

REMARKS ON THE SPORE ENVELOPES IN FISH COCCIDIA

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Abstract. Walls of oocysts and spores of two species of fish coccidia, *Eimeria subepithelialis* and *E. spleni*, have been studied with the electron microscope. The wall of the mature oocyst is a delicate membrane, about 130 Å thick, and is easily disrupted after the oocysts have been freed from the tissue. The spore envelope is composed of two valves. Its wall consists of two layers - a thin (100-200 Å) outer one and a thicker (700-800 Å) inner one. Both valves are joined along a slightly protruding suture line; ruffled collar-like membranes girdle the spore along this line. Adaptive meaning and taxonomic implications of these features are discussed. While located inside an intact spore, sporozoites of both species reveal a remarkable cross striation of the anterior end, which disappears in sporozoites freed from their envelopes.

There have been approximately a hundred coccidian species recorded as fish parasites. In spite of the fact that some species are serious pathogens of commercially important freshwater and marine hosts, our knowledge of fish coccidia, including taxonomy and morphology, is incomplete. This paper is a contribution to the morphology of fish coccidia, with special reference to the structure of the spore envelope.

MATERIALS AND METHODS

Two year old carp (*Cyprinus carpio*) infected with *Eimeria subepithelialis* Moroff and Fiebiger were obtained through the courtesy of Dr. Jan Tesárek from the Fisheries Research Institute in Vodňany, Czechoslovakia. *E. spleni* DeGiusti and Hnath (1968, 1971) was obtained from natural infections in the stoneroller (*Campostoma anomalum*) collected from Flemings Creek, Washtenaw County, Michigan, U.S.A. Both species were observed and photographed in the living state. For scanning electron microscope observation, the material was prepared according to the technique developed by Marszałek and Small (1968) using the Parduez mixture of $HgCl_2$ and OsO_4 as fixative. The preparations were examined with the Cambridge stereoscan electron microscope model Mark III, operated at accelerating voltages of 10 and 20 Kv. For transmission electron microscopy, *Eimeria*-infected tissue was fixed with OsO_4 fixative using Palade's buffer as vehicle, or glutaraldehyde (Polysciences, Pennsylvania) with postosmification. Sections of Epon-araldite embedded material, double stained with uranyl acetate and lead citrate, were examined with the Hitachi 11B electron microscope operated at 75 Kv accelerating voltage.

OBSERVATIONS

1. Oocyst and spore envelopes: Perfect preservation of mature sporozoites was not achieved in the present study, and the structure of the sporozoite could not be clearly

resolved, however, the structure of the spore envelopes could be resolved in the majority of oocysts.

Eimeria subepithelialis Moroff et Fieb'ger, 1906. The elongated spores have double-layered walls. The outer wall is finely granular, about 200 Å thick, and probably represents the modified original cell membrane. The inner layer appears to be homogeneous and about 700 Å thick. These two layers are separated by an electron lucent space, 200–300 Å wide. The spore envelope consists of two valves joined along a slightly protruding suture line (Fig. 1; Pl. I, Fig. 3). At this junction there is a definite structure of electron lucent and electron dense layers. Along the suture there is a membranous structure composed of two membranes of the unit membrane type, about 130 Å thick, so arranged as to form a ruffled collar-like structure girdling the spore (Pl. I, Fig. 3). These membranes merge at the spore suture and enter the electron lucent space which separates the outer and inner layers of the spore envelope. Their continuity could not be traced further. The collar membranes extend from the spore suture approximately 3 µ into the oocyst space but show no evidence of being continuous with the oocyst wall. The oocyst wall lines the space the parasite occupies within the host cells and appears to be of a delicate nature. Its structure and thickness is the same as that of the spore collar membranes. This could explain the extreme ease with which the spores are freed from their thin oocyst walls. The sporozoites and the spore residuum adhere closely to the surface of the inner spore wall layer. As previously stated, the structure of the sporozoites and the residual body was not satisfactorily preserved in the mature oocyst to permit detailed observation.

Eimeria spleni DeGiusti et Hnath, 1968. The oocyst of this species, located in the spleen tissue of the stone-roller (Pl. II, Fig. 1) appears to be similar to the oocyst of *E. subepithelialis*. The oocyst membrane appears to be delicate and subject to easy deformations as it is released from the host tissue (Pl. III, Figs. 2, 3). This species does, however, show some marked differences in the sporozoites and the residual body. These structures do not adhere to the spore walls, but are freely located within the spore, sporozoites laterally arranged in the spore with the residual body between them.

