Figure 4-1. Double immunodiffusion test. Precipitation lines developed between "soluble products" of second generation schizonts (sa) using anti-oocyst serum (S1) and antiserum prepared from orally infected chickens ($S_2$) that received the intraperitoneal injection of adjuvant.

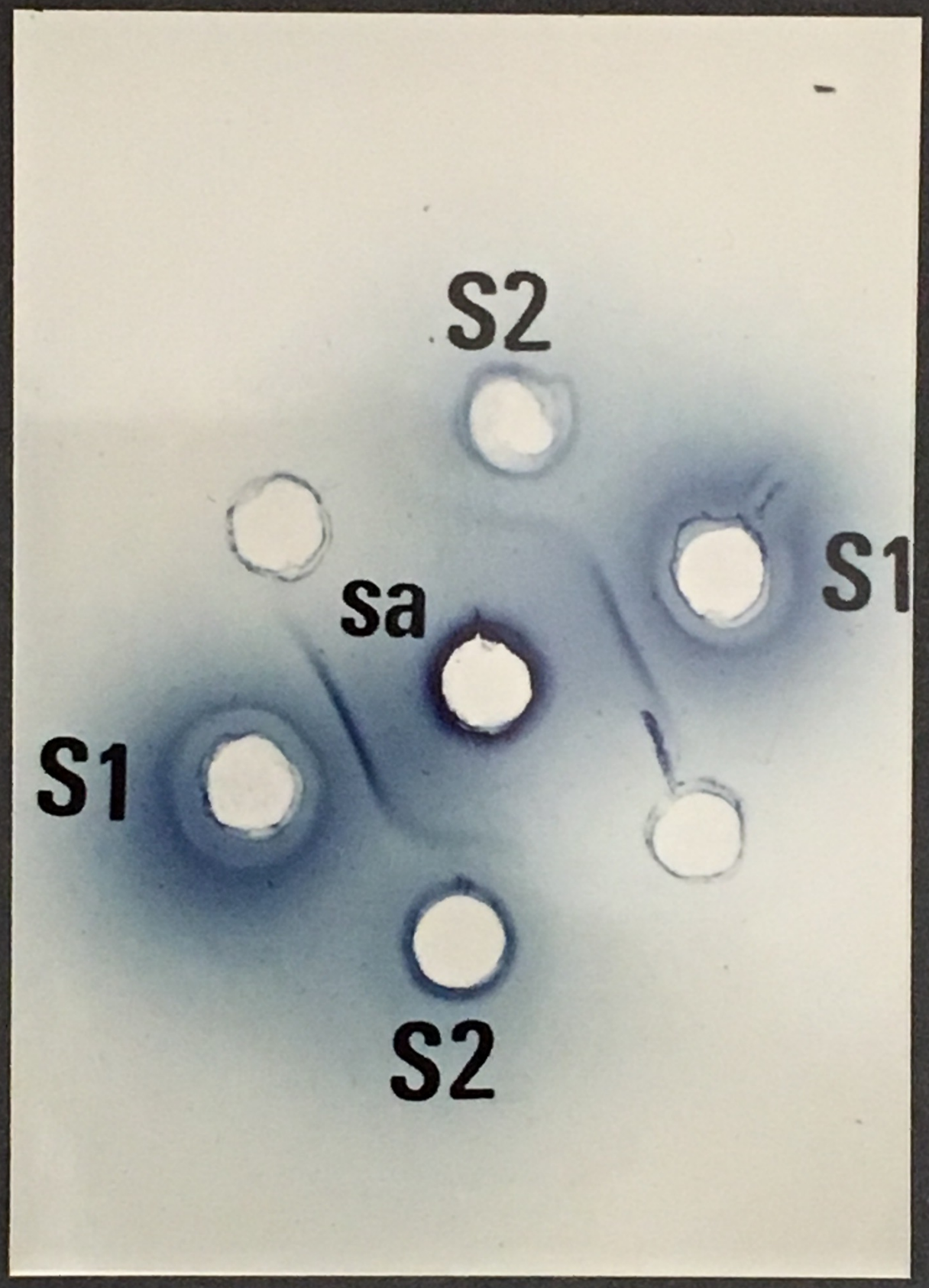
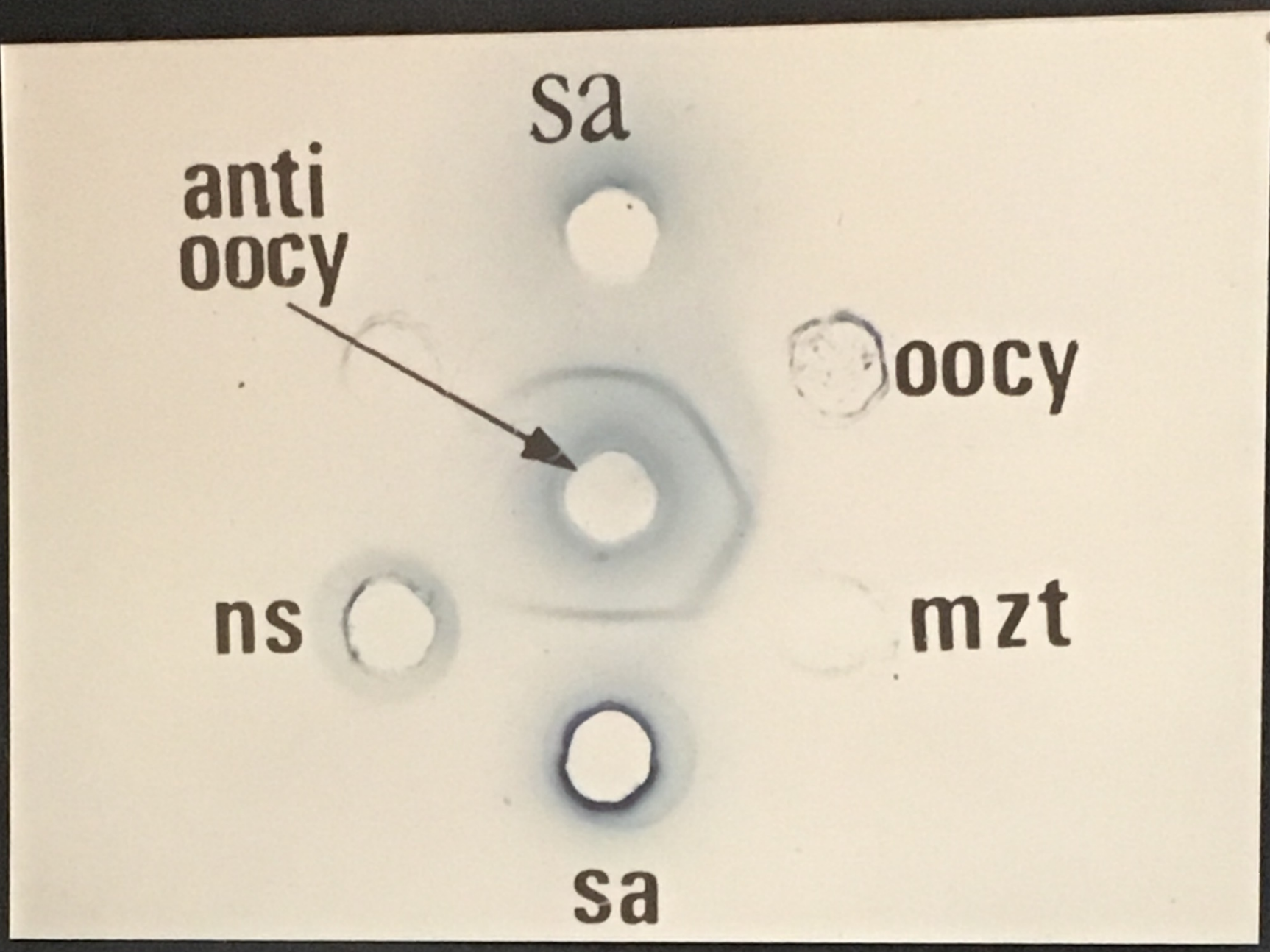

