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EXTRA-TISSULAR *SCHISTOSOMA MANSONI* EGG GRANULOMATA IN THE PERITONEAL CAVITY OF MICE

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The presence of Schistosoma mansoni *eggs surrounded by inflammatory cells were detected within the peritoneal cavity of experimentally infected mice. The histological and ultrastructural analysis revealed the predominantly macrophagic composition of these structures. The presence of epithelioid cells, macrophages in different stages of activation and the architectural pattern of the cells, characterize these structures as extra-tissular true granulomas. Granulomas much similar to those observed in the peritoneal cavity of infected mice were also detected after the intraperitoneal injection of viable eggs in non-infected mice. Collagen fibers were observed in between the inflammatory cells of granulomas obtained 10 weeks after infection and 48 hours after the injection of viable eggs into the peritoneal cavity. In later times of infection or injection the amount of collagen fibers increases resulting in a typical pattern of healed schistosoma egg granulomas. The possible influence of the immune response on the genesis of the granulomatous reaction as well as the influence of the vascularized connective tissue on this process is discussed.*

The concept that the induction and modulation of *Schistosoma mansoni* egg granulomata is a T cell dependent phenomenon is generally accepted (Adams, 1983). However, evidences that granuloma formation may be a more simple and immune independent reaction are described. For instance, BCG is able to induce a typical granulomatous reaction in nude mice and the transfer of *S. mansoni* egg granulomata from nude mice to heterozygotic mice develops into typical granulomatous reaction (Epstein et al., 1987). These results suggest, that granuloma formation is basically a macrophagic rather than an immune manifestation (Spector & Mariano, 1975).

The observation of *S. mansoni* eggs in the peritoneal cavity of infected mice followed by a peculiar cellular reaction surrounding them, is described in this paper and used as a model to discuss the above controvertial concept of granuloma formation.

MATERIAL AND METHODS

Animals and infection – Albino Swiss mice of both sexes, 2 to 3 weeks of age, were submitted to infection with 75 cercariae of a BH-Ressaca strain of *S. mansoni*, by percutaneous method of exposure to cercarial suspension.

Isolation of S. mansoni *eggs* – Eggs were isolated from the liver of hamsters infected 45 days previously with 300 cercariae of *S. mansoni* by a modified method of Coker and von Lichtenberg (1956).

Induction of synchronous intra-cavitary granulomas – Eggs were suspended in saline 0.85% at a concentration of 600 egg/ml and 150 eggs were injected into the peritoneal cavity of male mice weighing 20-25 g.

Peritoneal wash – Mice were killed by inducing ethilic ether narcosis at different time intervals after infection or after intra-peritoneal injection of eggs. The peritoneal cavity was washed with PBS containing heparin, the aspirate colected in glass petri dishes and examined under a dissecting microscope. Free floating structures composed by eggs surrounded by cell colections were removed by aspiration with a Pasteur pipet and processed for histologic and electron micropipette study.

Microscopic study – Histologic sections were made of hepatic, intestinal and omental tissues, and of periovular structures colected from the peritoneal wash, previously fixed in aqueous Bouin's fluid and embedded in paraffin. The sections were stained with hematoxylin and eosin (H & E). For electron microscopic study, periovular structures collected from the peritoneal wash were fixed in 2% glutaraldehyde and post-fixed in 4% osmium tetroxide, contrasted by uranyl acetate and embedded in

Araldite. Sections were obtained by using a Sorwall-MT-5000 ultra-microtome with glass knife and observed with a Philips EM201 microscope.

RESULTS

Analysis of the peritoneal wash of infected mice – Groups of 10 animals each were sacrified after 5, 6, 8, 10, 18 and 28 weeks post infection and the peritoneal cavity washed with PBS. In none of the animals signs of peritonites such as peritoneum opacity or adherence was observed.

When the peritoneal wash was examined under a dissecting microscope, small white round structures with more than 300 μm in diameter, always showing eggs in the center were observed (Fig. 1). The movement of the miracidium flame cells observed in some of these structures showed that these eggs were viable. These structures were colected with the aid of a Pasteur pipet and processed for optic and electron microscopy observation. Fig. 2 shows the number of eggs surrounded by cell colections obtained from the peritoneal cavity of each mouse at different time intervals.

Microscopic observations – When observed under the light microscope, the small white round structures obtained from the peritoneal cavity of mice with 6 weeks of infection, showed eggs surrounded by multiple layers of cells. These cells were predominantly mononuclear, but eosinophils, and rare mast cells and typical lymphocites were also observed. The cellular aggregate which surrounded eggs colected after 8 weeks post-infection were also mononuclear cells, with morphologic characteristics of macrophages disposed in a concentric array (Figs 3-4). The cells closer to the eggs were more aggregate to each other and when observed under the electron microscope showed morphological characteristics of epithelioid cells such as the typical interdigitation of cytoplasmatic projections (Fig. 5).