Fig. 1. Sections through the suture of the two spore valves; simplified.

E — external spore membrane,
I — internal spore membrane,
L — membranaceous collar.

not adhere to the spore walls, but are freely located within the spore, sporozoites laterally arranged in the spore with the residual body between them.

The spore wall is composed of two layers similar in structure to those described for *E. subepithelialis*. The outer layer is about 300 Å thick, and the inner layer 700 to 800 Å thick. The outer layer does not show evidence of a unit membrane structure and is a finely granular structure. The thickness of the membranes along the suture is about 100 Å and they appear as a ruffled collar around the spore (Pl. I, Figs. 1, 2). In some micrographs (Pl. II, Fig. 1) the thick inner layer of the spore envelope appears, when cut at a certain angle, to consist of strands arranged parallel to the suture line. The residual body consists of a central corpuscle, about 2–4 µ in diameter, of a low density homogeneous material surrounded by oval electron dense bodies ranging in size to 1.6 µ. The boundary of the residual body, which in electron microscope preparations averages 5–6 µ in diameter, is formed by a simple thin membrane.

2. Structure of the sporozoite: In the oocyst of *E. subepithelialis*, the sporozoites, together with the large residual body, completely fill the spore space (Pl. III, Fig. 1). This is clearly seen in oocysts located in host tissue as well as in those dissected from the gut mucosa and maintained for a period of time in the refrigerator (+4 °C). The

sporozoites do not appear to be bent within the spore, but rather slightly arched along its walls, as originally described by Moroff and Fiebiger (1905). The thicker anterior end of each sporozoite reveals a conspicuous cross striation. If the spores are activated by exposure to 1.4% NaHCO_3 solution saturated with CO_2 , about 10% of the spores excyst. The released sporozoites move freely displaying typical sporozoite movement. The remaining spores contain sporozoites which have an evidently changed form. They become longer and more slender so that their anterior end appears to bend backwards, thereby enabling the spherical residual body to be clearly delimited. In both freed and unfreed sporozoites (the latter still in spores treated as indicated above), no striation of the anterior end could be observed.

In *E. spleni* the sporozoites display the same striation of the anterior end while in the spore shell (Pl. III, Fig. 3). When released, they lose this character, assuming a slender shape similar to that observed in *E. subepithelialis*.

DISCUSSION

The electron micrographs reveal that in both *E. subepithelialis* and *E. spleni*, the oocyst walls are composed of a simple thin membrane. Upon sporulation the oocyst becomes difficult to stain; its walls appearing to be quite solid, are, in fact, very delicate. In the majority of immature oocysts of *E. subepithelialis*, isolated from the host tissue, the oocyst membrane maintains its regular spherical shape. In most mature oocysts, however, it breaks open very easily, releasing the solid walled spores. Thin oocyst membranes, similar to these described, are also found in a new species of *Eimeria* to be described from *Macrourus berglax*, and in several species observed by this author from freshwater fishes. Similarly thin oocyst membranes seem to occur at least in a great part of fish Coccidia thus contrasting sharply with eimeriids from homoiothermic vertebrates. In the latter the fine outer oocyst membrane is reinforced by the formation of thicker inner layers (Scholtyseck and Weissenfels 1956, Löser and Gönnert 1965). In the rabbit coccidian *Eimeria perforans*, Scholtyseck and Voigt (1964) described the process of formation beneath the original macrogamete membrane of 4 additional membranes reinforced by secreted material, to form the well-known firm layers of the oocyst shell, plus an additional outer fine membrane. The same configuration was found in *E. larimerensis* from ground squirrels (Roberts et al. 1970). Sometimes, the thick layer of mammalian eimeriids have even a further substructuring of the two layers (*E. callospermophili* — Roberts et al. 1970, Nyberg and Knapp 1970 b). Thick multilayered protective envelopes are known also in adeleid oocysts from terrestrial hosts. E. g., *Klossia helicina* from terrestrial pulmonates (Volkmann 1967) produces a thick mucous envelope plus three other later partly fusing membranes. The insignificant thickness of the oocyst wall in fish eimeriids evidently reflects the aquatic environment. Observations indicate that these oocysts do not require protection against mechanical insults or desiccation as those living in terrestrial hosts.

