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From the Department of Internal Medicine I, Division of Parasitology, Norwegian College of Veterinary Medicine, Oslo, Norway.

ULTRASTRUCTURE OF THE CYSTS OF SARCOCYSTIS TARANDIVULPES FROM SKELETAL MUSCLE OF REINDEER (RANGIFER TARANDUS TARANDUS)

By
Bjørn Gjerde

GJERDE, B.: *Ultrastructure of the cysts of Sarcocystis tarandivulpes from skeletal muscle of reindeer (Rangifer tarandus tarandus).* Acta vet. scand. 1985, *26*, 91—104. — The cysts of S. tarandivulpes were found to be limited by a unit membrane which has been called the cyst membrane. The surface of the cysts was covered by closely packed and hexagonally arranged knob-like protrusions. The protrusions were 0.6—1.2 μm long and had an elliptical cross section. At the base of and between the bases of the protrusions the cyst membrane was raised into low anastomosing folds which delineated shallow compartments. Between the folds the cyst membrane formed small vesicle-like invaginations into the cyst. On the apical part of the protrusions the cyst membrane had a smooth contour and was underlined by 2 layers of electron-dense material. Cyst ground substance divided the interior of the cyst into compartments containing either metrocytes or cystozoites. Cystozoites undergoing endodyogeny were present among the nondividing cystozoites. Some new terms were introduced to denote structures at the border of the cyst. The old terms are reviewed and the structural resemblance between S. tarandivulpes and S. odocoileocanis from Odocoileus virginianus is discussed.

cyst membrane; submembranal dense layer; reinforced cyst membrane; cyst wall; cyst surface process; endodyogeny.

Recently a Sarcocystis sp. with microcysts in skeletal muscle of domestic and wild reindeer (Rangifer tarandus tarandus) in Norway was described. The cysts measured about 900 μm in length and 60 μm in diameter and displayed very short knob-like surface process (*Gjerde* 1984a, b). After the fox had been found to be a definitive host for this species, it was named Sarco-

cystis tarandivulpes (*Gjerde* 1984c). In a previous paper the ultrastructure of S. grueneri, another species with a reindeer/Canidae life cycle, was described, using the established terminology in the description of the cysts (*Gjerde* 1985). In the present paper the ultrastructure of the cysts of S. tarandivulpes from reindeer is described and a somewhat different terminology is used.

MATERIALS AND METHODS

Samples of the abdominal muscles were collected from several adult domestic reindeer slaughtered at abattoirs in Kautokeino and Karasjok in northern Norway. After arrival at the laboratory 2—3 days later, the muscle samples were examined for sarcocysts both grossly and under a stereoscopic microscope. Sarcocysts, together with a small amount of the surrounding tissue, were excised with scissors and fixed in Karnovski's fixative. The specimens were further processed for electron microscopy as described previously (*Gjerde* 1985). Ultrathin sections, cut with glass knives, were examined in a JEOL JEM-100S transmission electron microscope.

RESULTS

The fusiform cysts of S. tarandivulpes were situated within skeletal muscle fibers and were surrounded by a thin layer of host cell material consisting of nuclei and sarcoplasm with a few myofibrils and numerous mitochondria (Figs. 1, 2, 3). The host tissue adjacent to the parasitized muscle fibers was unaltered.

Surface and interior of cyst

The entire cyst was limited by a unit membrane, 6—7 nm thick, which demarcated the cyst from the surrounding host cell sarcoplasm (Fig. 4). This trilaminar limiting membrane or surface membrane of the cyst will from now on be referred to as the cyst membrane. The surface of the cyst was covered by closely packed, short, knob-like processes or protrusions (Figs. 1—3). The surface protrusions had an elliptical to rectangular cross section (Fig. 2), which measured about 2.7—3.7 μm (long diameter) by 1.4—1.8 μm in the middle of the protrusions. The projections were 0.6—1.2 μm long and had a flattened or convex apical surface.

The protrusions were hexagonally arranged and were aligned in longitudinal, parallel rows with their long diameter orientated in the transverse direction (Fig. 2). The minimum distance between them was about 40—100 nm. Because of their elliptical cross section and hexagonal distribution, wider angular spaces were formed between the diverging surfaces of adjacent protrusions. Moreover, the appearance of the protrusions in sections cut perpendicularly to the surface of the cyst became highly dependent on how the plane of section had passed through the linear rows of protrusions, i.e. in which plane the individual protrusions had been sectioned (compare Fig. 1 and Fig. 4). Thus, when the plane of section had passed obliquely through several rows, the protrusions appeared as a row of irregularly shaped and sized profiles (Fig. 1), giving a very misleading impression of their real size and shape.

In a narrow zone in the proximal part of each protrusion and in the spaces between their bases, the cyst membrane was raised into low anastomosing folds or ridges (Figs. 2, 5, 6). The low ridges delineated innumerable round to hexagonal shallow compartments. In surface view the folds displayed a reticular pattern, while they appeared as short villiform projections in vertical sections (Figs. 5, 6). The folds were from 100—200 nm high, about 50 nm thick and were lying about 150—180 nm apart. At the bottom of each of the shallow pits delineated by the folds, the cyst membrane formed a few vesicle-like invaginations into the interior of the cyst (Figs. 5, 6). The invaginations were about 50—70 nm deep and had a diameter of about 60—70 nm. Because of the invaginations, the protrusions were slightly constricted at their bases. The area with invaginations and folds seemed to be demarcated from the remaining, distal portion of each protrusion by a low fold which encircled the entire protrusion. Distal to this encircling fold each protrusion was lined by a smooth-contoured cyst membrane (Fig. 3).