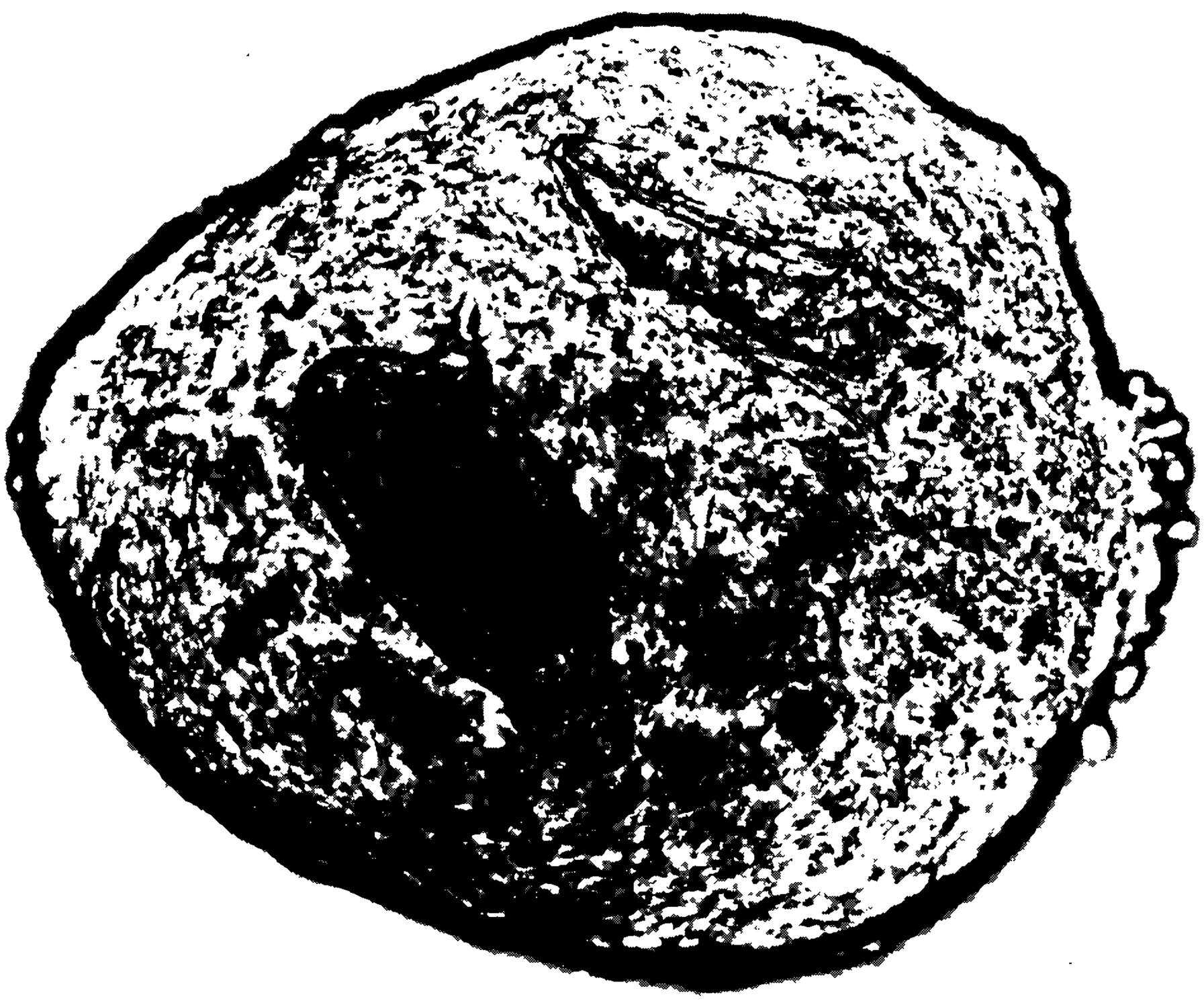

Fig. 1: photomicrography of a viable *Schistosoma mansoni* egg and an egg shell surrounded by cells, collected from the peritoneal cavity of mouse infected for 18 weeks. X 200.

The ultrastructural analysis of these structures obtained after 10 weeks of infection showed the presence of collagen fibers in between the cellular elements.

The histologic observations of the different organs examined was not sufficient to determine the route taken by the eggs to reach the peritoneal cavity. The morphological differences observed between granulomas developed in the liver and intestine was the same as those described by others (Weinstock & Boros, 1981).

Induction of periovular cell aggregates by egg inoculation — A group of 40 normal mice were intraperitonealy injected with a suspension of 150 *S. mansoni* eggs in 0.25 ml of saline. The animals were divided in groups of five and sacrified after 1, 2, 5, 8, 16, 24 and 30 days. Ten mice were used in the last group.