The spore wall of both *E. subepithelialis* and *E. spleni* shows remarkable similarities in structure and dimensions. The membranaceous girdle around the oocysts is a feature markedly contrasting with the egg-like, smooth appearance of oocysts of *Eimeria* of warm-blooded vertebrates, seen in *Eimeria tenella* with the scanning electron microscope (Nyberg and Knapp 1970a). Also, the walls of spores in other coccidian species are double (e.g., *E. callospermophili* — Roberts et al. 1970). The thickness relation of both layers in sporocysts of *E. subepithelialis* and *E. spleni*, however, may be at variance with that observed in other species. In *Eucoccidinium dinophili* (Bardele 1966) the outer layer is the thicker one and in addition to that, it is quite electron lucent.

However, in the latter species the spore shell is clearly bivalvular much in the same way as in *E. subepithelialis*. This paper presents the first record of the bivalve character of the spore in this species. Being restricted to several fish-invading species of the genus *Fimeria*, bivalve spores are quite common feature among other members of *Eimeriina* (e.g., *Cyclospora*, *Barrouxia*, *Urobarrouxia*, *Aggregata*) and also in *Adeleina* (e.g., *Adelina*). Further studies might show that bivalve spores are characteristic of fish-invading species of *Eimeria*.

In a bivalve spore, the walls open as the two parts of a pod, so that an apical opening plugged by a *Stieda* body is not necessary. It would be worth while to examine carefully fish species of *Eimeria* possessing a knob or concentric elevation at one pole (e.g., *E. rutili*, *E. associ*, *E. cottii*), for the possible presence of a *Stieda* body.

The striated appearance of the anterior end of sporozoites is a rather uncommon feature among *Eimeria* species, although (Hammond — personal communication) it has already been observed (infrequently) in species from warm-blooded vertebrates. In fish coccidia we could observe the striation also in some other species with at least medium sized spores. Such striation is distinct in *Eimeria* sp. from the gut of *Tautoglabrus adspersus* or in the already mentioned species from the gut of *Macrourus berglax*. This striation could be the result of transverse folding of the pellicle of a "contracted" quiescent sporozoite and does not reflect any important cell structure. As soon as an activated sporozoite assumes a longer form, the striation disappears.

Within the vast assemblage of *Eimeria* species those invading fish are characterized by 1. sporulation accomplished within the host tissue and 2. by a very delicate oocyst membrane and a bivalve spore, thusfar observed in a considerable number of species. These differences are probably not sufficient enough to constitute a basis for their separation as an independent genus, but according to our opinion they warrant the preservation of the taxon *Goussia* Labbé as a subgenus of *Eimeria*.

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REFERENCES

BARDELE C. F., Elektronenmikroskopische Untersuchung an dem Sporozoon *Encocciidium dinophili* Grell. Z. Zellforsch. 74: 559-595, 1966.

DEGIUSTI D. L., HNATH J. G., An eimerian (Coccidia) of fish with oocyst formation in the spleen. Trans. Amer. Mier. Soc. 87: 126, 1968.

—, —. 1971. (In press.)

LÖSER E., GÖNNERT R., Zur Bildung der Sklerotinhülle der Oocysten einiger Cocciden. Z. Parasitenk. 25: 597-605, 1965.

MARSZALEK D. S., SMALL E. B., Preparation of soft biological specimens for scanning-electron microscopy. In: Scanning Electron Microscopy. 1969. Proc. 2nd An. Scanning Electron Microscope Symposium. IIT Res. Inst., Chicago, 1969.

MOROFF T., FIEBIGER J., Über *Eimeria subepithelialis* n. sp. Arch. Protistenk. 6: 166-174, 1905.

NYBERG P. A., KNAPP S. E., Scanning electron microscopy of *Eimeria tenella* oocysts. Proc. Helminth. Soc. Wash. 37: 29-32, 1970a.

—, —. Effect of sodium hypochlorite on the oocyst wall of *Eimeria tenella* as shown by electron microscopy. Proc. Helminth. Soc. Wash. 37: 32-36, 1970b.

ROBERTS W. L., HAMMOND D. M., SPEER C. A., Ultrastructural study of the intra- and extracellular sporozoites of *Eimeria callospermophili*. J. Parasit. 56: 907-917, 1970.

