VIA MEDICA

ORIGINAL ARTICLE

Folia Morphol.
Vol. 64, No. 4, pp. 282–286

ISSN 0015–5659
www.fm.viamedica.pl

An electron microscopic study of the phosphatases in the ciliate *Balantidium coli*

Bogumiła Skotarczak[1], Lidia Kołodziejczyk[2]

[1]*Department of Genetics, Szczecin University, Szczecin, Poland*
[2]*Department of Biology and Medical Parasitology, Pomeranian Medical Academy, Szczecin, Poland*

[Received 10 December 2004; Accepted 22 July 2005]

The localisation and activity of D glucose-6-phosphatase (G-6-Pase) and alkaline phosphatase (AlP) in the trophozoites of Balantidium coli *isolated from pig intestine content were investigated using ultrastructural and cytochemical methods. The activity of G-6-Pase was demonstrated on the membranes of the endoplasmic reticulum, particularly in the cortical part of the trophozoites. In addition, the product of the reaction to G-6-Pase was concentrated in the vesicular structures, which were distributed along the reticular membranes. These structures were described as vesicles similar to glycosomes, containing enzymes of glycogenolysis. It is very likely that hydrolases in* B. coli *are formed on the rough reticular membranes without the involvement of cisterns of the Golgi complex. The ultrastructural deposits of the reaction to G-6-Pase and AlP in the trophozoites of* B. coli *described here indicate that some membranes of the rough endoplasmic reticulum and small vacuoles with a strong reaction to these enzymes can play a similar role to the Golgi complex.*

Key words: Ciliata, ultrastructure, phosphatases, cytochemistry

INTRODUCTION

Balantidium coli is the only representative of Ciliata to live as a parasite in humans. The domestic pig is considered the most frequent reservoir for this species and human cases are therefore found mostly in rural areas. Trophozoites of *B. coli* can cause an ulcerative colitis, but the symptoms can be of variable magnitude. The course of balantidiosis has been found to be dependent on the bacterial flora [16]. *B. coli* virulence towards the host's tissues may be a secondary effect of the changes in the biological, chemical, and physical conditions of the parasite's biotope.

Among the species of the genus *Balantidium* there are many fish parasites, and the most recent records show an intestinal symbiont inhabiting the intestines of the surgeonfish from the Tuvalu Islands in the Pacific Ocean [2].

To learn the biology and physiology of trophozoites and cysts of *B. coli* a number of studies based on cytoenzymatic methods have been undertaken, enabling the structures associated with the function and activity of intracellular enzymes to be identified. Phosphatases, including alkaline and acid phosphatase, have been detected with cytoenzymatic methods and employing light microscopy techniques in trophozoites of *B. coli* from *in vitro* cultures [13] and in those isolated from the intestines and tissues of experimentally infected hamsters [4, 14]. The ultrastructural localisation of hydrolases in trophozoites of this protozoan were found only in relation to acid phosphatase [15] and ATP-ase, isolated from intestines of pigs with symptomatic and asymptomatic balantidiosis [19, 20]. Among the results of cytoenzymatic studies of these enzymes were the

Address for correspondence: Prof. dr hab. med. Bogumiła Skotarczak, Department of Genetics, Szczecin University, Al. Piastów 40B, 71–215 Szczecin, Poland, tel: +48 91 444 27 82, fax: +48 91 444 27 80, e-mail: Bogumila_Skotarczak@univ.szczecin.pl

identification of mucocysts and the description of the formation process of primary and secondary lysosomes in this species. On the other hand, the ultrastructural study of peroxidase enabled peroxisomes to be identified for the first time in this species [17].

In this paper we would like to present the of alkaline phosphatase, the marker of the rough endoplasmic reticulum, and D glucose-6-phosphatase, the marker of the smooth endoplasmic reticulum.

MATERIAL AND METHODS

Pig rectum content was obtained from the municipal slaughter house. Trophozoites of *B. coli* were isolated from the caecum content of healthy individuals. The sediment of the intestine content was, after several washes in physiological fluid (0.9% NaCl), subsequently controlled in the light microscope. Suspensions with a plenitude of trophozoites constituted the material for further morphological examination in electron microscopy.

