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Ultrastructure of the Cortex of the Rumen Ciliate *Ophryoscolex purkynjei* Stein, 1858 (Entodiniomorphida: Ophryoscolecidae)

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Abstract: The ultrastructure of the cortex of *Ophryoscolex purkynjei,* an entodiniomorphid ciliate obtained from domesticated cattle rumens, was studied and found to have five layers. A particulate and/or filamentous glycocalyx layer, triple membrane layer, homogeneous epiplasmic layer with very fine grains, microtubular layer characterized with a double row of microtubule bundles each containing at most 6 units, and a compact microfilamentous layer were identified. In addition, scarce and disorganized kinetosomes and related microtubules were found to lie underneath all of these. These observations are discussed with reference to the findings of previous studies of other entodiniomorphid ciliates.

Key Words: Ultrastructure, Cortex, *Ophryoscolex purkynjei,* Rumen Ciliate.

İşkembe Siliyatı *Ophryoscolex purkynjei* Stein, 1858'nin (Entodiniomorphida: Ophryoscolecidae) Korteks İnce Yapısı

Özet: Evcil sığırların işkembesinden elde edilen entodiniomorphid siliyat *Ophryoscolex purkynjei*'nin korteks ince yapısı çalışılmış ve beş tabakadan oluştuğu gösterilmiştir. Korteks dıştan içe doğru sırasıyla, partiküler ve/veya filamentoz bir glikokaliks tabakası, üçlü yapı gösteren bir zar tabakası, homojen ince granüllü bir epiplazma tabakası, bunun altında iki sıra halinde düzenlenmiş en fazla 6 mikrotübülden oluşmuş paketlerin meydana getirdiği bir mikrotübüler tabaka ve en içte yoğun bir mikrofilament tabakasından oluşur. Bu tabaka altında az sayıda ve düzensiz dağılmış körelmiş kinetozomlar ve bunlarla ilişkili bazal mikrotübüller gözlenmiştir. Elde edilen bulgular diğer bazı entodiniomorphid siliyatlardan elde edilenlerle karşılaştırılarak tartışılmıştır.

Anahtar Sözcükler: İnce Yapı, Korteks, *Ophryoscolex purkynjei,* İşkembe Siliyatı.

Introduction

The genus *Ophryoscolex* is one of the well-known entodiniomorphid ciliates (order Entodiniomorphida) that live endocommensally in the rumen of ruminant mammals. This genus was previously defined as containing two species: *Ophryoscolex purkynjei* (1) and *O. caudatus* (2), but it was later reported that it should be considered as only one species, named *O. purkynje*, according to taxonomical revisions and new statistical analysis (3, 4). By the consideration of structures seen by light microscopy, the general morphology was detailed by Göçmen (3, 4). However, electron microscopy studies of rumen entodiniomorphid ciliates are largely limited to certain structures of some species because of the many difficulties in achieving isolation and cultivation (5-19). The ultrastructures of many entodiniomorphid ciliates have been clarified, especially by Furness & Butles (10-14). *O. purkynjei,* however, has been described ultrastructurally by Noirot-Timothée (16), Roth & Shigeneka (17) and Schrenk & Bardele (19). However, no information on the ultrastructure of the cortex in the non-ciliated region of this species is available.

The aim of the present study was to reveal the ultrastructure of the cortical and subcortical regions of *Ophryoscolex purkynjei*, for the purpose of shedding further light on the phylogeny of these ciliates by comparing the current results with previous findings.

Material and Methods

Rumen fluids were obtained from recently slaughtered cattle (*Bos taurus* L.) in the Izmir

Metropolitan Abattoir according to the method described previously (20, 21).

Ophryoscolex purkynjei samples were collected with a capillary micropipette from the mixture of ciliates in rumen fluid under a light microscope. The concentrated samples were fixed by the addition of equal volumes of 5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) and postfixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer. Then, the samples were embedded in 0.13% agar by microinclusion followed by routine electron microscopic applications, and were finally embedded in Epon-812 resin.

Semi-thin sections (0.5-10 µm) were mounted on slides and were stained with 2% methylen blue in 2% aqueous borax over a flame at 60 °C (8).