S2
S1
Sa
S1
S2

Figure 4-2. Double immunodiffusion test. Precipitation lines developed between antioocyst serum (antioocy) and "soluble products" (sa), oocyst (S1) and second generation merozoite (mzt) antigens. Normal chicken serum (ns).

Figure 4-3. Double immunodiffusion test using infected (+ intes) and uninfected (- intes) intestinal tissues. Antioocyst serum (S1); "soluble products" (sa).


sa
anti
oocy
oocy
ns
mzt
sa

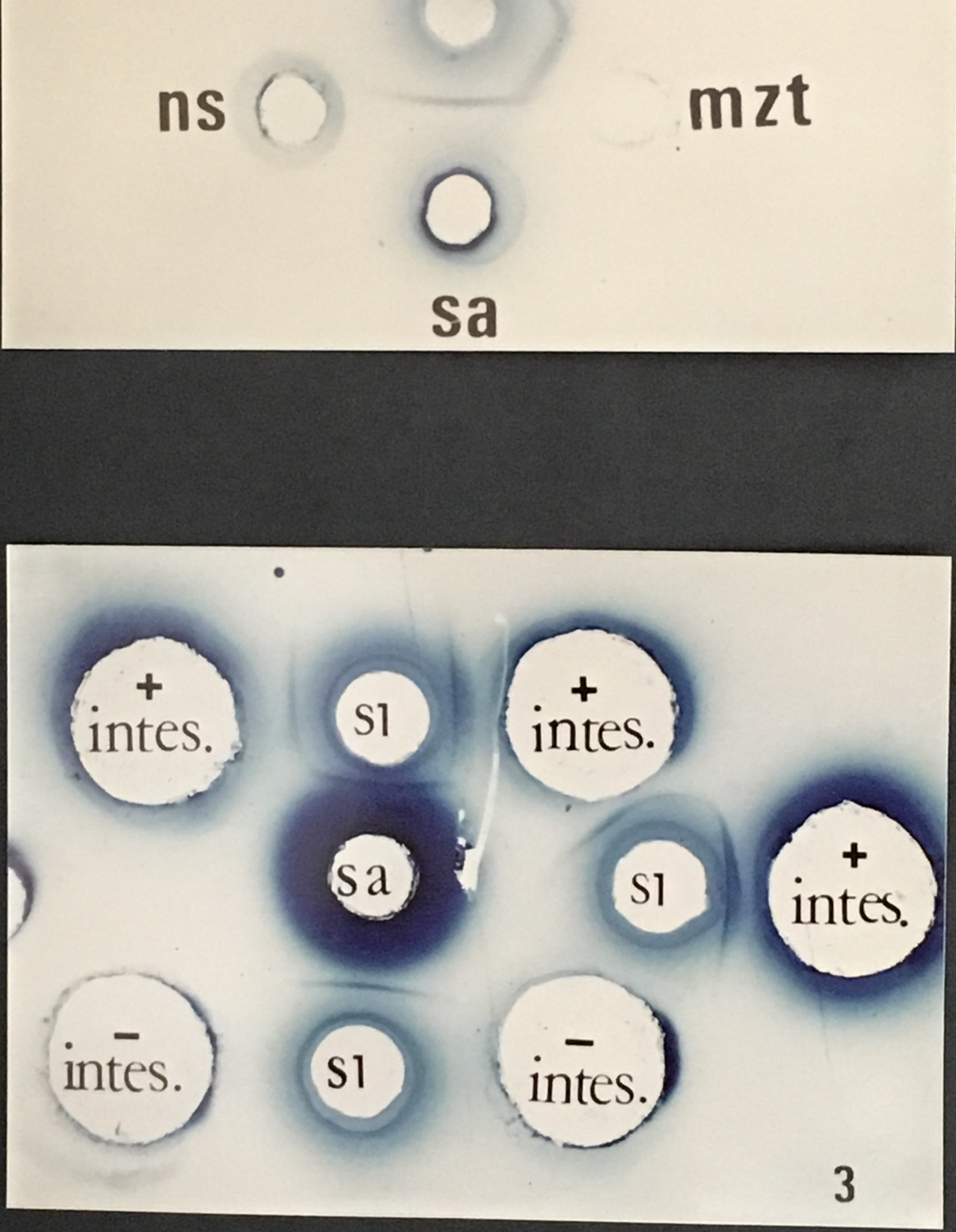
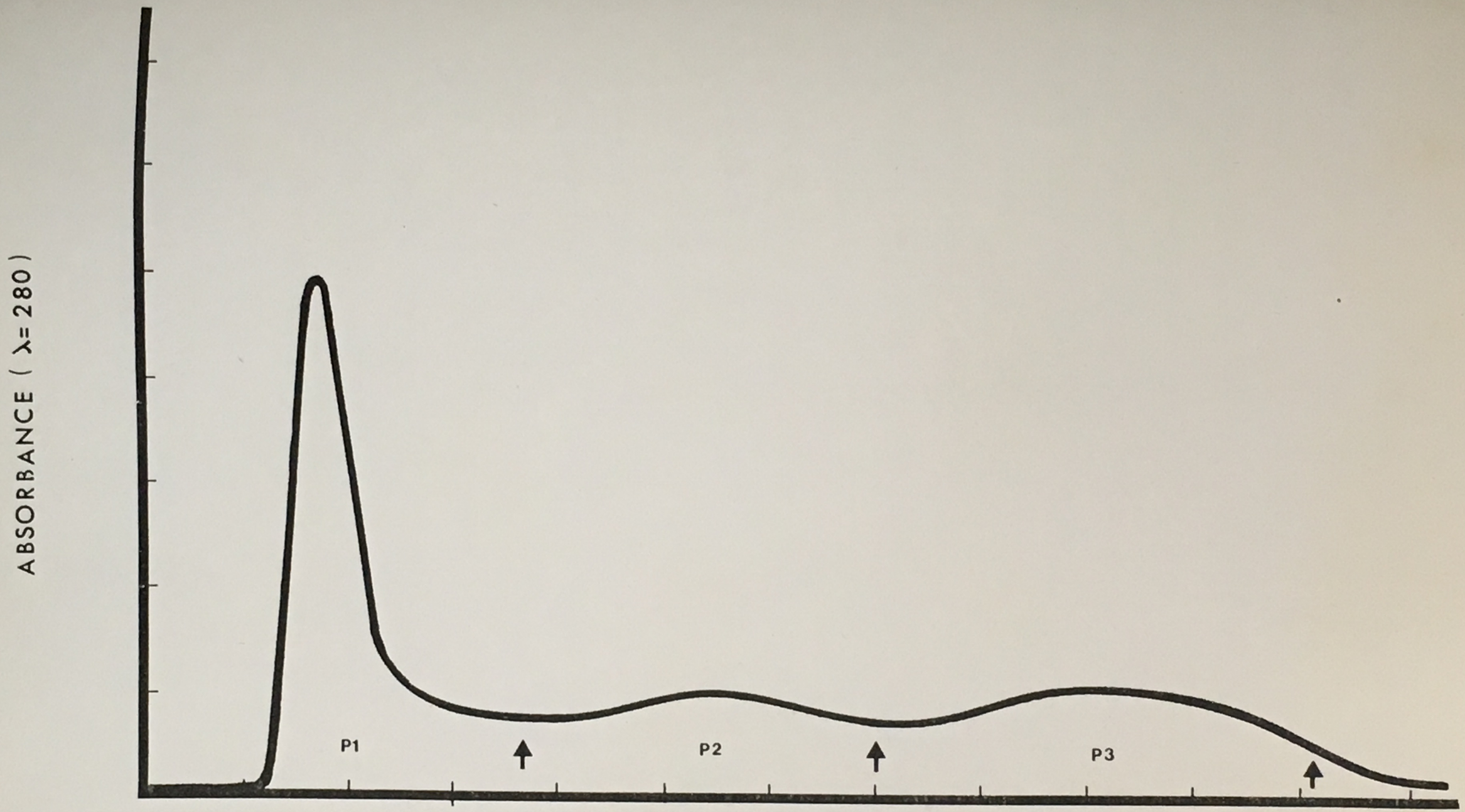