The cells of *B. coli* were washed in 0.9% NaCl, fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer and stored for 24 hours at a temperature of 4°C. After several rinses in cacodylate buffer the trophozoites were fixed in buffered 1% OsO_4 for 2 hours, and than dehydrated in a graded series of ethanol and embedded in Spurr (resin). Ultrathin sections were stained with uranyl acetate and examined under the JEM 1200 EX transmission electron microscope. The material for cytochemical detection was the same as for the morphological studies but without the staining in OsO_4.

D glucose-6-phosphatase (G-6Pase, EC 3.1.3.9) was detected with the Wachstein and Meisel method for electron microscopy [7].

The cytoenzymatic assay for the presence of alkaline phosphatase (EC 3.1.3.1, AlP) was carried out using the Gomori technique in electron microscopic modification [7]. In both cases control reactions were conducted in an incubation medium without substrates.

RESULTS

Observations under an electron microscope of ultrathin sections postfixed with osmium tetroxide enabled to distinguish all the principal structures of the trophozoites of *B. coli,* identified earlier [15, 17–20]. The product of the reaction to G-6-Pase in trophozoites of *B. coli* was observed along the endoplasmic reticulum and in the small vesicular structures (0.1–0.2 μm) situated on the reticular membranes (Figs. 1–3). The reaction product either filled the entire space of these vesicles (Fig. 2) or was concentrated only in their central part (Fig. 3). The product of the reaction to alkaline phosphatase (AlP) was visible in plasmalemma, in cilliary membrane, in kinetosomes, and along the endoplasmic reticulum (Figs. 4–7).

DISCUSSION

Phosphatases are hydrolytic enzymes; in other words they decompose respective substrates into prime ingredients and simultaneously accept water molecules. They catalyse hydrolysis of phosphate esters by releasing inorganic phosphate. The presence of G-6-Pase has been detected in various cells of many species of mammals, reptiles, insects, plants and also micro-organisms. In mammals its presence has been observed in, among other sites, the liver, the epithelial cells of the kidney and the small intestine, haematopoietic cells of the bone marrow, fibroblasts, cells of the smooth muscles, and in cells of the blood vessels [7]. G-6-phosphatase is one of the basic enzymes involved in the metabolism of carbohydrates. In the case of energy demand in cells, glycogen is broken down into glucose-1-phosphate, which is transformed in the cytoplasm into glucose-6-phosphate. The reaction of glucose release from glucose-6-phosphate through the removal of orthophosphate is catalysed by glucose-6-phosphatase. This is the terminal step of glycogenolysis. Large reserves of this enzyme are usually found in the liver cells of mammals in the form of small granules attached to the external surfaces of the smooth endoplasmic reticulum membranes [1]. As a result, glucose-6-phosphatase has been considered a characteristic enzymatic marker of the smooth reticulum. Glucose-6-phosphatase in the membranes of the smooth endoplasmic reticulum is responsible for removal of the phosphate group and glucose transport to the free space within the smooth endoplasmic reticulum. Its activity within the membranes of the reticulum of the rat and within the nuclear envelope was detected with cytoenzymatic methods.

In *B. coli* the product of a reaction to G-6-Pase was observed along the endoplasmic reticulum and in the small vesicular structures (0.1–0.2 μm) situated on the reticular membranes, particularly in the cortical parts of the trophozoites. In some of these vesicular structures the reaction product filled up the entire space, while in others it was concentrated only in their central part. This picture of the granulations evidencing G-6-Pase activity could have been affect-