—, SPEER C. A., HAMMOND D. M., Electron and light microscope studies of the oocyst walls, sporocysts, and exocyst sporozoites of *Eimeria callospermophili* and *E. torimerensis*. J. Parasit. 56: 918, 1970.

SCHOLTYSECK E., WEISSENFELS N., Elektronenmikroskopische Untersuchungen von Sporozoen. I. Die Oocystenmembran des Hühnereocerids *Eimeria tenella*. Arch. Protistenk. 101: 215-222, 1956.

—, VOIGT W. H., Die Bildung der Oocystenhülle bei *Eimeria perforans* (Sporozoa). Z. Zellforsch. 62: 279-292, 1964.

VOLKMANN B., Vergleichende elektronenmikroskopische und lichtmikroskopische Untersuchungen an verschiedenen Entwicklungsstadien von *Klossia helicina* (Coccidia Adeleidea). Z. Parasitenk. 29: 159-208, 1967.

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80th birthday of Professor Dr. Witold Stefański

Professor Dr. Witold Stefański, Member of the Polish Academy of Sciences, celebrated his 80th birthday on July 25th, 1971. He is a distinguished Polish parasitologist, founder of the Polish school of veterinary parasitology. He was born in Kielce and completed secondary school there. Then he studied in Geneva, and in 1914 he graduated as docteur-ès-sciences at the Faculté des Sciences Naturelles of the Geneva University. Up to 1917 he lectured on zoology at the same university as a docent. After his return to Poland he worked as assistant at the Zoology Chair of the Warsaw University and took the degree of docent in 1920. In 1925 he was appointed Head of the Chair of Zoology and Parasitology, Veterinary Faculty of the Warsaw University (after World War II the Faculty was transferred to the Warsaw Central School of Agriculture). He occupied this post up to 1961 as associate professor and later professor. In 1954—1961, he was appointed Director of the Parasitology Institute of the Polish Academy of Sciences, which he himself organized. Up to this day he has been Chairman of the Scientific Council of the Institute and its active contributor despite his retirement in 1961. Since 1944, Professor Stefański has closely co-operated with the Department of Parasitology and Infectious Diseases of the State Veterinary Institute, Pulawy.

The professional career of Professor Stefański was begun with his research work on the free-living Nematodes; at that time he published several works dealing with their systematics, ecology and faunistics; he has made a name for himself in the world as an authority on these subjects. When directing his Chair at the Veterinary Faculty, he naturally took interest in studies on parasitic Nematodes, their systematics, development, as well as methods of controlling parasitic diseases. After the liberation of Poland from the Nazi occupation, which, of course, caused a break in Professor Stefański's research, he and his numerous students and assistants focused their efforts on solving the most urgent parasitological problems of the time which were: controlling the mange of domestic animals, cattle hypodermatosis and dourine in horses. The relevant studies and their practical effects, which have brought Professor Stefański Scientific State Prize, allowed to eradicate the above-quoted parasitic

diseases of domestic animals, widely spread in the first post-war years. In later stage of his research work (which has been carried on until now), Professor Stefański has studied relationship between parasitic infections and the bacterial flora of their hosts. The results of his studies, published in numerous papers, have aroused interest in the world of science, and his work has been continued by other scientists.

The great didactic talent of Professor Stefański is best illustrated by the fact that at present all veterinary parasitology chairs in Poland are headed by his pupils; many others occupy responsible posts in various research centres. Professor Stefański's activity as a teacher was crowned by the publication of his two volume textbook for university students, "Veterinary Parasitology" (Vol. I, 1963, both volumes 1968). In 1952, he was appointed Corresponding Member and later Ordinary Member of the Polish Academy of Sciences, and for many years now he has been Chairman of the Parasitology Committee of the Polish Academy of Sciences which co-ordinates parasitological studies all over the country. On account of his scientific achievements and social activity during his long and distinguished career, a lot of honours were conferred on Professor Stefański. He has been awarded many high State Distinctions. As a result of his work on the international scale, he was invited to be Chairman of the European Federation of Parasitologists; he was appointed Foreign Member by the French Academy of Sciences. He is also holder of a number of foreign distinctions and honours. As the scope of his scientific interests is wide, he has also conducted research in biological sciences. For a long time he acted as Scientific Secretary of the Division of Biological Sciences of the Polish Academy of Sciences, and later he became Vice-President of the Academy. He is Chairman of the Polish Committee of the International Biological Union.

