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Ultrastructure of the salivary glands in *Lithobius forficatus* (Myriapoda, Chilopoda, Lithobiidae) according to seasonal and circadian rhythms

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ABSTRACT

The salivary glands (mandibular epidermal glands) of adult males and females of *Lithobius forficatus* (Myriapoda, Chilopoda) were isolated during spring, summer and autumn. In addition, the organs were isolated at different times of the day – at about 12:00 (noon) and about 00:00 (midnight). The ultrastructure of these organs depending on seasonal and circadian rhythms was analyzed using transmission and scanning electron microscopy and histochemical methods. The paired salivary glands of *L. forficatus* are situated in the vicinity of the foregut and they are formed by numerous acini that are surrounded by the fat body, hemocytes and tracheolae. The salivary glands are composed of a terminal acinar component and a system of tubular ducts that are lined with a cuticle. The glandular part is composed of secretory epithelial cells that are at various stages of their secretory activity. The saliva that is produced by the secretory cells of the acini is secreted into the salivary ducts, which are lined with a simple epithelium that is based on the non-cellular basal lamina. The ultrastructural variations suggest that salivary glands function differently depending on seasonal rhythms and prepare the animal for overwintering. Therefore, the salivary glands of the centipedes that were analyzed participate in the accumulation of proteins, lipids and polysaccharides during the spring, summer and autumn. Subtle differences in the ultrastructure of the secretory cells of the salivary glands during the circadian cycle must be related to the physiological reactions of the organism. The salivary ducts showed no differences in the specimens that were analyzed during the day/night cycle or during the seasonal cycle.

1. Introduction

Besides processing food, the digestive system of myriapods and other terrestrial arthropods is the first line of defense against toxic substances and plays a role in the elimination of pathogens and other harmful materials that originate from food. Toxic metals and reserve material are accumulated in the digestive epithelia in myriapods (Lewis, 1965, 1981; Hopkin et al., 1985; Hopkin and Read, 1992; Fontanetti et al., 2001, 2015; Chajec et al., 2012). The salivary glands of myriapods, which are organs that belong to the digestive system, take part in the above-mentioned processes, together with the synthesis, accumulation and secretion of many substances, e.g. enzymes (Lewis, 1981). Because they synthesize enzymes (e.g. amylase, phosphatase, invertase), the salivary glands are responsible for the preliminary digestion. The salivary glands of arthropods play an important additional role in osmotic regulation and are also involved in the secretion of anticoagulants (Coons and Roshdy, 1973). Therefore, it is a good study model for analyzing all kinds of changes that occur according to any factors, stressors or even their circadian and seasonal rhythms. However, four types of head epidermal glands have been described in centipedes: buccal, mandibular and the first and second maxillary glands. In the majority of myriapods, the second maxillary glands have no contact with the digestive system and do not play the role of salivary glands, while the remaining glands are joined to the alimentary canal and fulfill the role of the salivary glands (Desbalmes, 1992; Hilken and Rosenberg, 2006; Rosenberg et al., 2011).

Centipedes are nocturnal animals that hunt and feed during the night, while they hide under stones, dried leaves, barks or in crevices to avoid the light during the day (Lewis, 1981; Hopkin and

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Read, 1992; Minelli, 1993, 2011; Brusca and Brusca, 2003). This means, as it does in many invertebrates, that numerous processes in their body are linked to the day/night cycle (Bano and Krishnamoorthy, 1979; Andersson, 2006; Tuf et al., 2006). These circadian cycles of activity play an important role in the maintenance of homeostasis in the organism for example. Although most of the data concerning the circadian and seasonal rhythms primarily originate from studies on vertebrates (Lee and Sancar, 2011a), most studies on the circadian and seasonal rhythms in myriapods have been conducted on two centipedes – *Lithobius forficatus* and *Scolopendra cingulata,* in which the digestive epithelia of their midgut was main target (Chajec et al., 2012, 2014; Rost-Roszkowska et al., 2015, 2016). The physiological reaction to the length of the day and night cycle can affect cell proliferation and tissue/organ regeneration, the synthesis and secretion of many substances, etc. It can be demonstrated that the digestive system in insects together with the nervous system functions as an internal biological clock that controls numerous physiological processes (e.g. feeding, hormone synthesis, reproduction, etc.) (Wang et al., 2013; Park et al., 2013). Although we can find information regarding the digestive epithelia of myriapods and their functioning in relation to the circadian rhythm in the literature (Chajec et al., 2012, 2014; Rost-Roszkowska et al., 2015, 2016), data related to the functioning of the secretory epithelia in relation to seasonal and circadian rhythms, are rather scarce. Therefore, we decided to analyze the organization and ultrastructure of the salivary glands of an overwintering centipede – *L. forficatus*, as it is a species that is easy to collect, easy to breed and is common in the European fauna. We present changes that occur in the secretory epithelium (salivary glands) in relation to the day/night cycle and seasonal rhythms. *L. forficatus* is mainly active during the summer season; it begins its activity during spring and it prepares the organism for overwintering during autumn.