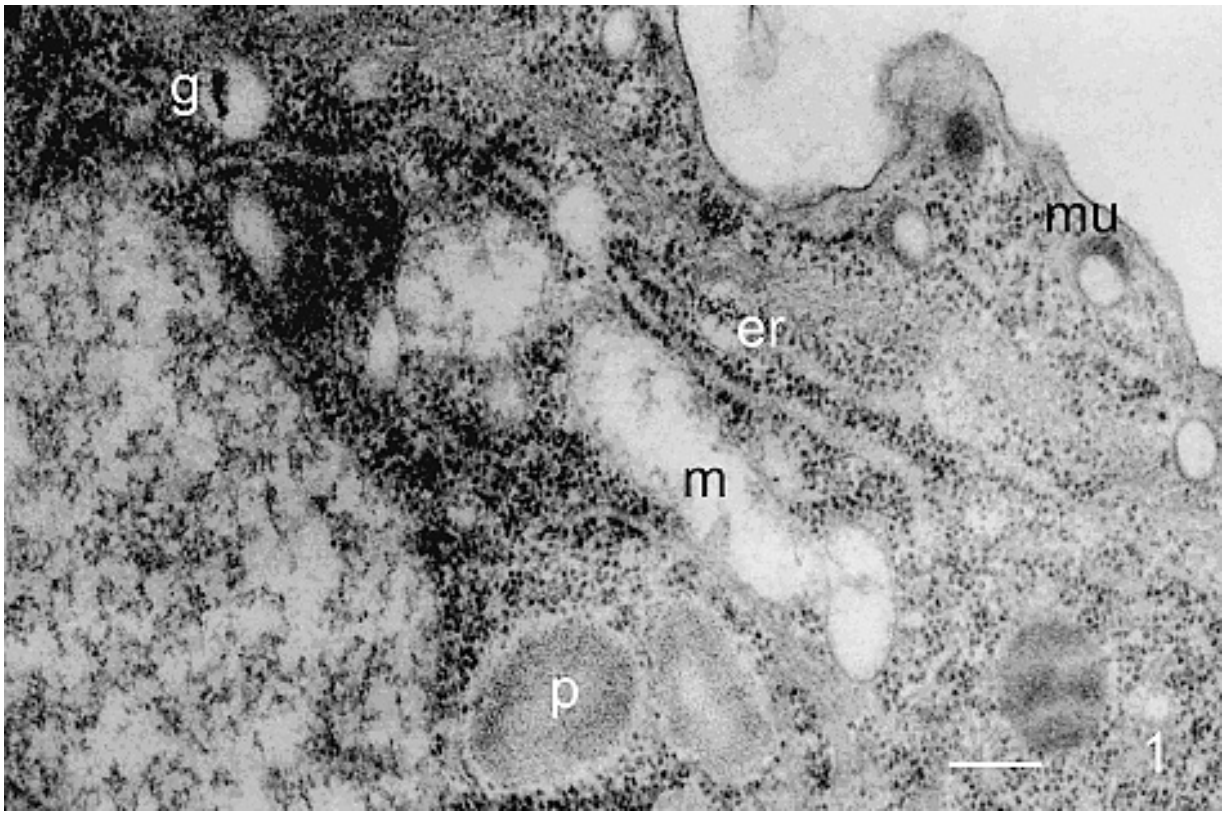


Figure 1. G-6-Pase reaction in the cortical part of *B. coli* trophozoite: endoplasmic reticulum (er) and glycosome (g) show the presence of phosphatase reaction product. Mitochondria (m), mucocyts (mu) and peroxisomes (p) are also visible. Bar = 0.1 μm.

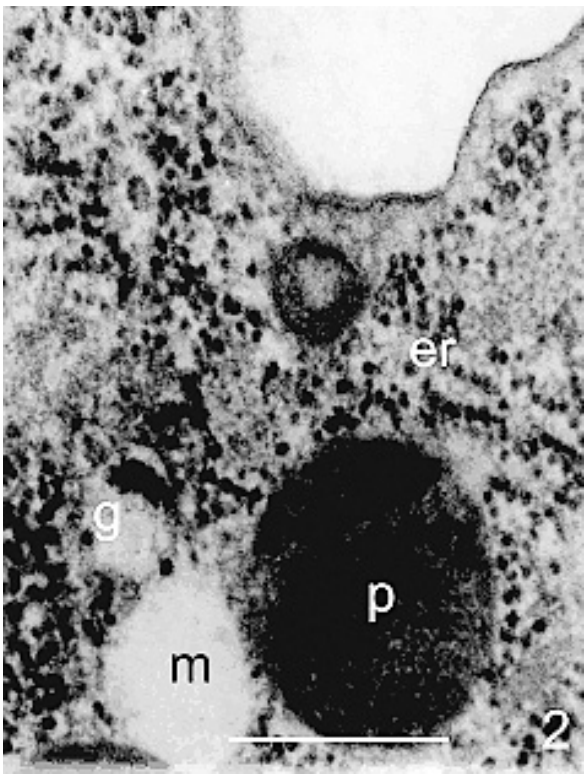


Figure 2. G-6-Pase reaction in the cortical part of *B. coli* trophozoite: glycosome (g), endoplasmic reticulum (er) and cytoplasma show reaction product. Mitochondrium (m) and peroxisomes (p) are also visible. Bar = 0.1 μm.