Subjacent to the cyst membrane there was an osmiophilic or electron-dense zone apart from at the points where the cyst membrane formed invaginations. At the latter points the invaginated cyst membrane bordered directly on the electron-lucent cyst ground substance. Between the invaginations the electron-dense zone consisted of a single layer of dense material, which was about 50—60 nm thick. This material also filled the core of the thin folds. Distal to the encircling fold on each pro-

trusion, the electron-dense zone consisted of 2 layers of dense material, which were more or less clearly separated from each other by an up to 12 nm wide electron-lucent interspace (Figs. 3—6). The exterior layer, immediately beneath the cyst membrane, was 10—12 nm thick and of high electron density, whereas the inner layer was about 16—17 nm thick and of intermediate electron density . The electron-dense layer(s) immediately beneath the cyst membrane will be referred to as the submembranal electron-dense layer(s). The complex consisting of the cyst membrane and the submembranal dense layer is called the primary cyst wall by many investigators, but will here instead be referred to as the reinforced cyst membrane.

Coarsely granular cyst ground substance filled the core of the protrusions (Figs. 3, 5, 6) and merged with the somewhat more finely granular substance that formed a continuous 270—550 nm thick layer at the periphery of the cyst beneath the protrusions. From the peripheral layer, the ground substance extended inwardly to form 160—450 nm thick partitions or septa, which divided the interior of the cyst into many rather small and irregularly shaped compartments (Fig. 1). The compartments contained tightly packed metrocytes or cystozoites and scattered masses of debris, which mainly consisted of membranous material.

Metrocytes and cystozoites

The metrocytes (Figs. 1, 7) were lying within small compartments located at the periphery of the cyst. There were usually only a few cells in each compartment. The metrocytes had an ovoid shape and measured up to 7.3 μm in length and 4.7 μm in width. They were limited by a typical coccidian pellicle, consisting of an outer membrane and 2 closely apposed inner membranes. At the anterior end there was a conoid, posterior to which a few rod-shaped, microneme-like structures were lying. However, with dimensions of about 120×30 nm they were considerably smaller than the typical micronemes of the cystozoites, which measured about 350×60 nm. Subpellicular microtubules extended posteriorly from the anterior end of the cell. The electron-lucent cytoplasm contained relatively few ribosomes, 1 or more tubular mitochondria with tubular cristae, large lipid-like bodies, and several membrane-bounded circular to crescentic structures containing a granular material of intermediate elec-

tron density. A large spherical nucleus (diameter about 4 μm) was located centrally in the cell and contained a nucleolus and plaques of electron-dense chromatin.

The cystozoites were elongated, slightly curved cells, which measured about 15 μm by 4 μm (Fig. 1). They were limited by a pellicle consisting of 3 membranes. At the anterior end the cystozoites had a conoid and a polar ring from which 22 subpellicular microtubules extended posteriorly. Numerous micronemes, a micropore and occasionally 1 or 2 membrane-bounded vacuoles were located in the anterior third of the cell. The anterior half of the cystozoites contained several rounded electron-dense bodies. Most of these structures probably represented free globules, or so-called dense granules, and not profiles of cross-sectioned rhoptries. In none of the cystozoites could profiles of more than 2 rhoptries be seen. An elongated tubular, and at times bifurcating mitochondrion with tubular cristae was found in the middle third of the cell surrounded by amylopectin granules, which were also distributed in the posterior third of the cystozoite. Most of this part of the cell was, however, occupied by the rounded nucleus, which contained a nucleolus and plaques of chromatin. The numerous ribosomes either occurred free in the cytoplasm, or were attached to the membranes of the endoplasmic reticulum. The ribosomes were usually evenly spaced along the membranes and collections of ribosomes, i.e. polyribosomes, regularly formed linear arrays. Moreover, in most cystozoites polyribosomes with a helical configuration and ribosome crystals occurred, especially along the nuclear membrane (Figs. 8, 9). The ribosome crystals seemed to arise from a parallel alignment of several helical polyribosomes.

Within the compartments containing the typical cystozoites described above, cells with a somewhat different structure occasionally occurred. There was usually only a single profile of this cell type in each compartment. These cystozoite-like cells represented cells undergoing endodyogeny, i.e. the formation of 2 daughter cells within a mother cell or parent cell. The mother cells measured about 15 μm by 4.5 μm (Figs. 1, 10, 11). They were limited by a three-layered pellicle and had a conoid at the anterior end. Micronemes of the same size and shape as those found in the cystozoites were distributed throughout the cell, either in larger aggregations, or in smaller, separate groups. The cytoplasm contained several rounded electron-dense structures, which probably

were dense granules since they also occurred in the posterior region of the cell. The cytoplasm also contained a few amylopectin granules, 1 or 2 mitochondria and numerous ribosomes. The ongoing endodyogeny was evident from the presence of profiles of 1 or 2 daughter cells within the mother cell (Fig. 11). Based on their location among the cystozoites and their structural resemblance to them (the presence of dense granules, micronemes, amylopectin granules and relatively many ribosomes) and their dissimilarity to the metrocytes at the periphery of the cyst, these mother cells are considered to represent cystozoites undergoing endodyogeny.

DISCUSSION

Terminology

In the preceding paper, in which the ultrastructure of the cysts of S. grueneri was described (*Gjerde* 1985), the established terminology was used in the description of the surface and the border of the cyst. However, it was felt that some of the terms were inappropriate and therefore complicated the description and understanding of the boundary and surface topography of the cyst. In the present paper, therefore, some alternative terms have been introduced and the emphasis has been put on the limiting unit membrane of the cyst, which has been named the cyst membrane. The new terms are equally suitable for the description of the cysts of other cyst-forming genera.