+
intes.
s1
+
intes.
s a
s1
+
intes.
–
intes.
s1
–
intes.
3

Figure 4-4. Chromatographic separation of soluble antigen of second generation schizont on Sephadex G-200.

P1 - Peak 1

P2 - Peak 2

P3 - Peak 3


ABSORBANCE ( λ= 280 )
P1
P2
P3
FRACTION NUMBER

DISCUSSION

The results of the experiments described here show that a soluble substance found in infected intestines 4 days post infection is immunogenic as suggested by the presence of precipitating antibody against this product in the serum of orally infected chickens that also received an intraperitoneal injection of adjuvent. No precipitating antibodies could be detected in sera from orally infected chickens without intraperitoneal injection of adjuvant as described by Rose and Long (1962). Davis et al. (1978) were also unable to obtain precipitating antibodies using the procedure described by Rose and Long (1962) and found that intraperitoneal injection of Freund's Complete Adjuvant necessary for the production of such antibodies. Using infected intestinal tissue in the special immunodiffusion test described by Taylor (1978) it was found that this substance is diffusable and may be produced in large amounts. The cross reaction between this product and antigens prepared either from oocysts or from purified second generation merozoites, indicates that it is a parasite antigen and that it is common to the stages tested. Soluble antigen prepared from saline extracts of infected tissue have been reported from E. tenella, E. necatrix, E. maxima and E. acervulina (Pierce et al., 1962; Rose and Long 1962). These antigens were used in the agar double immunodiffusion test only to detect precipitating serum antibody after oral immunization with the particular species studied.

In Eimeria spp. that develop deep in the mucosa such as E. necatrix and E. tenella such diffusable antigens may play a part in the pathogenesis of the disease. In those Eimeria sp. infecting the superficial villous epithelial cells, acute inflammation and tissue

damage may be prevented by the diffusion of their metabolic products into the intestinal lumen, rather than through the basal lamina into interstitial tissue.

According to Cochrane (1977) any local source of antigen will initiate immunological lesions once antibody is formed. As soon as the antigen-antibody complexes capable of activating complement are formed, polymorphonuclear leucocytes will accumulate, leading to the release of injurious constituents causing injury to blood vessel walls, giving rise to edema and hemorrhage. Goodwin (1976) suggested that pathogenic protozoa cause inflammatory reactions in the host, probably as a result of the release of their metabolites, enzymes or toxins in addition to their foreign proteins, which act as antigens and result in hypersensitivity reactions.

Attempts to purify the antigenic component of the crude "soluble products" using affinity chromatography were unsuccessful. This may be related to the sensitivity of these products to the very low pH (2.6) used to dissociate the antigen-antibody complexes.

# SUMMARY AND CONCLUSIONS

An important finding of this study was the detection of a diffusable antigen in intestinal mucosa 4 days post infection. This antigen was found to cross-react with antigens prepared from purified oocyst and second generation merozoites indicating that it is common among these stages. The role of such diffusable antigens in tissue damage seen during the development of second generation schizonts is not known. This antigen may act as a chemoattractant for leucocyte infiltration into the lamina propria leading to degranulation and release of their lysosomal enzymes. It was documented that the early development of second generation schizonts was accompanied by massive infiltration of heterophils. These heterophils probably play a role in tissue damage and in breaking the basal lamina of infected crypts during their migration towards the crypt lumen. Also, the break up of the basal lamina may help the migration of infected cells into the lamina propria. However, the use of the anti-heterophil serum was of no value in confirming this hypothesis, possibly because of its low efficiency in reducing the number of circulating heterophils. In conclusion, the tissue damage associated with E. necatrix could possibly be the result of a combined effect of the leaking out of the metabolic products of second generation schizonts and the massive leucocyte infiltration accompanying their development.

Another finding reported here in relation to the leucocyte response to E. necatrix infection was the eosinophilia observed during the development of sexual stages of the life cycle. Factors responsible for this stimulation of eosinophils may be related to specific antigenic

stimulation or to the pathogenetic mechanisms of the disease.

The type of cell in which first generation merozoites develop into second generation schizonts was confirmed to be crypt epithelial cells. It was also found that once these parasitized cells migrate into the lamina propria they acquired phagocytic activity.

Intraperitoneal injection of irritants was found to prolong the prepatent period of E. necatrix. Accordingly, many experiments were conducted to understand this effect of the intraperitoneal injection on the life cycle of the parasite. The results of these experiments indicate that the cells responsible for the transport of sporozoites from villous to crypt epithelial cells were affected by such injections. Electron microscopic examination revealed that sporozoites are transported by intraepithelial lymphocytes and not macrophages as reported by earlier researchers.

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