The incessant research activity developed by Professor Stefański allows us to expect that for many years to come he will successfully contribute to the development of his favourite scientific disciplines.

*Professor Dr. W. Michajlow
Ordinary Member of the Polish
Academy of Sciences*

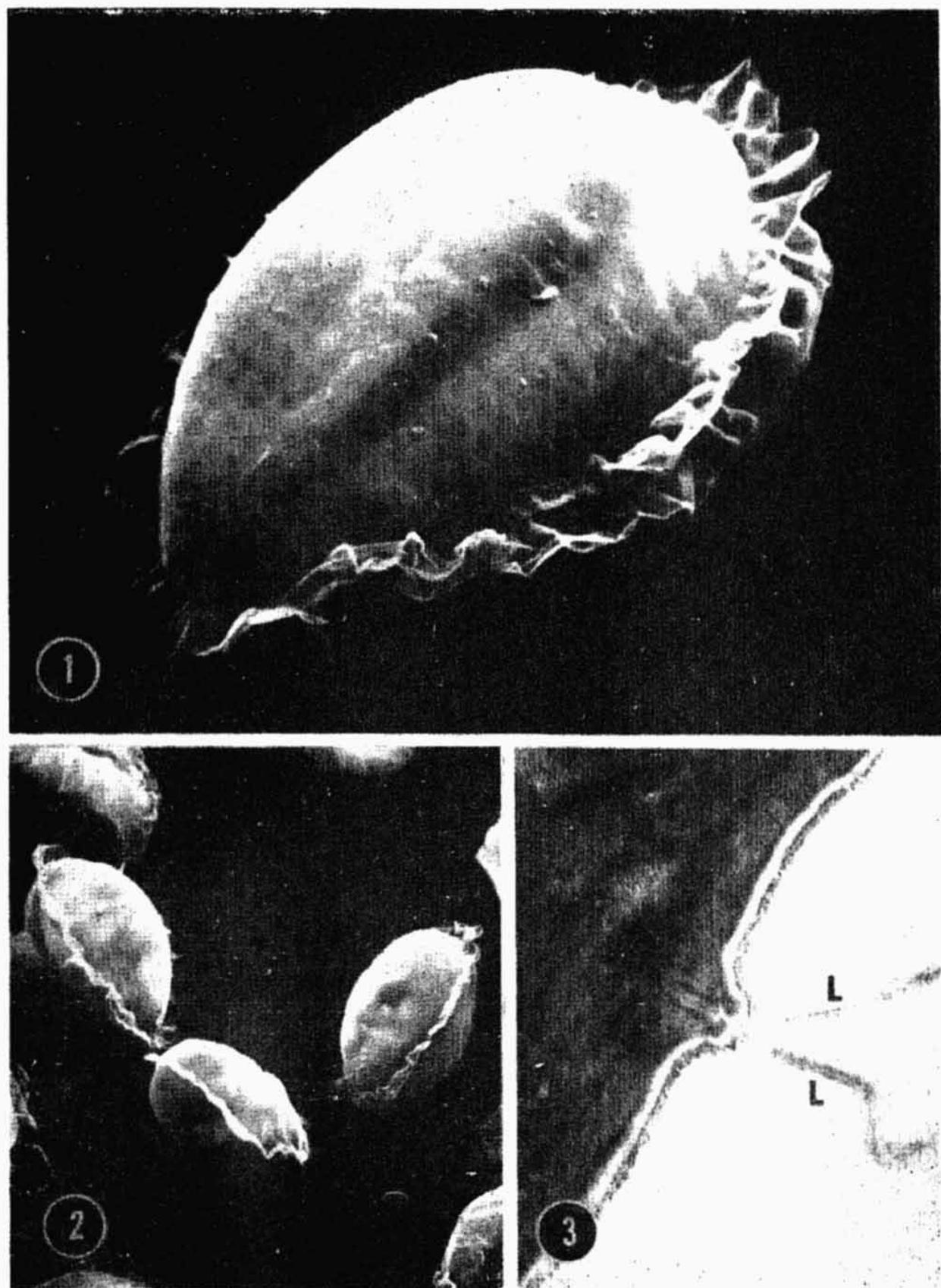


Fig. 1. Isolated mature spore of *E. spleni* with membranous collar along the suture line (Scanning electron micrograph, $\times 8500$).