There is considerable confusion in the literature concerning the designation of the border of the tissue cysts. Various authors either refer to a given structure by different terms, or they use a given term to designate different structures. There are several reasons for this confusion in the terminology. Some authors seem to disregard, or are not aware of, the definitions of the terms they use, and may for instance use the terms cyst wall and primary cyst wall interchangeably in the same paper. The erroneous application of the terms may also indicate that the available terms are somewhat inappropriate and should be replaced by terms that are more self-explanatory or descriptive, even though the introduction of new terms may temporarily add to the confusion.

In most of the papers published recently on the ultrastructure of sarcocysts, the established terms cyst wall and primary cyst wall are used to denote structures at the border of the cysts.

Figures 1—11. Transmission electron micrographs of Sarcocystis tarandivulpes cysts from skeletal muscle of reindeer.

Abbreviations: A = Amylopectin granules; Ac = Adjacent muscle cell; C = Conoid; Cm = Cyst membrane; Cy = Cystozoite; D1 and D2 = Outer and inner submembranal dense layers; Dc = Daughter cell; Dg = Dense granule; Dm = Degenerated material; F = Surface folds; Gs = Ground substance; In = Invagination of cyst membrane; Mc = Metrocyte; Mi = Mitochondrion; Mn = Micronemes; Moc = Mother cell; N = Nucleus; Pr = Surface protrusion; R = Ribosomes; Rcm = Reinforced cyst membrane; Se = Septum; Sp = Sarcoplasm of host cell.

Figure 1. Marginal part of a cyst with short knob-like surface protrusions (Pr) adjoining a thin layer of host cell sarcoplasm (Sp). Septa (Se) divide the interior of the cyst into compartments containing metrocytes (Mc), cystozoites (Cy) and masses of degenerated material (Dm). A mother cell (Moc) is lying among the cystozoites in one compartment. × 3,250.

Figure 2. Tangential section through the cyst wall toward the end of a cyst. Note the hexagonal arrangement of the protrusions (Pr). Numerous host cell mitochondria (Mi) are lying adjacent to the protrusions. × 5,250.

Figure 3. Marginal part of cyst, showing closely spaced protrusions (Pr) bounded by the cyst membrane with underlying dense material (Rcm). × 12,000.

Figure 4. Detail of the cyst border on the lateral aspect of two adjacent protrusions (Pr). The cyst membrane (Cm) is underlined by two layers of dense material (D1 and D2). × 120,000.

Figure 5. Tangential section through the spaces between the bases of the protrusions (Pr). The cyst membrane forms anastomosing folds (F) delineating shallow compartments, from the bottom of which invaginations of the cyst membrane (In) into the cyst arise. × 35,000.

Figure 6. Vertical section through a protrusion (Pr) and the space between 2 protrusions showing the folds (F) and invaginations (In) in profile. × 50,000.

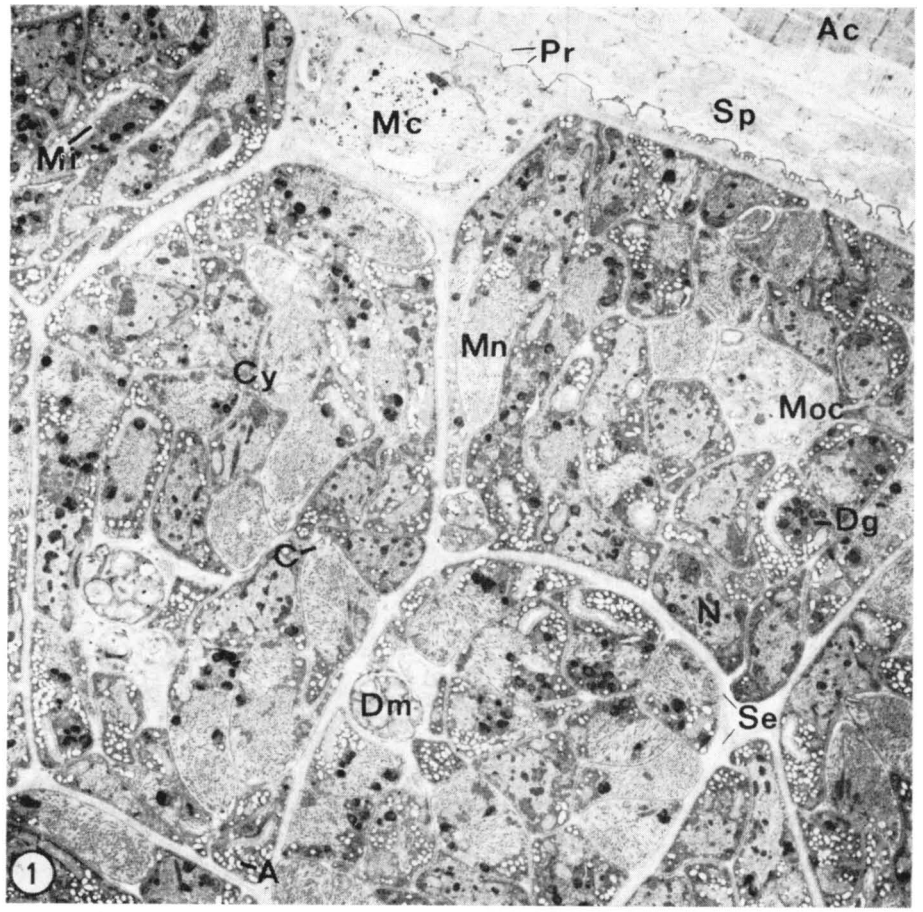
Figure 7. Three metrocytes in a compartment at the periphery of a cyst. × 10,500.

Figure 8. Tangential section through a ribosome crystal on the surface of the nucleus. Note densely packed and hexagonally arranged ribosomes (R). × 87,500.

Figure 9. Transverse section through a stack of 3 helical polyribosomes. × 87,500.