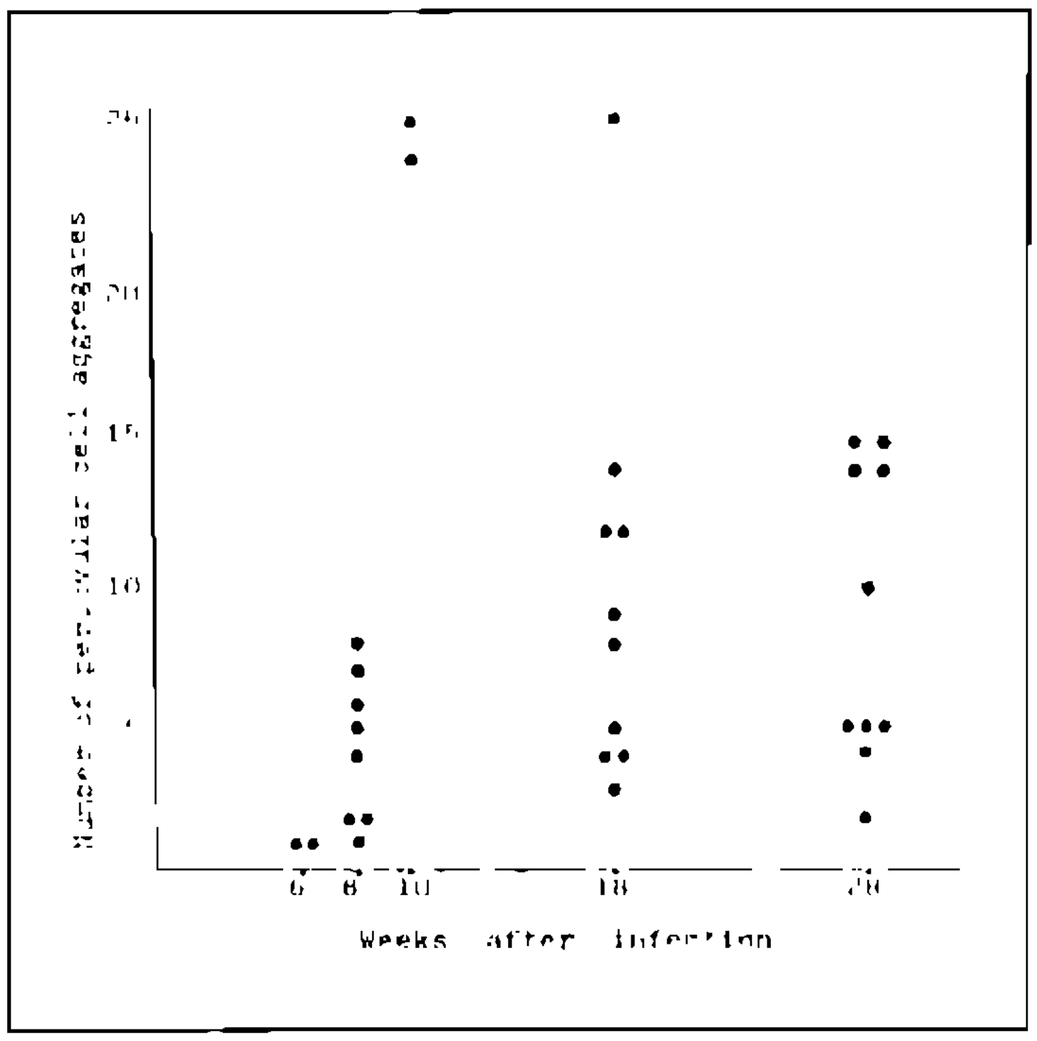

Fig. 2: number of periovular aggregates collected from the peritoneal cavity of mice infected with *Schistosoma mansoni* after different times.