Fig. 2. A group of isolated mature spores of *E. spleni* (S.e.m., $\times 2500$).

Fig. 3. Suture of the spore shell valves of *E. subepithelialis* in cross section (Transmission electron micrograph, $\times 114\,000$). L — membranous layer.

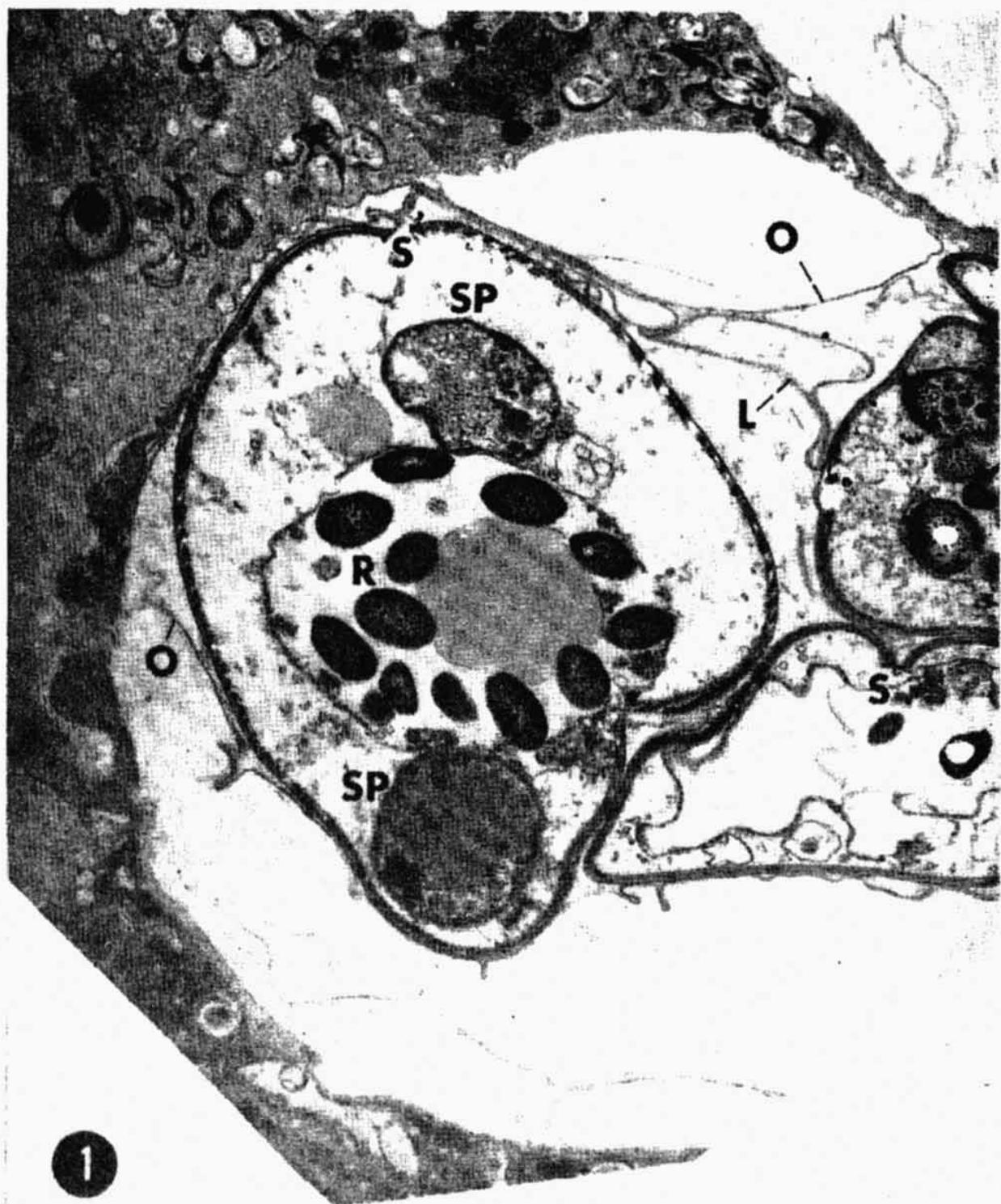


Fig. 1. A mature sporulated oocyst of *E. spleni* in situ in the infected spleen. The shrinkage of the oocyst membrane (O) and distorted shape of the spores is due to fixation artifact. The almost regularly globular wall of the cavity occupied by the oocyst is lined only with the thin oocyst membrane. Note the changes in the surrounding host cell cytoplasm (T.e.m., $\times 7000$).