Figure 10. Longitudinal section of a dividing cystozoite with redistributed organelles. Note the presence of a conoid (C), micronemes (Mn), amylopectin granules (A), dense granules (Dg), numerous free ribosomes (R), and the location of the nucleus (N) in the anterior half of the cell. × 9,000.

Figure 11. Section through a mother cystozoite with the profile of a daughter cell (Dc) at an early stage of development. × 12,000.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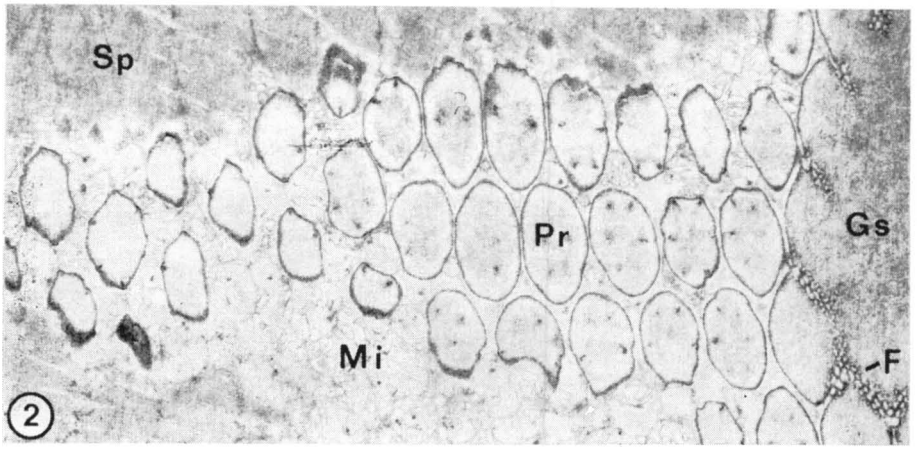
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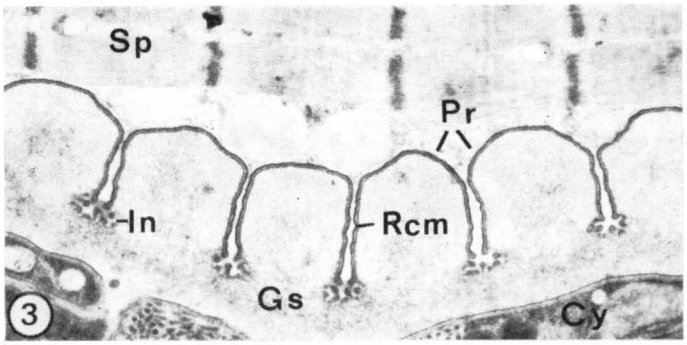
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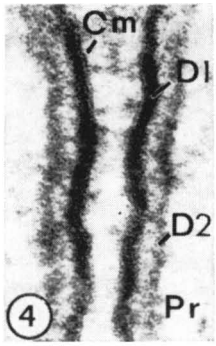
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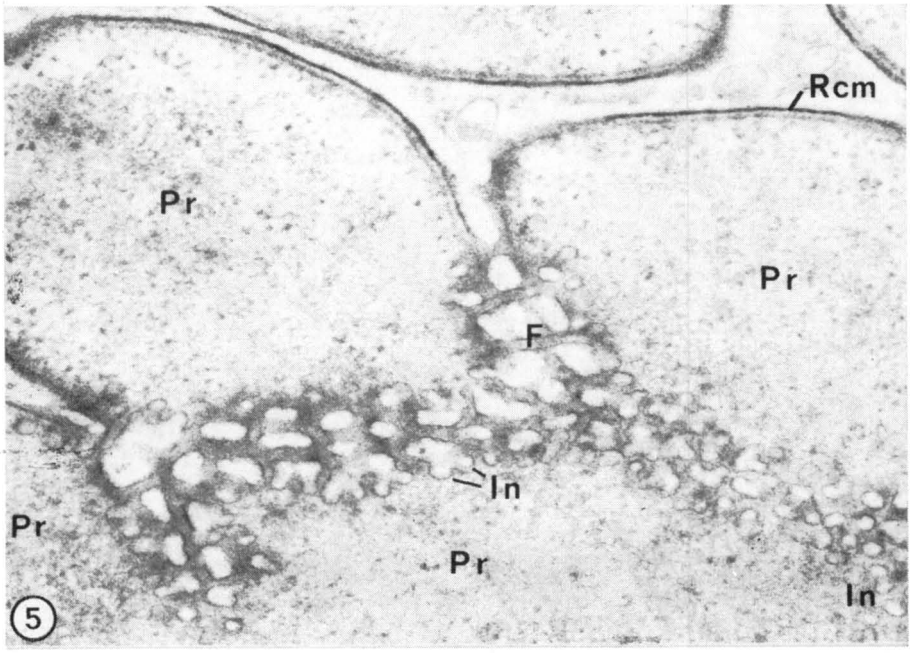
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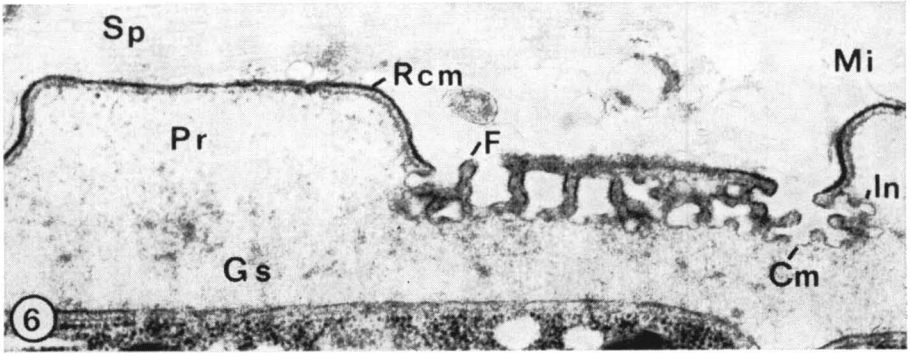
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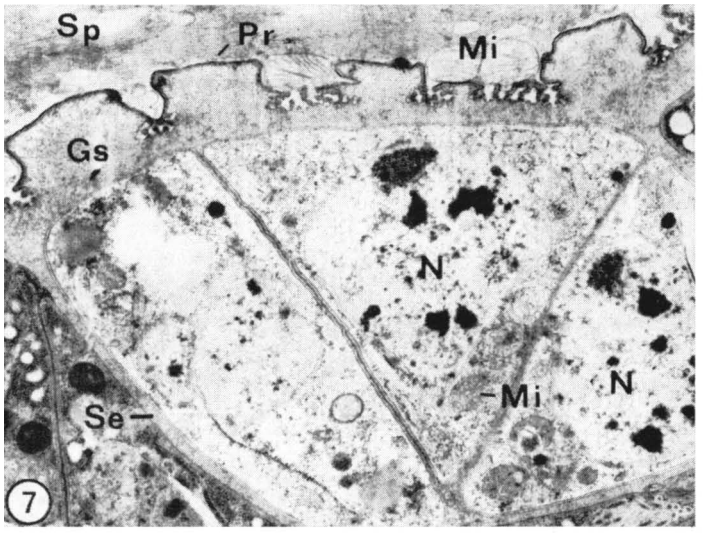
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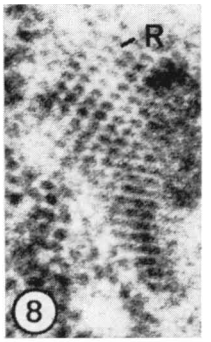
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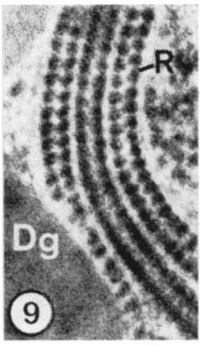
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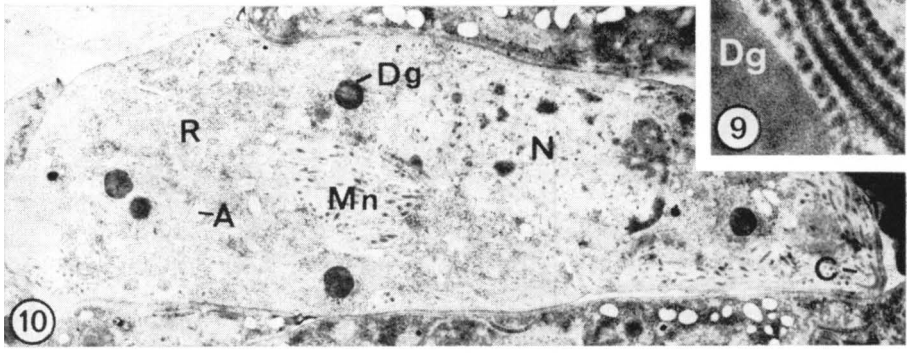
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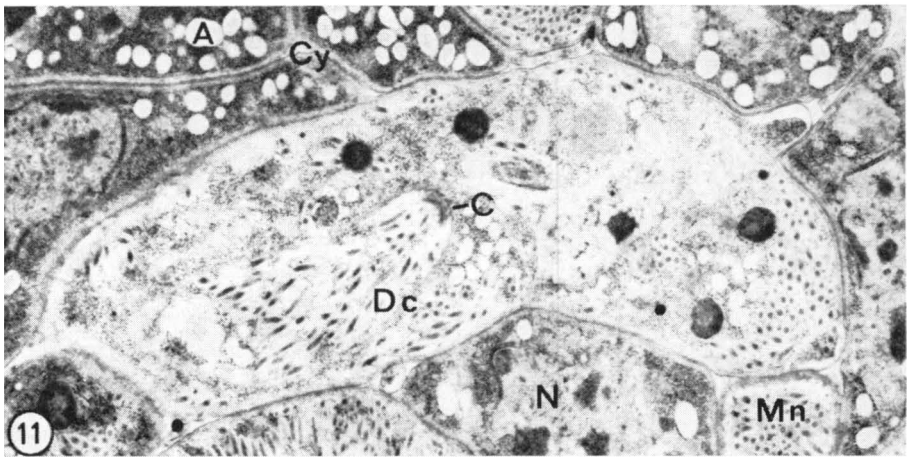
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When the light microscopic structure of cysts is described from their appearance in stained sections, the term cyst wall is usually used to denote the material or layer that separates the organisms within the cyst from the host cell cytoplasm adjacent to the cyst. Owing to the limited resolving power of the light microscope the cyst wall appear in such sections as a single and homogeneous structural entity. Some cysts apparently have a thin cyst wall, whereas others have a thick and radially striated wall. However, transmission electron microscopy has revealed that the classical cyst wall is a compound structure composed of the outer cyst membrane, the submembranal dense layer and cyst ground substance at the periphery of the cyst. Moreover, for cysts with surface processes, the host cell cytoplasm between the protrusions cannot be discerned with the light microscope and is perceived as a part of the cyst wall. Thus, cysts with tightly packed, upright, villus-like surface processes appear as thick-walled cysts, whereas cysts with no protrusions, with very short protrusions (e.g. S. tarandivulpes), or with delicate, flexible projections lying close to the surface of the cyst (e.g. S. grueneri), all appear as thin-walled cysts.