These sections were double-stained in 2% aqueous uranyl acetate and 2% aqueous lead citrate on 400-mesh copper grids (22). Then, they were examined in a Jeol 100 CTEM. The terminology used in the structural descriptions is the same as that used by Furness & Butler (10) and Grain (23).

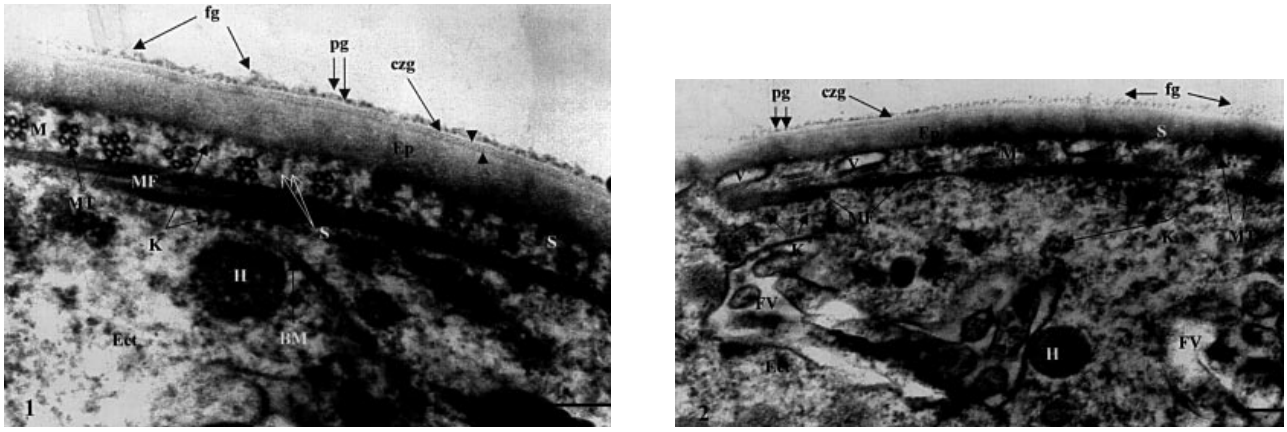
Results

The cortex of *Ophryoscolex purkynjei* is fairly thick (450-600 nm) and is composed of five layers. The Glycocalyx, the most peripheral layer, is about 25-120 nm in thickness. In transverse and tangential sections, the glycocalyx (20-40 nm) in the anterior half of the cell is laminated into two equally lean parts. The upper layer is particulated and/or filamentous and the subjacent layer was found to be lightly stained (Figures 1& 2). In the posterior half of the cell, the double-layered appearance is lost and the glycocalyx broadens to 120 nm (Figure 3).

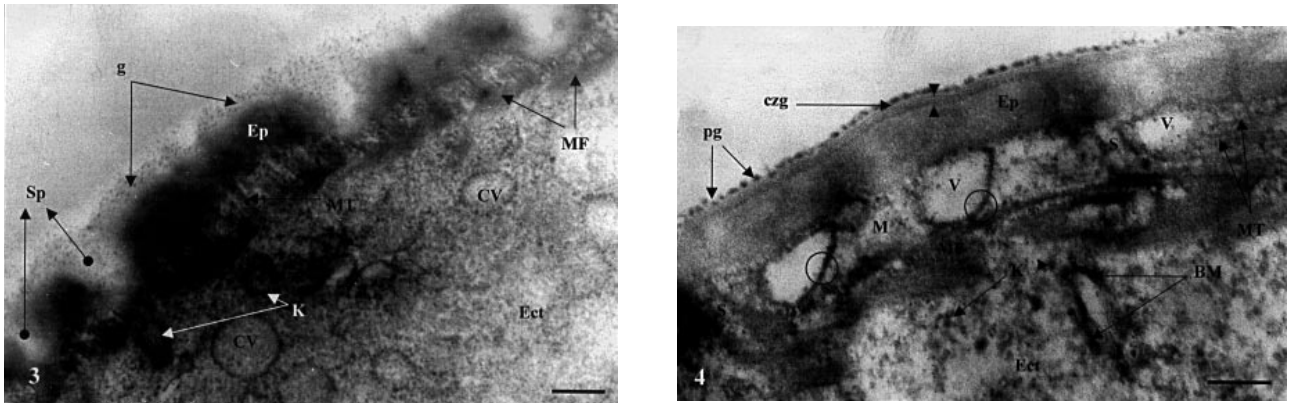
A triple-membrane layer of 20-30 nm underlies the gylcocalyx. Although the most external membrane appears less dense, all three are regularly spaced.