2. Materials and methods

2.1. Materials

Adult specimens of *L. forficatus* were collected in forests, gardens and yards in southern Poland – Katowice (19°00′E, 50°15′N) and Żywiec (19°12′E, 49°42′N).

The salivary glands were isolated from adult males and females during three seasons – spring, summer and autumn (during the winter centipedes are hidden deeply in the soil where they hibernate). Additionally, the organs were isolated at different times of the day – at about 12:00 (noon) and about 00:00 (midnight). The temperatures were typical for Poland – spring (about 20 °C), summer (about 25 °C) and autumn (about 15–18 °C). The material was prepared for the analysis using the histological and histochemical methods described in Table 1.

2.2. Methods

2.2.1. Scanning electron microscopy (SEM)

Salivary glands were isolated from males and females (Table 1) and fixed with 2.5% glutaraldehyde in a 0.1 M sodium phosphate buffer (pH 7.4, 4 °C, 2 h), postfixed in 2% osmium tetroxide in a 0.1 M phosphate buffer (4 °C, 30 min), dehydrated in a graded series of concentrations of ethanol (50%, 70%, 90%, 96%, 100%, each for 15 min), critical-point-dried using a Pelco CPD 2 and finally sputter-coated with gold using a Pelco SC6. Morphological analysis and imaging was performed using a Hitachi UHR FE-SEMSU 8010 scanning electron microscope.

2.2.2. Light microscopy and transmission electron microscopy (TEM)

Adult specimens of *L. forficatus* (36 males, 36 females) (Table 1) were decapitated and fixed with 2.5% glutaraldehyde in a 0.1 M sodium phosphate buffer (pH 7.4, 4 °C, 2 h), then the material was postfixed in 2% osmium tetroxide in a 0.1 M phosphate buffer (4 °C, 1.5 h). After washing in a 0.1 M phosphate buffer (2 × 15 min), the specimens were dehydrated in a graded series of ethanol (30%, 50%, 70%, 90%, 96% and 4 × 100%, each for 15 min), acetone (2 × 15 min) and embedded in epoxy resin (Epoxy Embedding Medium Kit; Sigma). Semi- and ultra-thin sections were cut on a Leica Ultracut UCT25 ultramicrotome. Semi-thin sections (0.8 μm thick) were stained with 1% methylene blue in 0.5% borax and observed using an Olympus BX60 light microscope. After staining with uranyl acetate and lead citrate (each for 20 min), ultra-thin sections (70 nm) were examined using a Hitachi H500 transmission electron microscope.

2.2.2.1. Histochemical methods for TEM

2.2.2.1.1. Detection of carbohydrates. The salivary glands from adult specimens (two males, two females at each season – Table 1) were prepared following the standard method for transmission electron microscopy described above. Epon blocks were cut on a Leica Ultracut UCT25 ultramicrotome. Ultra-thin sections (70 nm) were stained with 1% periodic acid (20 min, room temperature) and then washed in distilled water. Finally, the sections were transferred to a solution of 0.2% thiosemicarbazide for 20 min (room temperature) and washed in 10% acetic acid (Martínez et al., 2014). The material was examined using a Hitachi H500 transmission electron microscope.

2.2.2.1.2. Detection of mucopolysaccharides (ruthenium red staining). Fragments of the salivary glands that were isolated from

Table 1
Diagrammatic representation of the collection of adult specimens of *L. forficatus*.

Season of the year and time of day/night	Number of specimens analyzed		
	SEM	TEM + LM	Histochemical methods TEM
Autumn (October–November) noon (about 12:00)	2 males 2 females	6 males 6 females	2 males 2 females
Autumn (October–November) midnight (about 00:00)	2 males 2 females	6 males 6 females	2 males 2 females
Spring (April–June) noon (about 12:00)	2 males 2 females	6 males 6 females	2 males 2 females
Spring (April–June) midnight (about 00:00)	2 males 2 females	6 males 6 females	2 males 2 females
Summer (July–September) noon (about 12:00)	2 males 2 females	6 males 6 females	2 males 2 females
Summer (July–September) midnight (about 00:00)	2 males 2 females	6 males 6 females	2 males 2 females

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