Using a disposable Pasteur pipette 1 millilitre of the final mixture was placed in a 15 ml graduated conical centrifuge tube. Saturated sodium chloride was added up to 10 ml mark and the suspension was mixed very well by inverting the tube 20 times.

5. Some of the mixture was transferred to fill both sides of a McMaster counting chamber and the chamber allowed to sit for at least 5 minutes before counting.

6. All oocysts seen in the 1 centimeter square area of both sides of the McMaster counting chamber were counted and the average used to

calculate the total oocyst numbers according to the following formula:

Y x 10 x 6.6* x Z = Total number of oocysts in volume Z
y is the average number of oocysts counted
10 is the dilution factor
Z is the total volume of the mixture

* The volume under the ruled area of the McMaster chamber is 0.15 ml. To bring this volume to 1 ml a factor of 6.6 is used, which is obtained by dividing 1 by 0.15.

4. Intestinal lesion scores

Intestinal lesions were scored according to the severity of infection. The presence of an established infection could be demonstrated by the presence of white foci on the serosal surface of the intestine, representing second generation schizont nests. In most cases, petechial hemorrhages within schizont nests could be seen from the serosal surface but not from the mucosal surface of the intestine. The presence of small schizont nests and petechial hemorrhages represented the earliest lesion to be seen during second generation schizont development. Other pathological changes seen, depending on the severity and stage of infection, included: congestion, edematous thickening of the intestinal wall, ecchymotic hemorrhages, fibrin and excessive mucus production with or without large blood clots, and in the most severe cases when the chicken was near death, a blood cast filling the intestinal lumen. Scores were given as follows depending on severity of infection:

1 - a score of 1 for the presence of schizont nests and petechial hemorrhage as seen from the serosal surface of the intestine

2 - a score of 2 for the presence of congestion

3 - a score of 3 for the presence of intestinal thickening

4 - a score of 4 for the presence of ecchymotic hemorrhages

5 - a score of 5 for the presence of fibrin and excessive mucus production

6 - a score of 6 for the presence of blood clots

7 - a score of 7 for the presence of blood casts and ballooning of the intestine

Chickens with higher lesion scores always had the lesions represented by that score and those of chickens with lower lesion scores. For example, a chicken with a lesion score of 4 would have ecchymotic hemorrhages plus the lesions of scores 3, 2 and 1.

Unless otherwise stated, lesions were scored at 5 days post infection.

Histological Techniques

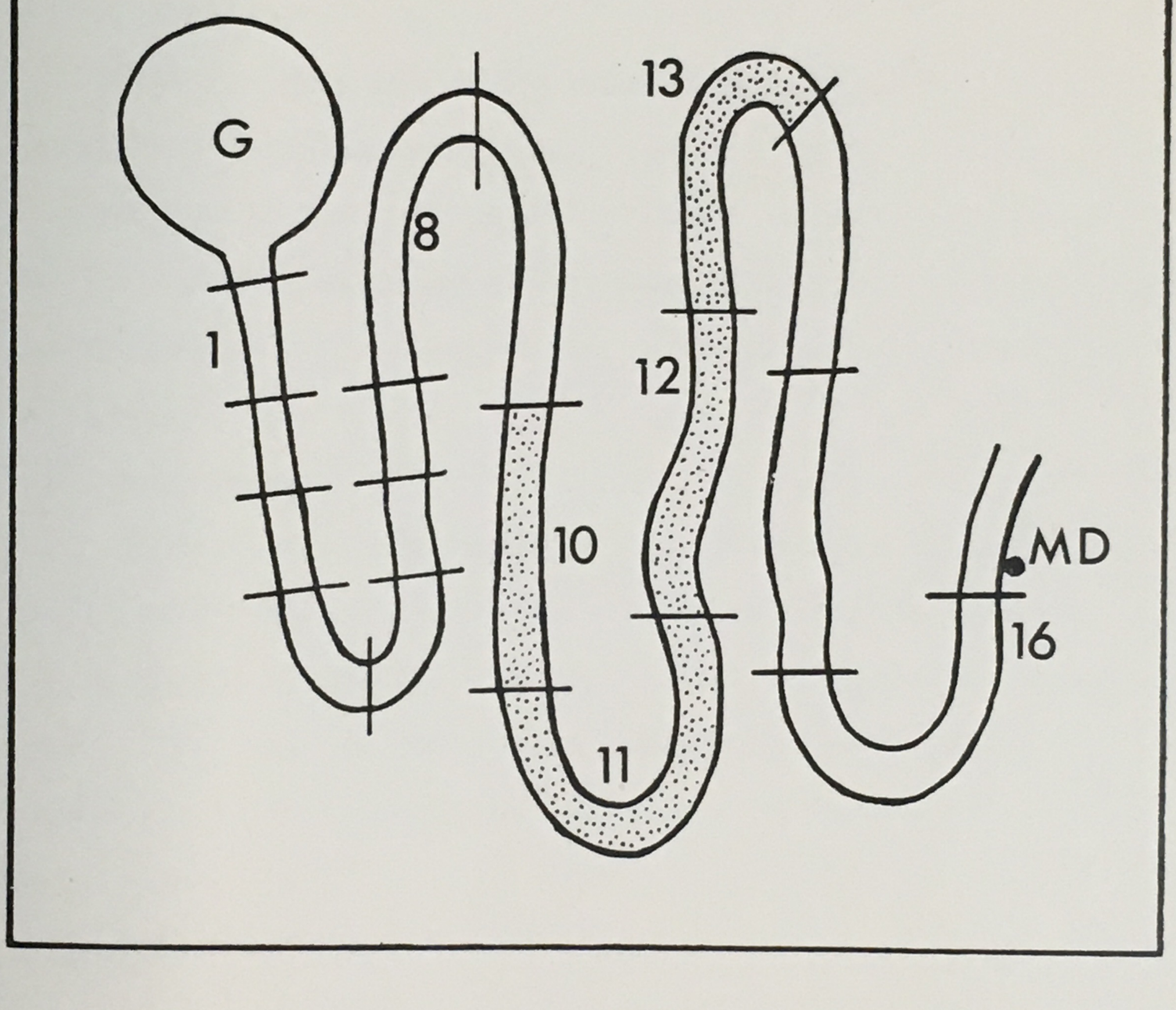
1. Light microscopy

Chickens were killed by cervical dislocation and designated parts of intestine (Fig. 1-1) were removed as soon after death as possible and placed in 10% formalin in phosphate buffered saline (PBS pH 7.4, 0.02 M) for 1 to 2 minutes. These pieces were opened longitudinally and returned to the fixative. Twenty four to 48 hours after fixation, two longitudinal segments from areas 10, 11 and 12 or 11, 12, and 13 (Fig. 1-1) were trimmed and submitted for paraffin embedding, sectioning and staining with hematoxylin and eosin.

2. Electron microscopy

i. Fixation

Pieces of intestine were removed as soon as possible after death, opened longitudinally and the mucosal surface washed with cold phosphate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