A homogenous epiplasmic layer (135-165 nm) with very fine granules (<5 nm) extends underneath the membrane layer. These granules become denser towards the inside of the cell (Figures 1 & 2). Below the homogenous layer, there is a thick electron-transparent region (13-155 nm) where microtubule bundles extend longitudinally. Each bundle contains two rows of 6 almost round-elliptical microtubules. The individual microtubules have 10-15 nm spacing and the bundles are separated by a thin layer of material (a septum) which sometimes exhibits a double-layered appearance and is oriented a a right angle to the barren kinetosomes. It therefore appears that the remains of the peripheral fibers project from the kinetosomes.

Ovoid or elliptical double-layered vacuoles were observed within the microtubular layer in transverse (Figure 2) and in longitudinal sections (Figure 4).



Figures 1-2. Ultrastructure of the cortex of *Ophryoscolex purkynjei* in the anterior of the cell in tranverse (1) and oblique longitudinal (2) sections. V=cortical vacuole, BM,basal microtubulees associated with kinetosomes, czg=agranulated and afilamentous clear or light zone of glycocalyx, Ep=epiplasm, Ect=ectoplasm (central periplasm), fg=filamentous glycocalyx, FV=food vacuole, H=hydrogenosome, K=barren kinetosomes, M=microtubular layer with microtubule bundles (MT), MF=microfilamentous layer, pg=particulate glycocalyx, S=septum. Arrowheads show the range of membranous layer. Bars: 150 nm.



Figures 3-4. Ultrastructure of the cortex in tangential section at anterior (3) and longitudinal sections in the caudal region (4) of *Ophryoscolex purkynjei*. V=cortical vacuole, BM=basal microtubulees associated with kinetosomes, CV=coated vesicles, czg=agranulated and afilamentous zone of glycocalyx, Ep=epiplasm, Ect=ectoplasm (central periplasm), fg=filamentous glycocalyx, g=glycocalyx, K=barren kinetosomes, MF=microfilamentous layer, M=microtubular layer with microtubule bundles (MT), pg=particulate glycocalyx, S=septum, Sp=superficial pits. The circles show the double membrane layer of the vacuoles. Arrowheads show the range of membranous layer. Bars: 150 nm.

The innermost layer of the cortex, the 55-200 nm zone, which is composed of 5 nm microfilaments, is arranged into large fibrous bundles vertical to the microtubule bundles, but anastomosing to form a network. The upper cortical layers are perforated to the level of the microtubular layer by facial porousness (Figure 3). There are sparsely distributed scant barren kinetosomes in this part of the ectoplasm. Sometimes the basal microtubules extend from the base of the barren kinetosomes deep into the ectoplasm.

Discussion

Rumen entodiniomorphid protozoa are classified into two ciliate families, Entodiniidae and Ophryoscolecidae (3, 4, 23). THe ultrastructure, especially of the non-ciliated cortical region, of the simplest form *Entodinium* (Entodiniidae) and the more complex forms *Eudiplodinium (=Metadinium)* and *Epidinium* (Ophryoscolecidae) was studied in detail and described accordingly by Furness & Butler (10-13).

Although *Ophryoscolex purkynjei* has been accepted morphologically and phyllogenetically as being one of the most complex ciliate species (14, 24-26), there have been no ultrastructural studies dealing exclusively with the cortex. A comparison between the results obtained in the present study and other findings in the literature is given in Table 1.

The cortex of *Ophryoscolex purkynjei* is described as Type I because it lacks a longitudinal dense ribbon in between the microfilamentous and microtubular layers and so resembles other rumenentodiniomorphid ciliates (Entodiniidae and Ophryoscolecidae) (12,23).

