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Paraldehyde Fuchsin Staining and Secretion of Rumen Ciliates of Cattle

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Abstract: Secretory processes in cattle - rumen ciliates have been studied using Paraldehyde Fuchsin (PAF) stain at the light microscope level. It has been shown that while in Entodinimorphida the secretion is primarily in the endoplasm, in Trichostomatida, the secretory products are located in the ectoplasm. The observations obtained in this study have indicated that secretory processes may function in digestion, attachment, and ciliary movement in relation to the metabolic niches that the two ciliate groups occupy in the rumen ecosystem.

Key Words: Rumen Ciliates, Secretion, Paraldehyde Fuchsin Staining.

Sığır İşkembe Siliyatlarında Paralhedit Fuksin Boyaması ve Sekresyon

Özet: Sığır işkembe siliyatlarındaki sekresyon işlemleri, Paraldehit Fuksin (PAF) boyaması kullanılarak ışık mikroskobu düzeyinde çalışılmıştır. Sekresyonun Entodinimorphida'da öncelikle endoplazmada olduğu, Trichostomatida'da ise Salgı ürünlerinin ektoplazmada yerleştiği gösterilmiştir. Çalışmada elde edilen gözlemler sekresyon işleminin, bu grupların işkembede işgal ettikleri metabolik nişleri ile ilişkili olarak sindirimde, yapışma işleminde ve sil hareketinde iş görebileceğini işaret eder.

Anahtar Kelimeler: İşkembe Siliyatları, Sekresyon, Paraldehit Fuksin Boyaması

Introduction

Cilliates which inhabit the rumen of cattle belong to two orders in the subclas Vestibulifera: Trichostomatida and Entodinimorpihda (8, 9). These two ciliate groups are utilized as protein sources by the host animals (5, 7); they also provide volatile fatty acids (VFA) which are released into the rumen as a result of their metabolism.

By the usual light microscopic techniques, the trichostomatid ciliate ectoplasm is usually homogeneous in appearance; whereas, in the entodinimorphids it appears heterogeneous due to the presence of amylopectin reserves, contractile vacuoles, and also partial nuclear material. In the trichostomatid ciliates, these organelles are located within the endoplasm. The endoplasm of entodinimorphid ciliates have a stomach function (9). The ectoplasm and endoplasm are separated by a fibrillar boundary in both groups (3, 9,14).

Ultrastructural studies have revealed some granules and pleomorphic organelles of unknown function in the ectoplasm of trichostomes (3, 10,14) and in the endoplasm of entodinimorphids (11), respectively. Because of their peripheral location, they were thought to be secretory in nature and may have a function in ciliary movement (10).

From the studies conducted recently, it was shown that exocellular carbohydrase (15) protease activities (12, 13) are present in the rumen ciliates. In this way, it could be considered that thay can contribute to the degradation of the host's protein (12, 13). and carbohydrate - containing foods (15). The two ciliate groups in the rumen ecosystem occupy different metabolic "niches." Trichostomes primarily utilize soluble carbohydrates; whereas, the endotinimorphid ciliates, in addition, ingest and ferment particulate material (16, 10).

In this study, we investigated the results of the application of PAF staining procedure, which is used to determine the secretion in insects, to show whether secretion in the rumen ciliates is present or not, to determine the location of possible secretory granules, and to find the direction of secretion in the two ciliate groups.

The paraldehyde fuchsin (PAF) which is extensively used in the study of secretory materials, selectively stains mucopolysaccharides in acidic structures, i.e., acidic musin materials depending on the presence of certain groups (cystein, sulphydryl, aldehydic, and dicarboxylic), since it is fundamentally basic in nature.