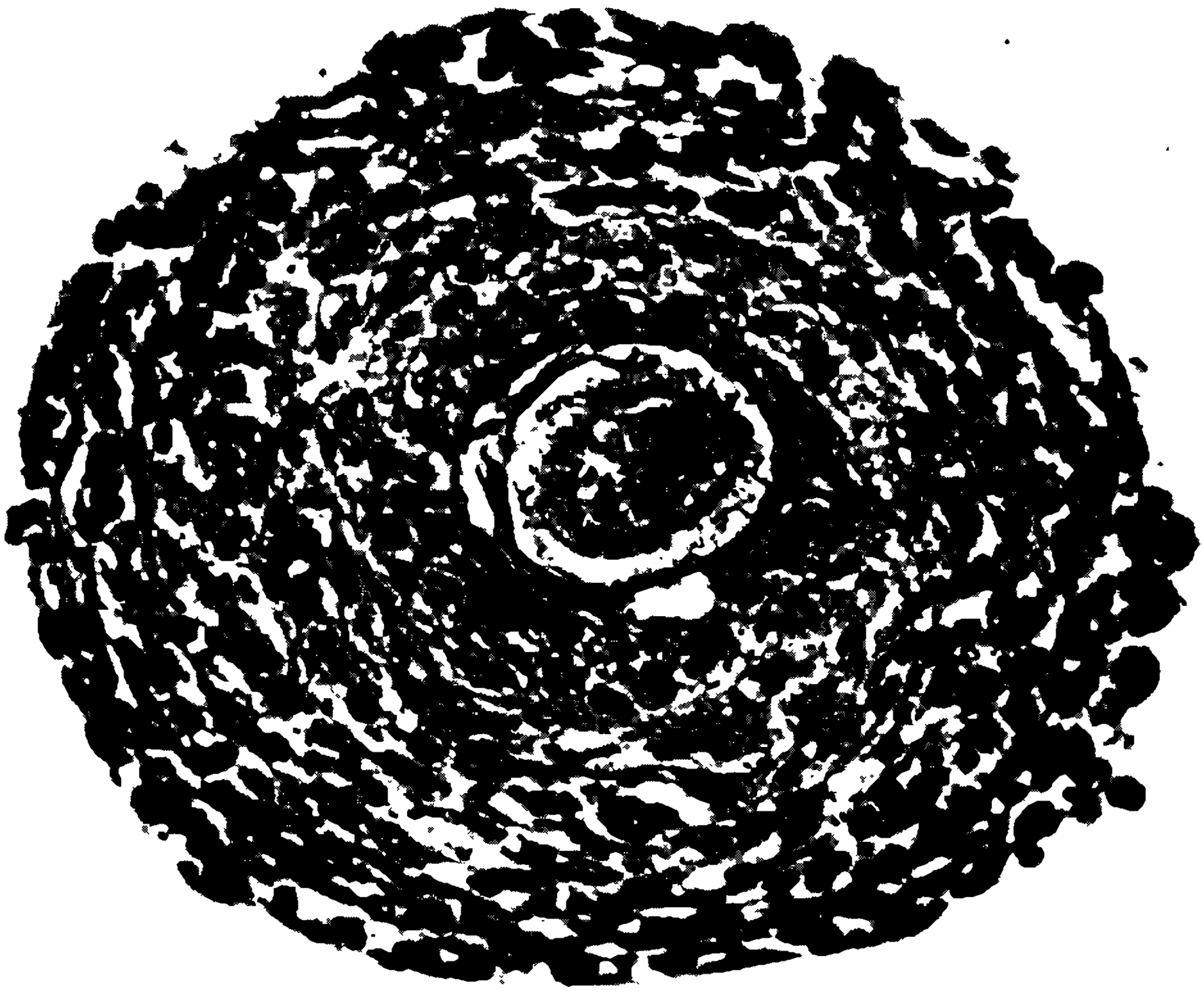

Fig. 3: photomicrography of a *Schistosoma* egg surrounded by inflammatory cells, collected from the peritoneal cavity of infected mouse after 18 weeks. Note the concentric distribution of the cells. H & E. X 500.

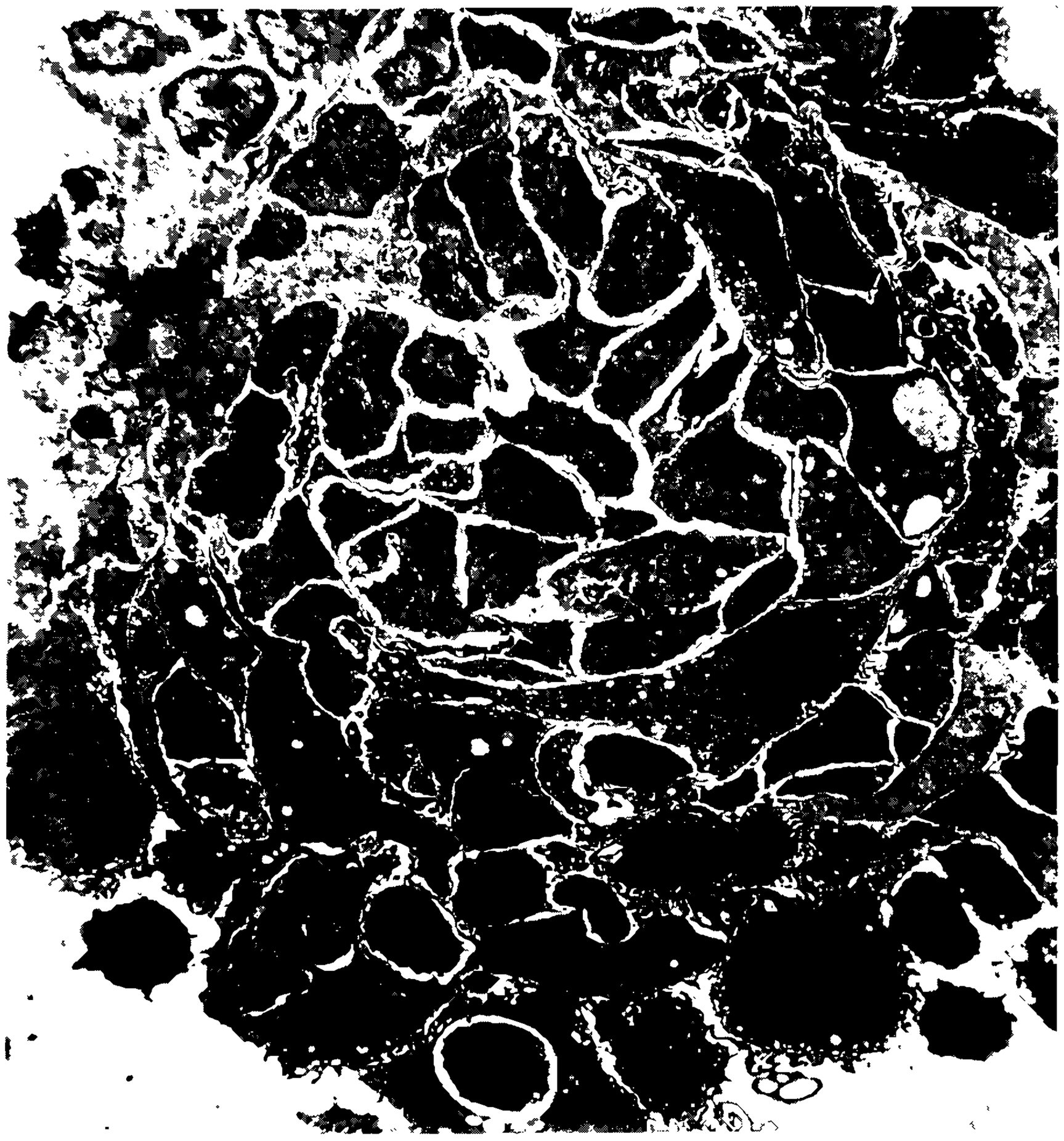

Fig. 4: electronmicrography of cells surrounding an *Schistosoma* egg (not seen) collected from the peritoneal cavity of infected mouse. X 3,100.