Abbreviations. L — membranaceous collar, O — oocyst membrane, R — residual body of the spore, S — suture of the spore valves, SP — sporozoite.

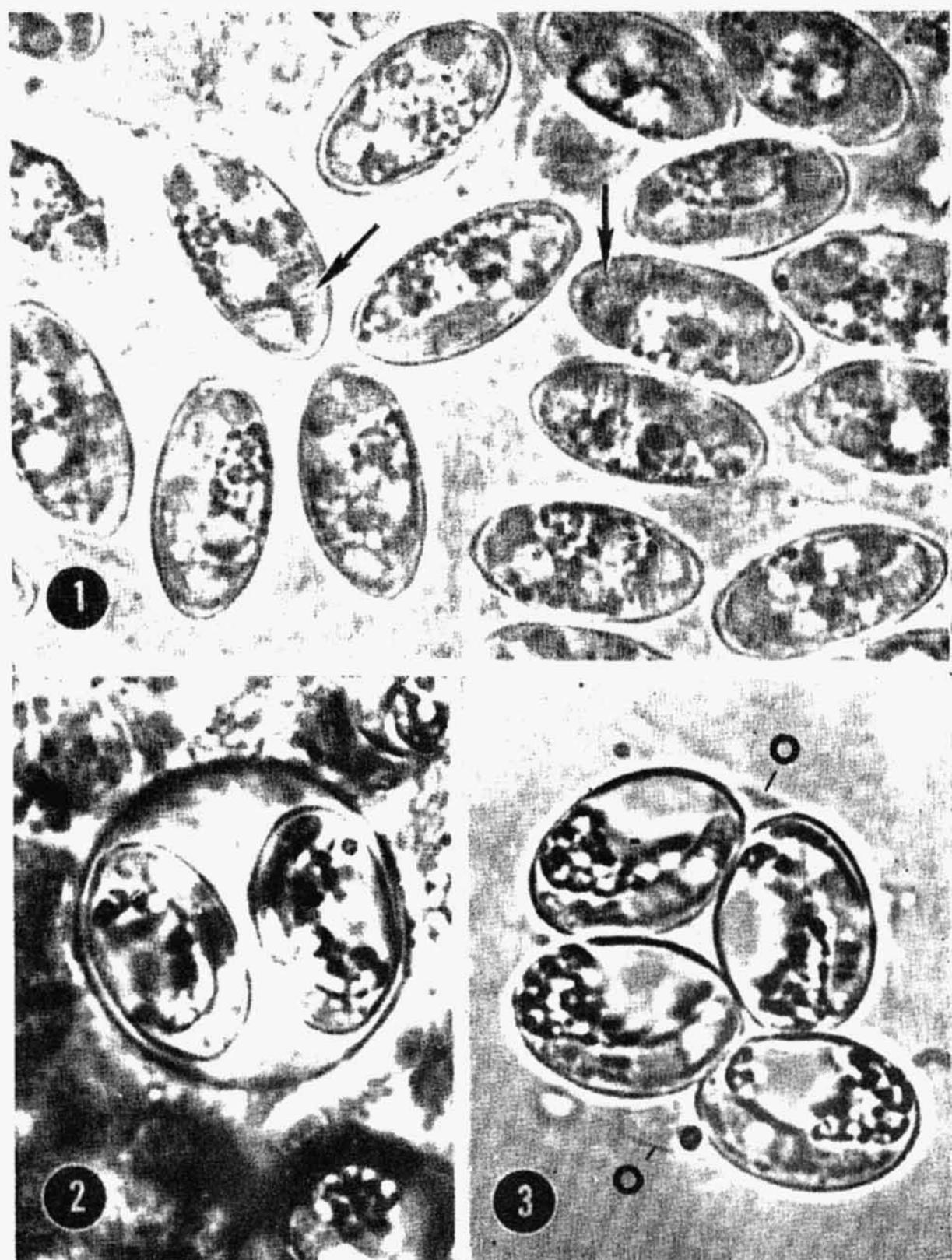


Fig. 1. Mature sporulated spores of *E. subepithelialis*, isolated from disrupted oocysts. Note the compact appearance of the sporozoites and residual bodies; arrows point at striated anterior ends of sporozoites ($\times 2500$).