Material and Methods

The rumen contents of a Holstein - type cow with a fistulated rumen, weighing about 650 kg, and fed 4 kg of mixed food (composed of 2 kg of oat hay, 1 kg clover, and 1 kg of white beet molasses) twice a day at 08^{00} and 16^{00} hours was used. Samples of rumen liquor were withdrawn 1 hr. after the 08^{00} hr. feed. The ciliates were strained through a double layer of cheesecloth, then selected by capillary pipettes in mixed preparations under a stereo microscope and diluted with an inorganic salt solution (NaCl, 6g; KH_2PO_4, 1g; $NaHCO_3$, 1 g; $CaCl_2$, 0.1 g; $MgSO_4.7H_2O$:, 0.05 g; Aq.d., 1000 cc.) They were fixed with Bouin's and Champy solutions. After microinclusion with 0.13 percent agar, paraffin blocks were prepared and serial sections 4, 5, and 7 µm were obtained. Then PAF staining procedure (4) was applied. For the staining process with basic aldehyde fuchsin, three acidic stains, light green, orange G, and chomotrope - 2R were used. Two types of staining procedures were performed. In the first type only light green was applied with aldehyde fuchsin; whereas, triplet mixture (Halmi Mixture) was carried out in the second type. The sections were examined by Jena "NF - binocular" microscope and Jena "MF" photomicrography accessory.

Results and Discussion

When the PAF was applied to a mixed preparation of the ciliates fixed with Bouin's and especially Champy, some dense granules which stained bluish purple appeared mainly in the ectoplasm and at the level of pellicle of trichostomatid ciliates, *Isotricha* spp., and mainly in the endoplasm of entodinimorphid ciliates (Fig. 1). Also, with this stain the interciliary regions of trichostomatid ciliates and the endoplasm of entodinimorphids were diffusely stained dark purple. This material exhibiting PAF - positive reaction indicates secretory substances. The regions where bluish purple granules accumulated and where the secretory material stained dark purple coincide with each other (Fig. 1). Bluish purple granules were located partially in the endoplasm of trichostomatids and also in the ectoplasm of entodinimorphids. The peresence of granules which stained bluish purple both in the ectoplasm and at the level of pellicle in trichostomatid ciliates strengthens our suggestion that these granules are secretory granules which transport secretory materials to the outside of cells, i.e., interciliary regions. The overlapping positions of the granules and the materials which stained diffusely dark purple in entodinimorphid ciliates (Fig. 1) indicates that the secretion materials are more often released into the endoplasm which serves as a stomach role in this group.

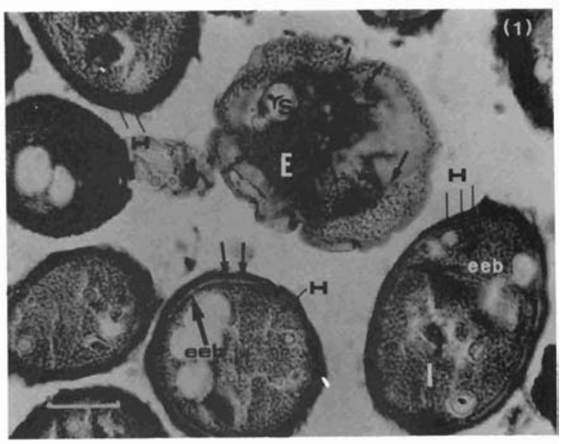

Figure 1. The PAF - Halmi mixture application after Champy fixation (Bar= 30µm). Bluish purple granules (i.e., the secretory granules) (arrows) are seen in the endoplasm of *Entodinium* sp. (E) and in the ectoplasm of *Isotricha* sp. (I) which belongs to Trichostomatida. The secretory material is diffusely stained dark purple, especially in interciliary regions of *Isotricha* sp. and in the endoplasm of *Entodinium* sp. S= Starch grains, eeb= ecto - endoplasmic boundary, H= Hydrogenosomes.

Our staining results show the presence of a developed secretion apparatus and its secretions which are primarily released into to the rumen environment at the level of pellicle in trichostomatids and into the endoplasm in entodinimorphids. The primary release of secretory material to the outside of trichostomatid cells is obviously observed with the interciliary accumulation of dark purple material in thick sections (Fig. 2).

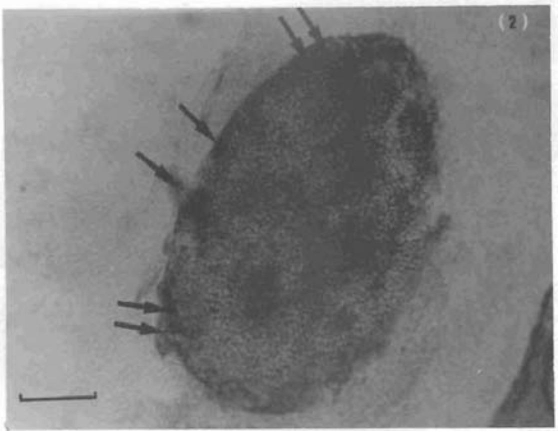

Figure 2. The PAF - Light Green application after being fixed with Bouin's solution in the oblique sections of *Isotricha prostoma* (Bar=20 µm). Arrows indicate dense secretory material in the interciliary regions.