The cyst wall described from electron micrographs should correspond to the cyst wall seen with the light microscope. However, it is obvious that the host cell cytoplasm between the protrusions cannot be included as a component of the cyst wall. The cyst wall should therefore include the material or region of the cyst that lies peripheral to (exterior to) the outermost organisms within the cyst and is limited on the outside by the cyst membrane. Since the protrusions are components of the cyst wall, they do not project from the cyst wall, but rather from the surface of the cyst. They should therefore preferably be referred to as cyst surface processes or cyst processes (protrusions, projections), which will be in accordance with the terminology used for similar structures in current textbooks of histology. The cyst surface processes of different Sarcocystis species consist of variously sized, shaped and arranged evaginations of the cyst membrane containing a core of cyst ground substance. They might also be described as extensions of the cyst ground substance covered by the cyst membrane. For cysts with surface processes it makes little sense to talk about the thickness of the cyst wall. Instead, the thickness of the cyst wall zone might be used. The thickness of the cyst wall zone represents the combined

thickness of the peripheral layer of ground substance and the zone of surface processes.

Like the cyst wall, the so-called primary cyst wall is a compound structure. The primary cyst wall has been defined as the thickened border of the transformed parasitophorous vacuole, i.e. the limiting unit membrane plus underlying osmiophilic meterial (*Mehlhorn et al.* 1976). According to the definition, a primary cyst wall is present only when both components of the primary cyst wall are present. Hence, the cysts of Besnoitia species do not have a primary cyst wall since the cyst membrane of these cysts is not reinforced by osmiophilic material (*Chobotar & Scholtyseck* 1982). Moreover, in the cysts of the other cyst-forming genera (Sarcocystis, Frenkelia, Toxoplasma and Hammondia) the submembranal osmiophilic layer is interrupted at numerous sites, coinciding with the points of invagination of the cyst membrane into the cysts. Since the primary wall has been defined as a compound structure, there is no primary cyst wall at the points of invagination where the cyst membrane remains unreinforced. It is therefore not correct to say, as it is frequently done, that the primary cyst wall has unthickened places at the points where the osmiophilic material is missing. It is also frequently said that the primary cyst wall limits the cysts. However, it is the outer unit membrane of the cyst, irrespective of whether it is reinforced or not by osmiophilic material, that limits the cyst and demarcates it from the surrounding host cell cytoplasm. When describing the cyst border it is usually easier to refer to the outer unit membrane and the underlying osmiophilic material separately than to refer to the complex that they form, especially in the areas of the cyst surface where the submembranal layer is interrupted by invaginations from the cyst membrane. The literal meaning of the term primary cyst wall also gives the erroneous impression that this is a rather thick structure, comparable to the cyst wall, and that the cysts also have a secondary cyst wall. However, only the cysts of a few species have a so-called secondary cyst wall (*Chobotar & Scholtyseck* 1982), which clearly is an inappropriate term, since this structure has been defined to represent fibrillar material surrounding the parasitized cell (*Mehlhorn et al.* 1976). The secondary cyst wall is therefore an extra envelope or capsule around the host cell rather than around the cyst within the cell.

Some investigators refer to the outer membrane of the cyst as the parasitophorous vacuolar membrane since this membrane

is derived from the original parasitophorous vacuolar membrane, which surrounded the merozoite that invaded the cell and initiated cyst formation. However, when the original parasitophorous vacuole has developed into a cyst, it is more appropriate to refer to the limiting membrane of the cyst as the cyst membrane. According to the prevailing opinion the original parasitophorous vacuolar membrane is formed by the host cell and not by the invading merozoite (*Chobotar & Scholtyseck* 1982, *Entzeroth* 1984). However, I think it is more likely that the invaded merozoite itself in part form the parasitophorous vacuolar membrane from material released from the dense granules or other organelles. If not, it is difficult to envisage how the cyst membrane of different species of Sarcocystis can form a species specific pattern of invaginations and surface processes on the cysts.

The term cyst membrane has been used previously. Thus, *Van der Zypen & Piekarski* (1966) called the outer boundary of the cysts of Toxoplasma gondii the cyst membrane. However, the structure referred to as the cyst membrane by them, was 15—20 nm thick and it has therefore probably comprised both the outer unit membrane and the submembranal dense material, which they have erroneously interpreted as a single structural entity. *Bergmann & Kinder* (1975), on the other hand, used the term cyst membrane to denote the outer limiting membrane of the cyst in the legend to the figures, but not in the text, in one of their papers. The term membrane should preferably be reserved to structures that by electron microscopy show the characteristic trilaminar appearance of a unit membrane. The cyst membrane was therefore defined as the limiting unit membrane of the cyst in the present paper.

The cysts of S. tarandivulpes

S. tarandivulpes was originally differentiated from S. grueneri on the basis of a slight difference in the appearance of the cyst surface when fresh preparations of the cysts were viewed with the light microscope (*Gjerde* 1984a). While S. grueneri had a rather smooth cyst surface with no visible protrusions, S. tarandivulpes had minute knob-like protrusions, giving the cysts a slightly indented outline. When examined with the electron microscope, the cyst membrane of S. grueneri was found to form numerous vesicle-like invaginations into the cyst and irregularly spaced, fine, ribbon-like protrusions (*Gjerde* 1985). The present investigation showed that the cysts of S. tarandivulpes were

covered by tightly packed, short projections and that the invaginations were confined to the areas around the bases of the protrusions. Thus, S. tarandivulpes differs markedly in cyst surface topography from S. grueneri; both with regard to the shape and arrangement of the protrusions and the distribution and number of invaginations.

S. tarandivulpes has a similar cyst wall structure as a Sarcocystis sp. reported from the white-tailed deer (Odocoileus virginianus) by *Entzeroth et al.* (1982) and by *Dubey & Lozier* (1983), and referred to as S. odocoileocanis in the latter paper. The brief and incomplete descriptions of the cyst border of the white-tailed deer species in both papers differ considerably from the present description of S. tarandivulpes. However, the accompanying electron micrographs show that the similarly shaped surface protrusions of the white-tailed deer species also have 2 submembranal layers of electron-dense material distally and a complex arrangement of invaginations and folds at their bases. The presence of 2 dense layers beneath the cyst membrane lining the protrusions seems to be a characteristic feature of these species.