Figs. 2, 3. Mature sporulated oocysts of *E. spleni*. Note the difference between the solid appearance of the oocyst wall while still in tissue (Fig. 2) and its real fineness demonstrated on an isolated oocyst (Fig. 3). Arrows point at the striation at the anterior end of the sporozoite ($\times 2000$). O — oocyst membrane.

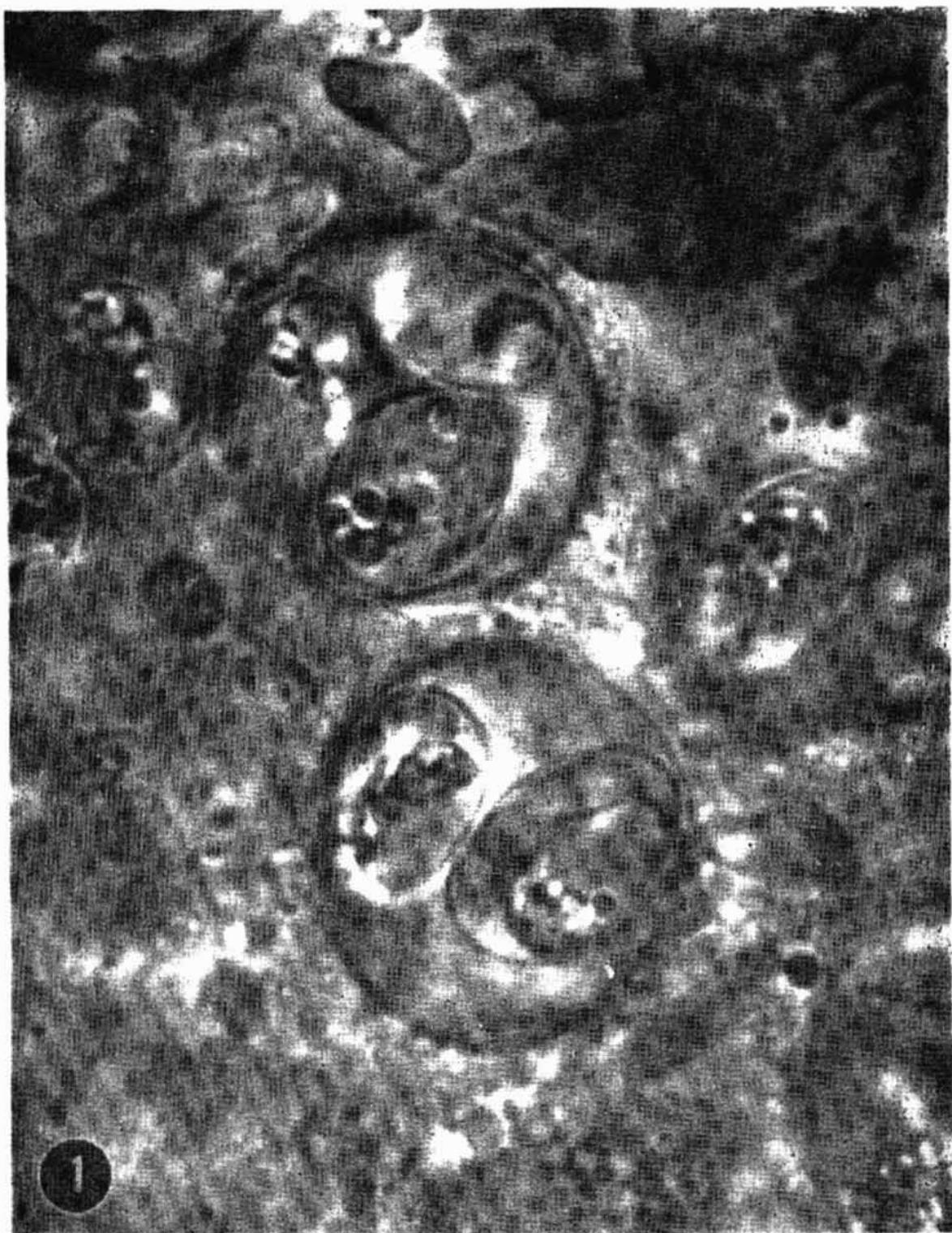


Fig. 1. Two oocysts of *E. spleni* in situ in the invaded tissue of the spleen ($\times 2000$).