Eggs surrounded by cells, in all similar to those observed in infected mice, were detected in the peritoneal cavity 24 hours after the inoculation. The main difference observed was that a variable number of eggs were seen in a single aggregate. As shown in Fig. 6 a small number of eggs was recovered from the peritoneal cavity of injected mice in all the times examined. Eggs free of the periovular cell aggregate were never observed.

Microscopic analysis – Twenty four hours after inoculation the eggs recovered from the peritoneal cavity were surrounded by different cell types. Under the electron microscope these were characterized as young macrophages and large number of polymorphonuclear neutrophils and eosinophils. Rare mast cells were also seen to compose the aggregates. A fibrilar material compatible with the morphology of fibrin was observed between the cells.

At 48 hours post-inoculation the eggs were surrounded by different cell types disposed to form a loose aggregate. Close to the egg shell, the electron microscope showed cells with cytoplasmatic projections which inter-digitate with the neighbours. In between the cells an abundant extracellular matrix composed by delicate filaments and typical collagen fibers were detected. Macrophages and cells with characteristic morphology of fibroblasts were observed.

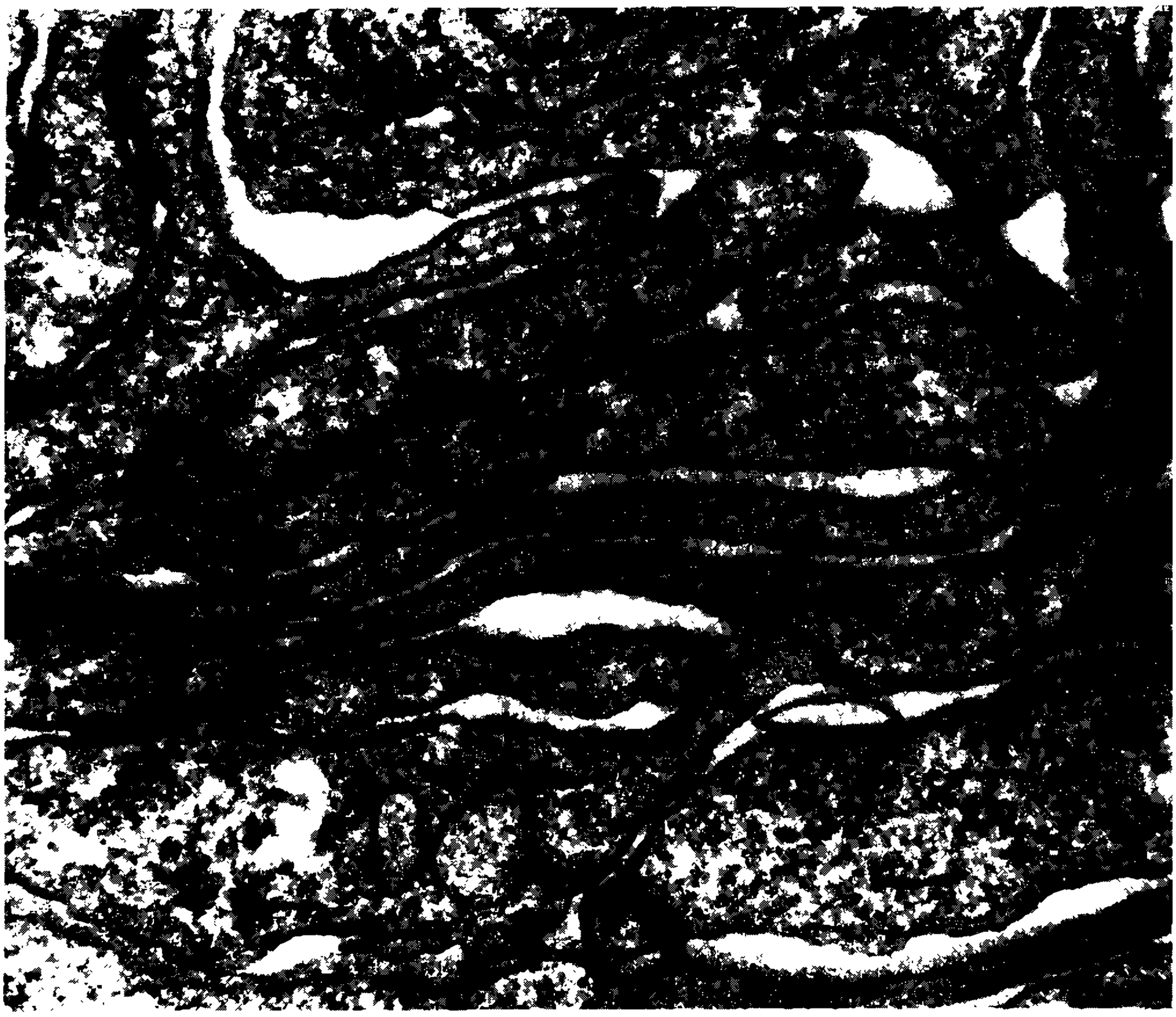

Fig. 5: electronmicrography showing typical interdigitation of epithelioid cells projections in cellular aggregate around *Schistosoma mansoni* egg. X 100,000.