S. tarandivulpes also has a similar light microscopic cyst surface configuration as a Sarcocystis sp. described from roe deer (Capreolus capreolus), and referred to as S. gracilis (*Erber et al.* 1978). This species has also been found in a roe deer from southern Norway (*Gjerde*, unpublished data). To my knowledge the fine structure of the cysts of S. gracilis has not been described. It is therefore not known whether the cysts of this species are ultrastructurally similar to S. tarandivulpes and S. odocoileocanis. However, all three species have a canine-cervid life cycle (*Erber et al.* 1978, *Crum et al.* 1981, *Gjerde* 1984c), and S. odocoileocanis was apparently transmitted to cattle and sheep (*Crum et al.* 1981). In a previous paper, the cysts of S. grueneri from reindeer were reported to be similarly structured as sarcocysts from red deer, roe deer and moose (*Gjerde* 1985). These findings suggest that the various cervid intermediate hosts may be infected by common species of Sarcocystis, which have a weak intermediate host specificity. Alternatively, each species of intermediate host may harbour different Sarcocystis species, which have morphologically indistinguishable cysts. Thus, it will probably be necessary to carry out extensive cross-transmission experiments in order to determine whether S. grueneri and S. ta-

randivulpes have other cervid intermediate hosts than the reindeer.

The occasional finding of only 1 or 2 elongate profiles of typical rhoptries within the cystozoites was in contrast to the regular finding of several rounded structures of similar or slightly higher electron density. These rounded structures have commonly been regarded as being sections of the posterior portions of club-shaped and presumably tortuous rhoptries (*Chobotar & Scholtyseck* 1982). However, in the present study there was no evidence of any connections between the rounded structures, not even in serial sections, suggesting that most of them were free glubules, rather than portions of rhoptries. Thus, it is likely that the actual number of rhoptries within the cystozoites of S. tarandivulpes is low, possibly not more than 2, while most of the rounded structures represent the so-called dense granules as described by *Dubremetz & Dissous* (1980) from the cystozoites of S. gigantea (ovine/feline life cycle), which contain only 2 rhoptries. Likewise, *Sheffield et al.* (1977) reported that the cystozoites of S. muris had few rhoptries, but several dense granules; a finding that was confirmed by *Entzeroth* (1984) who found that the cystozoites of this species contained 2 rhoptries and several dense granules. In cross sections of cystozoites it may be difficult, or even impossible, to distinguish between a cross-sectioned rhoptry and a dense granule.

The cystozoite-like mother cells described in the present paper were considered to represent cystozoites undergoing endodyogeny. This is in accordance with *Sénaud* (1967) who described similar cells from the cysts of S. gigantea from sheep and referred to them as endodyocytes (cystozoites) undergoing endodyogeny. He also described in detail the process by which 2 daughter cystozoites were formed from a parent cystozoite (see Fig. 32 in his paper). *Zaman & Colley* (1975) also referred to similar binucleate cells as cystozoites. However, in several papers published is recent years, such dividing cells have been described as metrocytes (*Göbel & Rommel* 1980, *Entzeroth et al.* 1982). In other papers parent cystozoites have been illustrated, but the authors have apparently not been aware that they differed in structure from the surrounding cystozoites. A cystozoite undergoing endodyogeny was also illustrated in the paper on S. grueneri (*Gjerde* 1985), but in that paper the dividing cystozoite was only referred to as a mother cell.

It is in fact somewhat strange that so few papers have described cystozoites undergoing endodyogeny since it has been reported in several papers that the cystozoites, like the metrocytes, multiply by endodyogeny. In the present investigation typical metrocytes, none of which was seen to divide, only occurred in small compartments at the periphery of the cysts, whereas the diving cystozoites were found among the nondividing cystozoites in compartments throughout the cyst. These findings show that cystozoites, after having arisen from the metrocytes, continue to divide by endodyogeny, and gradually increase in number, whereas the metrocytes apparently cease to divide. By a careful examination, multiplying cystozoites will probably be found in most cysts. However, they are probably rather few in number in mature cysts. Moreover, from their location and structural resemblance to the nondividing cystozoites, they may be rather difficult to detect. In the present study only a limited number of dividing cystozoites were examined, but the endodyogeny of these cells appeared to be similar to the endodyogeny described from typical metrocytes (*Heydorn et al.* 1975) and from the cystozoites of S. gigantea (*Sénaud* 1967).

The present description of helical polyribosomes and crystalline arrays of helical polyribosomes is, to my knowledge, the first report of such structures from the cystozoites of a Sarcocystis species. Similarly arranged ribosomes have, however, been found in a variety of cells from different organisms.

REFERENCES

Bergmann, V. & E. Kinder: Unterschiede in der Struktur der Zystenwand bei Sarkozysten des Schafes. (Sarcocystis in sheep. Differences in cyst wall structure). Mh. Vet.-Med. 1975, *30*, 772—774.

Chobotar, B. & E. Scholtyseck: Ultrastructure. *In:* Long, P. L. (ed): The Biology of the Coccidia. University Park Press, Baltimore 1982, pp. 101—165.

Crum, J. M., R. Fayer & A. K. Prestwood: Sarcocystis spp. in white-tailed deer I. Definitive and intermediate host spectrum with a description of Sarcocystis odocoileocanis n.sp.. J. Wildl. Dis. 1981, *17*, 567—579.