Two types of PAF - positive granules, apart from the bluish purple secretory granules, were also distinguished. The cytoplasmic localization and the morphological size of these granules indicate that they arė hydrogenosomes (the major organelles in carbon metabolism) and amylopectin reserves. Some of these stained light yellow in color (Fig. 1). These granules are localized both in the ectoplasm and endoplasm of Entodinimorphida; whereas, in Trichostomatida they are localized only in endoplasm. This location and also the large starch grains which stained in the same color (Fig. 1) show that these granules are amylopectin reserves. Some other granules are seen in brownish black (Fig. 1, 2). When we take into consideration their cytoplasmic locations and, particularly, their accumulation beneath the ecto - endoplasmic fibrillar boundary in trichostomatids, we are led to think that they are hydrogenosomes.

The aldehyde fuchsin usually applied with Halmi Mixture (which is acidic in nature) stains the ecto - endooplasmic boundary and its derivatives in the color of chromotrope - 2R, i.e., in red (Fig. 1). However, when only light green was applied with aldehyde fuchsin, they are stained green.

The data provided from the above - given staining characteristics and comparison of cytoplasmic localizations of various granules show that the PAF technique can also be used in protozoa to demonstrate the presence of secretory granules or materials, together with fibrillar systems by light microscopy.

When we take into considieariton that the rumen ciliates occupy different metabolic niches in the rumen ecosystem, especially in entodinimorphids, the suggestion that the secretory material may be related to the digestive enzymes is consistent with the cytoplasmic location determined in this study. The interspecific anatgonism (i.e., predator - prey interactions) observed in entodinimorphids (6) and the fact that they can ingest and ferment particulate material in endoplasm (16) supports this suggestion. The pleomorphic organelles which have been determined ultrastructurally in entodinimorphid ciliates (11) and the secretory granules revealed in this work may be identical structures. Since entodinimorphidis probably do not bear a barrier consisting of kineties (= ciliary rows), whether the secretion is directly discharged to the outer environment at the level of pellicle of the cells is not obvious.

On the other hand, it has been shown ultrastructurally that some granular organelles exist in the endoplasm of trichostomatid ciliates (3, 14, 10) and it was discussed that they may be secretory in nature; their peripheral location perhaps indicates a function in cilliary movement (10). Since extracellular material was detected on the surface of the cells and interciliary regions, and also since secretion granules were determined both in the ectoplasm and at the level of pellicle in the present study, this demonstrates that the secretion is derived from these granules. The demonstration of the existence of extracellular carbohydrase (15) and extracellular proteolytic enzymes (12, 13) makes us think that this secretion should be rich in lysosomal hydrolases (15) and act in extracellular digestion. Also, that these organisms show an attachment behavior during in vivo observations (2), indicates that the secretion may be associated with the attachment function (perhaps it contains some activating enzymes for the attachment organelle). On the other hand, the sequestration of protozoa and their attachment to each other in the reticulum(1) supports this idea. In this way, the extracellular secretion in trichostomatids is additionaly thought to be associated with digestion and with a role in attachment to each other and to mucosal epithelium of rumen - reticulum to aid their continuous retention in rumen.

The conclusion is that secretion exists in the rumen ciliates and that this process is towards the outside of the cells from the ectoplasm in trichostamatids and towards the endoplasm, which functions as a stomach, in entodinimorphids. The secretion granules are localized in the ectoplasm of trichostomatids and in the endoplasm of entodinimorphids.

From the ultrastructural studies (3, 10, 16), it is apparent that the developed golgi does not exist in these protozoans. Therefore, the presence of a widespread secretion process in the rumen ciliates indicates that this is performed by endoplasmic reticulum which is developed and widely distributed. It is thought that both ectoplasmic granular end endoplasmic pleomorphic organelles determined ultrastructurally by various investigators, are indentical in structure with the secretion granules revealed in this study.

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