The aggregates recovered after 5 days of egg inoculation were composed by macrophages, eosinophils, fibroblasts and rare mast cells. From this time on the cells were disposed in a concentric array and typical epithelioid macrophages interdigitate with the neighbour cells. The presence of an abundant extracellular matrix composed of amorphous material and typical collagen fibers was consistently observed and increased in older structures. At 30 days of egg inoculation the aggregates surrounding the eggs were composed by layers of cells and extracellular matrix with large amounts of collagen fibers (Fig. 7). Typical fibroblasts were constantly characterized in these structures under the electron microscope.

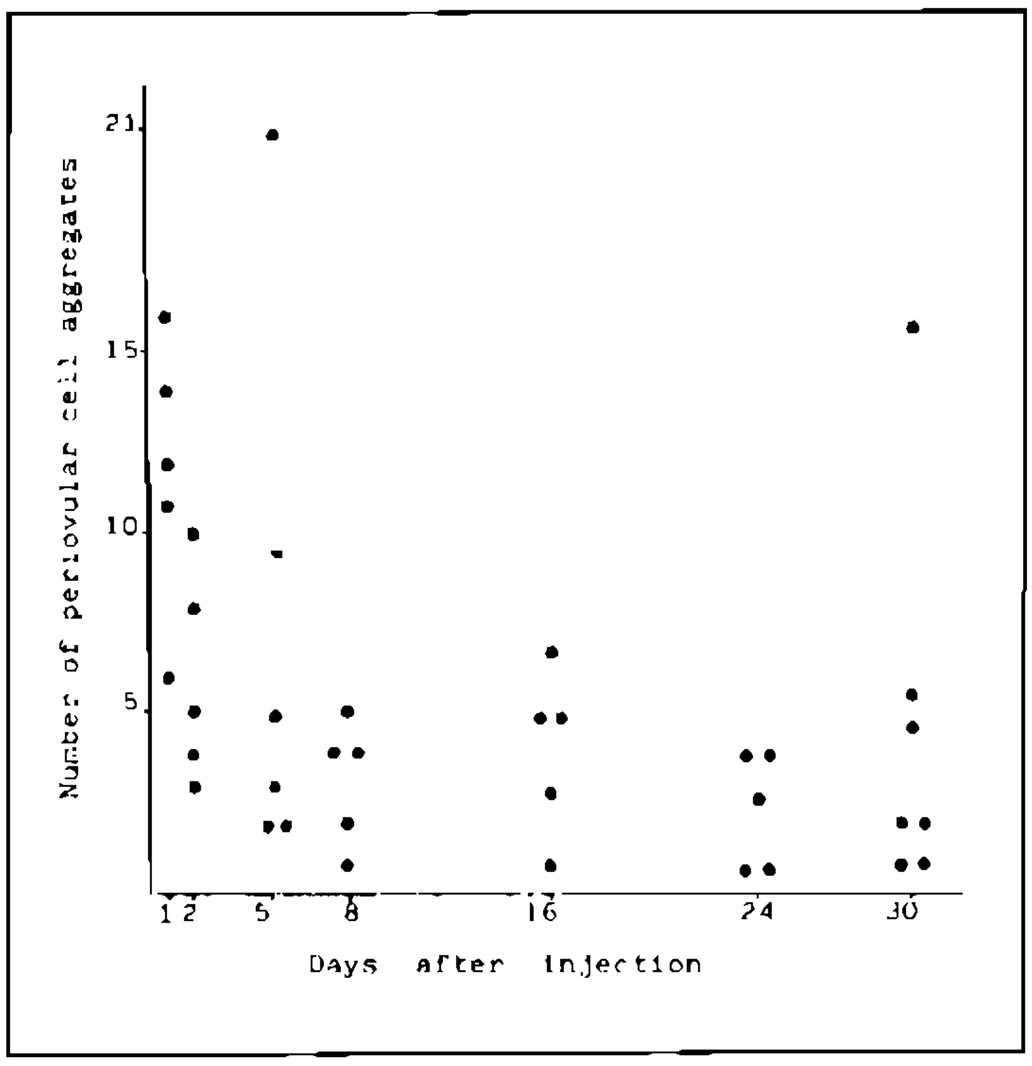

Fig. 6: number of periovular aggregates obtained after different times from the peritoneal cavity of normal mice injected with *Schistosoma mansoni* eggs.