Dubey, J. P. & S. M. Lozier: Sarcocystis infection in the white-tailed deer (Odocoileus virginianus) in Montana: Intensity and description of Sarcocystis odoi n. sp. Amer. J. vet. Res. 1983, *44*, 1738—1743.

Dubremetz, J. F. & C. Dissous: Characteristic proteins of micronemes and dense granules from Sarcocystis tenella zoites (Protozoa, Coccidia). Mol. Biochem. Parasitol. 1980, *1*, 279—289.

Entzeroth, R.: Electron microscope study of host-parasite interactions of Sarcocystis muris (Protozoa, Coccidia) in tissue culture and in vivo. Z. Parasitenkd. 1984, *70*, 131—134.

Entzeroth, R., B. Chobotar & E. Scholtyseck: Ultrastructure of Sarcocystis sp. from the muscle of a white-tailed deer (Odocoileus virginianus). Z. Parasitenkd. 1982, *68*, 33—38.

Erber, M., J. Boch & D. Barth: Drei Sarkosporidienarten des Rehwildes. (Three Sarcocystis species in the roe deer (Capreolus capreolus)). Berl. Münch. Tierärztl. Wschr. 1978, *91*, 482—486.

Gjerde, B.: A light microscopic comparison of the cysts of four species of Sarcocystis infecting the domestic reindeer (Rangifer tarandus) in northern Norway. Acta vet. scand. 1984a, *25*, 195—204.

Gjerde, B.: Sarcocystis infection in wild reindeer (Rangifer tarandus) from Hardangervidda in southern Norway: With a description of the cysts of Sarcocystis hardangeri n. sp.. Acta vet. scand. 1984b, *25*, 205—212.

Gjerde, B.: The fox as definitive host for Sarcocystis sp. Gjerde, 1984 from skeletal muscle of reindeer (Rangifer tarandus). With a proposal for Sarcocystis tarandivulpes n. sp. as replacement name. Acta vet. scand. 1984c, *25*, 403—410.

Gjerde, B.: Ultrastructure of the cysts of Sarcocystis grueneri from cardiac muscle of reindeer (Rangifer tarandus tarandus). Z. Parasitenkd. 1985, *71*, 189—198.

Göbel, E. & M. Rommel: Licht- und elektronenmikroskopische Untersucungen an Zysten von Sarcocystis equicanis in der Ösophagusmuskulatur von Pferden. (Light and electron microscopic study on cysts of Sarcocystis equicanis in the oesophageal musculature of horses). Berl. Münch. Tierärztl. Wschr. 1980, *93*, 41—47.

Heydorn, A. O., H. Mehlhorn & R. Gestrich: Licht und elektronenmikroskopische Untersuchungen an Cysten von Sarcocystis fusiformis in der Muskulatur von Kälbern nach eksperimenteller Infektion mit Oocysten und Sporocysten von Isospora hominis Raillet et Lucet, 1881. 2. Die Feinstruktur der Metrocyten und Merozoiten. (Light and electron microscope studies on cysts of Sarcocystis fusiformis in the muscles of calves infected experimentally with oocysts and sporocysts of Isospora hominis Raillet et Lucet, 1881. 2. Fine structure of metrocytes and merozoites). Zbl. Bakt. Hyg., I. Abt. Orig. A, 1975, *232*, 373—391.

Mehlhorn, H., W. J. Hartley & A. O. Heydorn: A comparative ultrastructural study of the cyst wall of 13 Sarcocystis species. Protistologica 1976, *12*, 451—467.

Sénaud, J.: Contribution a l'étude des sarcosporides et des toxoplasmes (Toxoplasmea). (Contribution to the knowledge of the sarcosporidia and the toxoplasms (Toxoplasmea)). Protistologica 1967, *3*, 167—232.

Sheffield, H. G., J. K. Frenkel & A. Ruiz: Ultrastructure of the cyst of Sarcocystis muris. J. Parasitol. 1977, *63*, 629—641.

Van der Zypen, E. & G. Piekarski: Zur Ultrastruktur der Cystenwand von Toxoplasma gondi im Gehirn der weissen Maus. (On the ultrastructure of the cyst wall of Toxoplasma gondii in the brain of the white mouse). Z. Parasitenkd. 1966, *28*, 45—59.

Zaman, V. & F. C. Colley: Light and electron microscopic observations on the life cycle of Sarcocystis orientalis sp. n. in the rat (Rattus norvegicus) and the Malaysian reticulated python (Python reticulatus). Z. Parasitenkd. 1975, *47*, 169—185.

SAMANDRAG

Ultrastrukturen til cyster av Sarcocystis tarandivulpes frå skjelettmuskulaturen hos rein (Rangifer tarandus tarandus).

Cystene var avgrensa av ein typisk elementærmembran som ein har kalla cystemembranen. Cysteoverflata var dekka av tettsitjande, utvekstar (projeksjonar) med eit ellipseforma tverrsnitt. Projeksjonane var frå 0.6—1.2 μm lange. Ved basis av projeksjonane og i romma mellom dei danna cystemembranen låge anastomoserande mikrofoldar og små invaginasjonar. Grunnsubstans danna eit lag perifert i cystene og delte det indre av cystene opp i talrike kammer som inneheldt anten metrocytar eller cystozoitar. I nokre av cystozoitane føregjekk det ein delingsprosess (endodyogeni). Cystene til S. tarandivulpes hadde ein liknande morfologi som cystene til S. odocoileocanis frå kvithalehjort. I omtalen av cystene er det delvis nytta ein ny terminologi, og denne vert samanlikna med den etablerte terminologien.

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Reprints may be requested fram: B. Gjerde, the Department of Internal Medicine I, Division of Parasitology, Norwegian College of Veterinary Medicine, P. O. Box 8146 Dep., N-0033 Oslo 1, Norway.