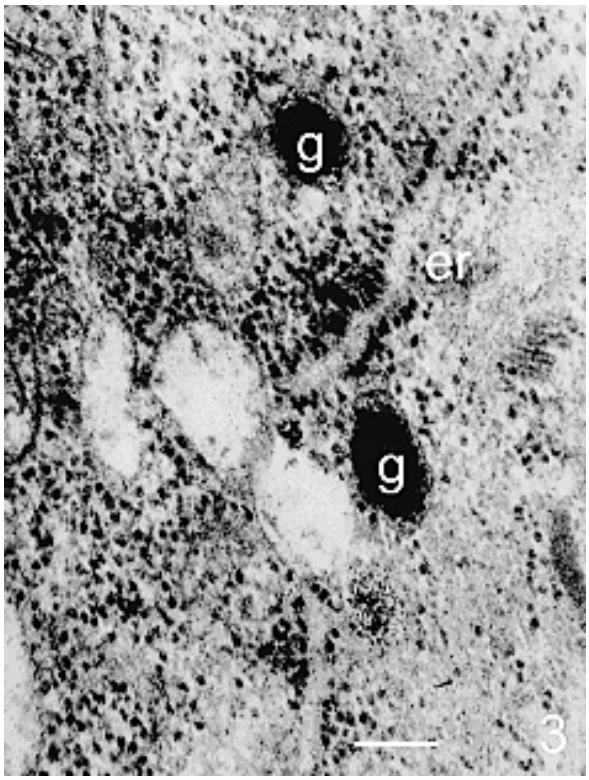


Figure 3. The product of G-6-Pase reaction in glycosome (g) and in endoplasmic reticulum (er). Bar = 0.1 μm.

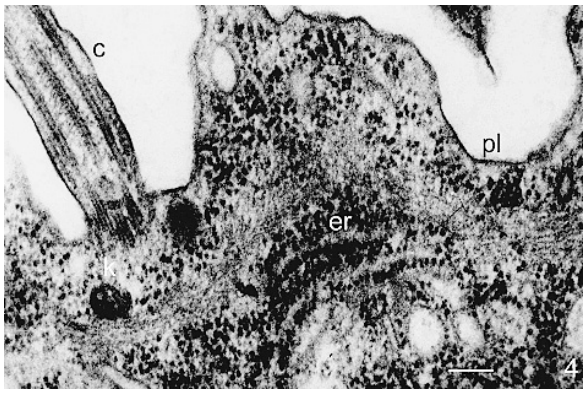


Figure 4. AlP reaction in the cortical part of *B. coli* trophozoite: plasmalemma (pl), endoplasmic reticulum (er), cytoplasma, membrane of cilia (c) and kinetosomes (k) show the presence of the phosphatase product of reaction. Bar = 0.1 μm.