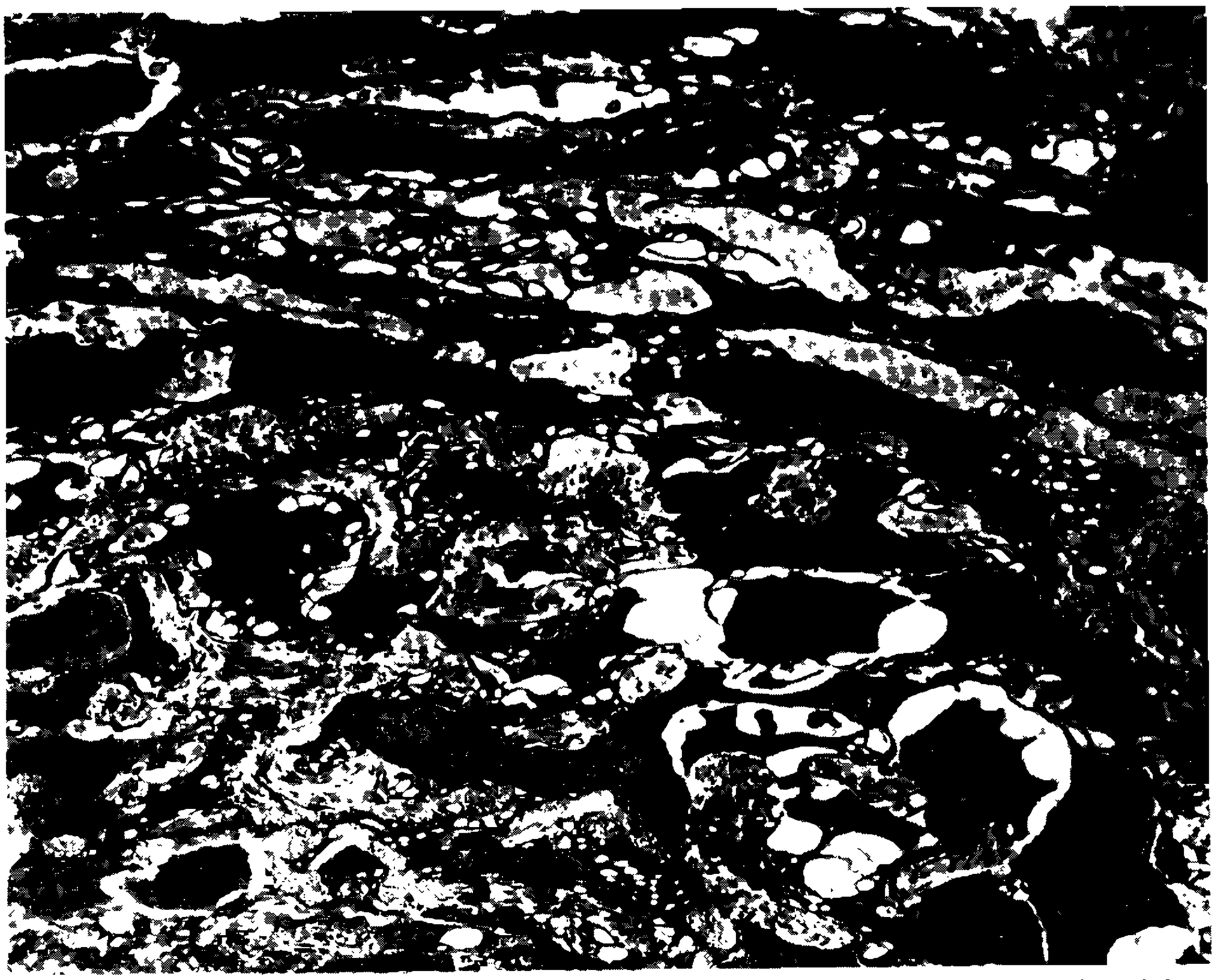

Fig. 7: electronmicrography of cells and extracellular matrix surrounding *Schistosoma mansoni* egg injected 24 days before into the peritoneal cavity of normal mouse. X 5,800.

DISCUSSION

The presence of Schistosoma eggs free in the peritoneal cavity of infected animals has not yet been reported.

Our histological analysis of the intestine of infected mice was not sufficient to demonstrate the route followed by the eggs to reach the peritoneal cavity. Two hypothesis could be considered to explain the origin of the cells which envelop the eggs. One that the eggs should migrate from the tissues already surrounded by inflammatory cells and the other that these cells originate from the peritoneal cavity. Considering that the histological analysis did not show eggs recovered by cells evidently budding from the intestinal serosa, and that the inoculation of eggs into the peritoneal cavity of non infected mice are promptly recovered by cells, the second hypothesis seems more reasonable.

The cell population surrounding the eggs collected at the early phases of infection was predominantly macrophagic although eosinophils, neutrophils, mast cells and rare cells with morphology of lymphocytes were present. Cells with typical morphology of epithelioid macrophages were always detected and another peculiar characteristic of these cellular aggregates was their concentric architecture. To our concept these morphological aspects characterize a granulomatous reaction (Spector & Mariano, 1975).

When the morphology of these extra-tissular granulomas is compared to the morphology of hepatic lesions the basic difference observed was the absence of the perilesional cuff of small round cells observed in the later. Confirming previous observation (Weinstock & Boros, 1983) we also have not detected the collection of small round cells surrounding egg granulomas in the ileum wall of infected mice and coincidentaly, the same authors have shown that these granulomas are not immune modulated. These observations suggest that the perilesional cuff of small round cells is not a necessary parameter to morphologically define the granulomatous reaction.