One of the most prominent features is the homogenous agranulated and afilamentous appearance of the inner side of the glycocalyx. This has never been reported in other entodiniomorphid ciliates, so its main role could not be explained.

Populous and very fine epiplasmic material of *Ophryoscolex purkynjei* was observed in the electron micrographs when they were compared with those from previous studies (5, 10-12; 16, 23). THe same layer was described as coarse-granulate in *Entodinium,* filamentous in *Polyplastron* and fibrogranular in *Eudiplodinium* and *Epidinium* (Table 1). Although the epiplasm of *Ophryoscolex purkynjei* appears to be homogenous, the side adjacent to the microtubular layer seems more compact. This observation is in accordance with Grain's (23) opinion that the purpose of the epiplasm is to maintain the shape of the cell, providing strength by fusing through microtubule bundles. The isolation of a special protein of 60 kDa among the microtubule bundles in the genera of *Entodinium, Eudiplodinium, Polyplastron* and *Epidinium* by Grain (23) supports this assessment. It could be clarified by further biochemical observations. THe structural differences, such as superficial cleavages (antimerization) and further spination in several

Table 1. A comparison of the results obtained in the present study with pre-existing knowledge of the ultrastructure of the cortex.

Ciliates	*Entodinium* spp.	*Eudiplodinium* sp.	*Epidiinium* sp.	*Ophryoscolex* sp.
(References)	(12, 23)	(11, 23)	(10, 23)	(Present Results)
Glycocalyx	40-50 nm filamentous	200 nm filamentous	<55 nm particulate or filamentous	25-120 nm two zone: the upper particulate and/or filamentous, the lower lightly stained
Membranes	2	3	3	3
Epiplasm	45-80 nm Two homogenous layers, granulated at different densities	125-400 nm Single homogeneous fibrogranular layer	60-280 nm Single homogeneous fibrogranular layer	135-165 nm In general a single homogenous layer of very fine accumulated granules but apparent with denser internal zone
Microtubular Bundles	65-110 Single layer of various numbers of microtubules	140nm Two layers of 4-6 microtubules	150nm Two layers of maximum 7 microtubules	135-155 nm Two layers of 4-6 microtubules
Microfilamentous Layer	40-70 nm	220 nm	100-200 nm	55-200 nm
Barren Kinetosomes at Subcortical Region	distinct, abundant and in coarsely-organized layers	distinct, abundant and in coarsely-organized layers	distinct, abundant and in coarsely-organized layers	indistinct, small-scale and disperse
Infraciliature components Associated with Barren Kinetosomes	Complete: 3 postciliary microtubules, 3-4 transverse microtubules and one striated fiber	One microtubule extending towards cortex, two basal microtubules and one striated fiber	Two basal microtubules and a striated fiber	Only two basal microtubules

entodiniomorphid ciliates, are accepted in terms of cellular firmness. The presence of vacuoles or vesicles in between the longitudinal microtubule bundles and epiplasm was first reported by Grain (23) in *Polyplastron multivesiculatum.* There is however, no evidence indicating whether these structures arise from the pits on the cell surface, as proposed by Grain (23), or directly from the ectoplasm (central periplasm). It was presumed that the location of these structures adds strength to the cortex and gives the cell its shape.

The presence of septa among the microtubular bundles or packages and the fact that there is a continuation of the barren kinetosomes in the subcortical region (Figure 1) suggest that they are supported by the dense material extending from the epiplasm.

The cortex acts as a toughening and shaping element in the order Entodiniomorphida, from the most primitive form *Entodinium* to the more advanced *Ophryoscolex. Isotricha* and *Dasytricha,* which are members of another primitive order, Trichostomatida, stay successfully within the rumen (10, 23, 27). The degradation of somatic cilia and related infraciliature may not be the result of environmental effects as postulated by Furness & Butler (10, 14). It may be the result of an evolutionary trend towards more complex formation, because the tendency towards less ciliature and increasing complexity is common in most free-living protozoa (28, 29). A comparison of the more scattered and scarce barren and non-ciliated kinetosomes in *Ophryoscolex purkynjei* with those of the simpler genera (*Entodinium, Eudiplodinium, Epidinium,* etc.) supports the assumptions given by Furness & Butler (10, 14).

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