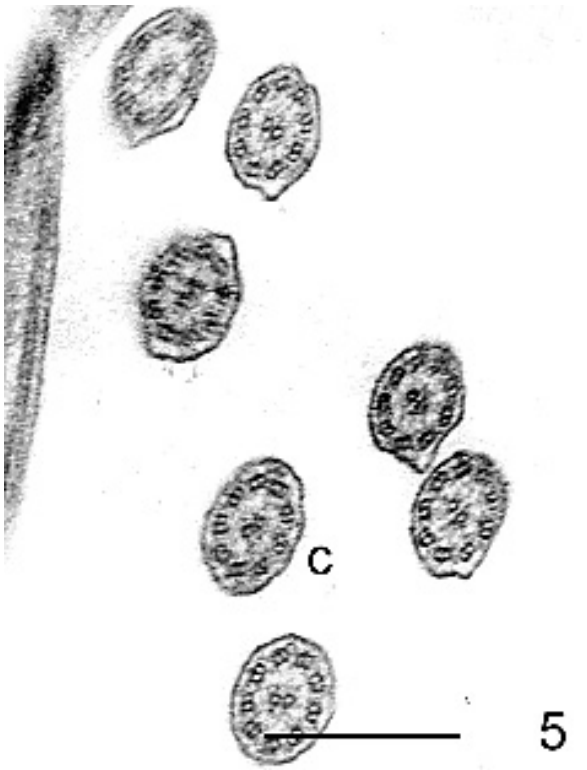
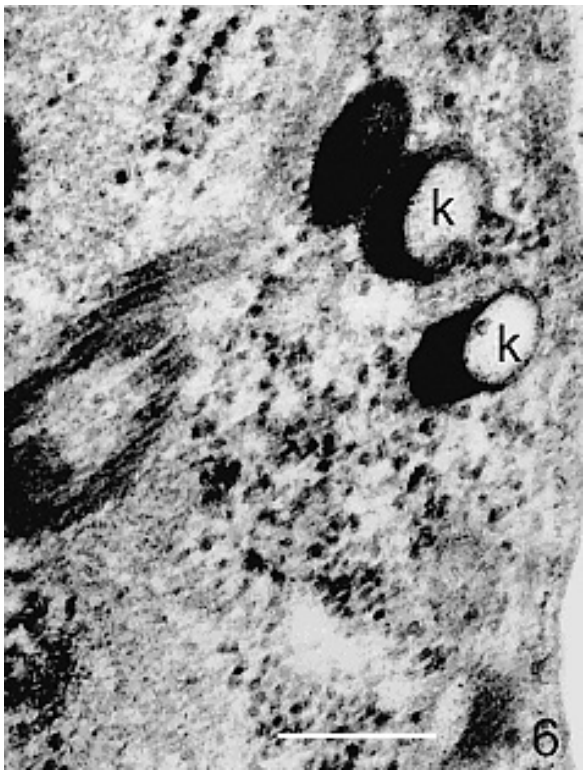
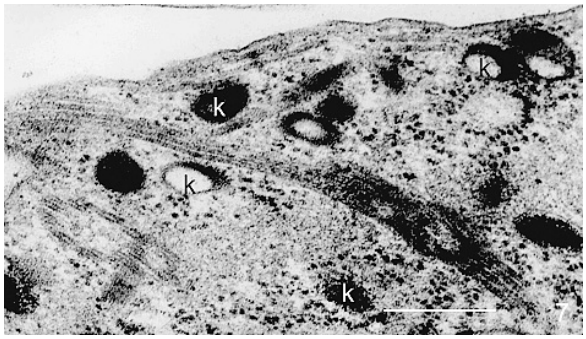


Figure 5. Membrane of cilia (c) shows the presence of AlP product of the reaction. Bar = 0.2 μm.





Figures 6 and 7. The product of AlP is visible in cytoplasma and in kinetosomes (k) of *B. coli.* Bar = 0.1 μm.

ed by the image with different cross-sections planes of the vesicles.

Protozoans of the genus *Trypanosoma*, a particular kind of microbody, were found to contain a system of glycolysis enzymes. Those which did not show the presence of catalase or peroxidase were labelled glycosomes [9, 11]. Their size was the same as the size of the vesicles observed in trophozoites of *B. coli* with the reaction product to G-6-Pase and therefore they may be structures analogous to glycosomes but containing enzymes of glycogenolysis. Because the process catalysed by G-6-Pase, consisting of glucose phosphorylation, is involved in the distribution of glucose in a cell, it is possible that its presence requires specialised cell structures. Moreover, some structures that are very similar in size and shape have previously been found and, because they exhibited acid phosphatase activity, were considered primary lysosomes [19].

Alkaline phosphatase is one of the enzymes of the heterogeneous hydrolytic enzyme group active

in an alkaline environment. Its activity is observed not only in the membranes of the nucleus, but also in the Golgi complex [5]. A role in cellular transport and in the processes of proliferation and differentiation of cells has therefore been attributed to it [12]. In earlier, light microscope, studies of *B. coli* trophozoites, the reaction to AlP was localised in the nucleus and in the area of the endoplasmic reticulum [13]. In the present study, in the images obtained with the aid of electron microscopy, a distinct reaction to AlP was visible in the plasmalemma, in the membrane of kinetosomes and in the cilia. Quantitative studies showed a concentration of alkaline phosphatase 6 times greater in the cilia of *Paramecium tetraurelia* than in the cell bodies [6]. A similar location of the product of cytochemical reaction to AlP was observed in the trophozoites of *Entamoeba histolytica* [8]. The product was located in, among other sites, the plasmalemma, in cytoplasmic structures (0.2 μm diameter) and in packets labelled as subpellicular bodies, and in *Hartmanella castellani* in the vicinity of the nucleus and reticular membranes [3]. Studies in *Paramecium* cells have also shown that alkaline phosphatase is involved in membrane fusion during exocytosis [10].