The basic question whether these lesions are initiated by specific immunological stimulus, is under dispute. Some *in vitro* data show that inflammatory cells migrate to schistosome eggs under the influence of T activated cells to form a "granuloma" (Doughty et al., 1984). Nevertheless, the basic morphological characteristics of the granulomatous lesions such as epithelioid transformation of macrophages and the concentric distribution of the cells around the egg was not demonstrate. In our opinion, these characteristics are fundamental to distinguish a focal inflammatory reaction from a granulomatous lesion.

The prompt cellular response around eggs inoculated into the peritoneal cavity of non infected mice support the hypothesis that the initiation of the cellular process is not dependent upon sensitized T cells. The eggs were recovered by inflammatory cells 24 hours after innoculation and at 48 hours interdigitated macrophages resembling epithelioid cells were seen close to the egg shell. Four to five days after inoculation typical granulomas were formed. These observations strongly suggest that the initiation and progress of granuloma formation depends on the chemical characteristic of the egg and their products rather than on the influence of locally activated T cells. The absence of typical lymphoid cells close to the eggs in the early phase of cell aggregation also corroborates this hypothesis. It does not imply, of course, that the immune system do not modulate and control the fate of these lesions (Andrade & Warren, 1964; Warren, 1976). In our opinion, the demonstration that granulomas can be formed in the absence of immune mechanisms opens and stimulates research on the characteristics of substances which are able to aggregate, modulate and organize macrophages to result in the granulomatous reaction.

The model here presented offers also the opportunity to investigate another fundamental aspect of the schistosome egg granuloma which is the colagenization of the process. Collagen fibers were observed in granulomas collected from the peritoneal cavity of mice with 10 weeks of infection. In later phases the number of periovular reactions and the amount of collagen deposition increased when "healed" granulomas were observed. Conversely, collagen deposition was detected in granulomas induced by the egg inoculation into the peritoneal cavity 48 hours before. Typical fibroblasts were detected in both structures although we were not able to demonstrate the origin of these cells. The prompt collagen deposition in granulomas induced by egg inoculation as compared to those developed in infected mice suggests that this phenomenon is under regulatory mechanisms which independ of the chemical characteristics of the egg. This data corroborates the hypothesis that the immune mechanism which modulates granulomas in infected animals is a restraining factor for fibrogenesis, since hepatic lesions increases fibrosis in phases of infection when suppresive factors decrease the size of granulomatous lesions (Andrade & Grimaud, 1986; Wyler et al., 1987).

Finally, our findings demonstrate that granulomatous reaction may occur without the influence of the surrounding vascularized connective tissue, mimicking the cellular response observed in the celomatic cavity of invertebrated animals (Barraco & Menezes, 1985). These structures, called "white bodies", are morphologicaly similar to the free floating granulomas here described. The study of these similarities in detail could contribute to the phylogenetic aspects of the granulomatous response.

REFERENCES

ADAMS, D. O., 1983. The Biology of Granuloma, p. 1-20. IN H. L. Ioachim, *Pathology of Granulomas.* Raven Press, New York.

ANDRADE, Z. A. & WARREN, K. S., 1964. Mild prolonged schistosomiasis in mice: alterations in host response with time and development of portal fibrosis. *Trans. R. Soc. Trop. Med. Hyg., 58:* 53-57.

ANDRADE, Z. A. & GRIMAUD, J. A., 1986. Evolution of the schistosomal hepatic lesions in mice after curative chemotherapy. *Am. J. Pathol., 124:* 59-65.

BARRACCO, MARGHERITA A. & MENEZES, H., 1985. Mecanismos celulares de defesa em insetos. *Ciência e Cultura, 37:* 237-250.

COKER, C. M. & LICHTENBERG, F., 1956. A revised method for isolation of *Schistosoma mansoni* eggs for biological experimentation. *Proc. Soc. Exp. Biol. Med., 92:* 780-782.

DOUGHTY, BARBARA L.; OTTESEN, E. A.; NASH, T. E. & PHILLIPS, S. M., 1984. Delayed hipersensitivity granuloma formation around *Schistosoma mansoni* eggs *in vitro. J. Immunol., 133:* 993-997.

EPSTEIN, W. L.; OKAMOTO, M.; SUYA, N. & FUKUYAMA, K., 1987. T cell independent transfer of organized granuloma formation. *Immunol. Letters, 14:* 59-63.

SPECTOR, W. G. & MARIANO, M., 1975. Macrophage behavior in experimental granulomas,

p. 927-942. IN R. van Furth, *Mononuclear Phagocytes in Immunity, Infection and Pathology.* Blackwell Scientific Publishers, Oxford.

WARREN, K. S., 1976. A Functional classification of granulomatous inflammation. *Ann. N. Y. Acad. Sci., 278:* 7-18.

WEINSTOCK, J. V. & BOROS, D. L., 1981. Heterogeneity of the granulomatous response in the liver, colon, ileum and ileal Peyer's patches to schistosome eggs in murine schistosomiasis mansoni. *J. Immunol., 127:* 1906-1909.

WEINSTOCK, J. V. & BOROS, D. L., 1983. Organ-dependent differences in composition and function observed in hepatic and intestinal granulomas isolated from mice with schistosomiasis mansoni. *J. Immunol., 130:* 418-422.

WYLER, D. J.; PRAKASH, S. & LIBBY, P., 1987. Mesenchymal target cell specificity of egg granuloma-derived fibroblast growth factor in schistosomiasis. *J. Infect. Dis., 155:* 728-736.