Hydrolytic enzymes are synthesised on the membranes of the rough endoplasmic reticulum and are subsequently transported to the cisterns of the Golgi complex. No Golgi structures have ever been found in *B. coli*. The unique deposit of the reaction to G-6-Pase and AlP described in the present paper indicates that some membranes of the endoplasmic reticulum and small vacuoles with a strong reaction to those enzymes can play an analogous role in this species to the Golgi complex in Metazoa. Similar structures were observed in *Entamoeba histolytica* [8]. Moreover, structures which were very similar in size, shape, and localisation have previously been found in trophozoites of *B. coli* labelled primary lysomes because they demonstrated the activity of acid phosphatase [20].

REFERENCES

1. De Jong A, Van Dujin P, Daems W (1976) Cytochemical model system for microsomal rat liver glucose-6--phosphatase. J Histochem Cytochem, 24: 643–651.
2. Grim JM, Clements KD, Byfield T (2002) New Species of *Balantidium* and *Paracichlidotherus* (Ciliophora) inhabiting the intestines of four surgeonfish species from the Tuvalu Islands, Pacific Ocean. J Eukaryot Microbioll, 2: 146–153.
3. Hryniewiecka-Szyfter Z, Kuźna-Grygiel W (1970) Histochemical localization of acid and alkaline phosphatases in throphozoites and cysts of amoeba *Hartmanella castellanii* Neff Bull Soc Sc Lettr (Poznań), 11: 31–38.
4. Humiczewska M, Skotarczak B (1984) The influence of *Balantidium coli* (Malmsten) on liver's morphology and metabolism of *Mesocricetus auratus* (Waterhouse). Wiad Parazytol, 5–6: 575–583.
5. Kierek-Jaszczuk D (1981) Heterogeneity of mammalian alkaline phosphatase (in Polish). Post Biochem, 27: 217–264.
6. Klumpp S, Schultz JE (1990) Alkaline phosphatase from *Paramecium* cilia and cell bodies: purification and characterization. Biochim Biophysis Acta, 1037: 233–239.
7. Krygier-Stojałowska A, Godlewski H (1982) Topochemiczne metody badań komórek i tkanek. Państwowe Wydawnictwo Naukowe, Warszawa, pp. 628–648.
8. Kuźna-Grygiel W (1994) Ultrastructural localization of enzymes in *Entamoeba histolytica.* I. Acid phosphatase, alkaline phosphatase and thiamine pyrophosphatae. Acta Protozool, 33: 233–238.
9. Lazarov PB, Fujiki Y (1989) Biogenesis of peroxisomes. Ann Rev Cell Biol, 1: 489–530.
10. Lumpert CJ, Kersken H, Gras U, Plattner H (1987) Screening of enzymatic mechanisms possibly involved in membrane fusion during exocytosis in *Paramecium* cells. Cell Biol Int Rep, 11: 405–414.
11. Opperdoes FR (1988) Glycosomes may provide clues to the import of peroxisomal proteins. TIBS, 13: 255–260.
12. Pearse AGE (1972) Histochemistry: theoretical and applied, vol. II, Churchill Livingstone, Edinburgh, pp. 808–826.
13. Skotarczak B (1980) Activity of phosphatases in *Balantidium coli.* I. Folia Biol (Kraków), 28: 63–67.
14. Skotarczak B (1986) Cyto- and histochemical studies on the enzymes activity in *Balantidium coli* (M) and *Mesocricetus auratus* (W) in experimental balantidiosis. I. Phosphatases. Acta Parasitol Pol, 29: 275–288.
15. Skotarczak B, Leonaldi R (1986) Localizzazione ultrastrutturale della fosfatasi acida in *Balantidium coli* (Malmsten). Esperienze di Medicina e Chirurgia, 2: 109–118.
16. Skotarczak B (1997) Bacterial flora in acute and symptom-free balantidiosis. Acta Parasitol, 42: 230–233.
17. Skotarczak B (1997) Ultrastructural and cytochemical identification of peroxisomes in *Balantidium coli,* Ciliophora. Folia Biol (Kraków), 45: 117–120.
18. Skotarczak B (1997) Ultrastructure of the nucleus system of *Balantidium coli.* J Protozool Res, 7: 127–132.
19. Skotarczak B (1999) Cytochemical identification of mucocysts in *Balantidium coli* trophozoites. Folia Biol (Kraków), 47: 61–65.
20. Skotarczak B (1999) The formation of primary and secondary lysosomes in *Balantidium coli*, Ciliata. Folia Histochem Cytobiol, 37: 261–265.