Age-dependent morphology and ultrastructure of the thoracic labial gland of *Apis mellifera carnica* Pollm. workers (Hymenoptera, Apidae)

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ABSTRACT. Exocrine glands are well developed in social Hymenoptera and play an important role in the social life of these insects. We studied the thoracic labial gland of *Apis mellifera carnica* Pollm. in relation to age. We observed two cell types in the secretory tubes of the thoracic salivary gland : parietal and secretory cells. In the secretory cells, two types of secretory vesicles occur. Abundant mitochondria, vesicles and apical microvilli indicate that parietal cells also have a secretory function. The size of the subcuticular space increases with age, probably by filling with secretion. We found differences in the numbers of secretory vesicles between summer and winter bees. Probably, these vesicles accumulate in winter bees, and glands do not discharge their secretion until spring.

KEY WORDS : honeybees, exocrine glands, thoracic labial gland, morphology, ultrastructure.

INTRODUCTION

A very striking characteristic of social insects is the extent and development of the exocrine system (BILLEN & MORGAN, 1998). These glands play an important role in the social life of these insects, e.g. maintaining task regulation, which is mainly based on age in worker honeybees. Age-dependent changes in exocrine glands can be expected, because of changing functions. Preliminary studies described these morphological changes in e.g. the wax gland (CRUZ LANDIM, 1963) and the hypopharyngeal gland (CRUZ LANDIM & HADEK, 1969), but only for functional task groups.

Another exocrine gland is the labial or salivary gland that appears in all Hymenoptera and is situated in the ventral part of the thorax, with ducts fusing in the head and opening near the labium (SNODGRASS, 1956). The thoracic part of the labial gland of the honeybee belongs to the tubular type (CRUZ-LANDIM, 1973). The secretory cells are of the epithelial type (NOIROT & QUENNEDEY, 1974; BILLEN, 1991). There is also a pair of postcerebral labial glands in the head of the honeybee, which are unique for the Apidae (SNODGRASS, 1956). The thoracic labial gland in the honeybee is well described by SCHÖNITZER & SEIFERT (1990), but not with respect to age.

This paper forms part of a general study that examines the age-dependent morphological and ultrastructural characteristics of the exocrine glands in the honeybee, of which we here focus on the thoracic labial gland.

MATERIAL AND METHODS

Workers of *Apis mellifera carnica* Pollm. were studied. The queen was placed under arrest on two combs to get a concentration of brood, each week on different combs for three weeks. We let workers emerge once a week in a breeding box, after which we marked the callow bees with different colours to know their age. Winter bees were taken on the combs, their age is not exactly known, but was at least two months. Three workers of each age were dissected in Ringer solution (Jolly), fixed in 2% glutaraldehyde buffered with 0.05 M sodium cacodylate. One percent osmium tetroxide was used for postfixation and was followed by dehydration in acetone and embedding in Araldite.

Semi-thin sections (1 µm) for light microscopy were made with a Reichert OmU2 microtome and stained with methylene blue and thionine. Thin sections (70 nm) for electron microscopy were made with a Reichert Ultracut E microtome and double-stained with uranyl acetate and lead citrate. They were examined with a Zeiss EM900 electron microscope.

Samples for scanning microscopy were dehydrated in formaldehyde dimethyl acetal, after which the glands were critical point dried. They were coated with gold and viewed with a Philips SEM 515 microscope.

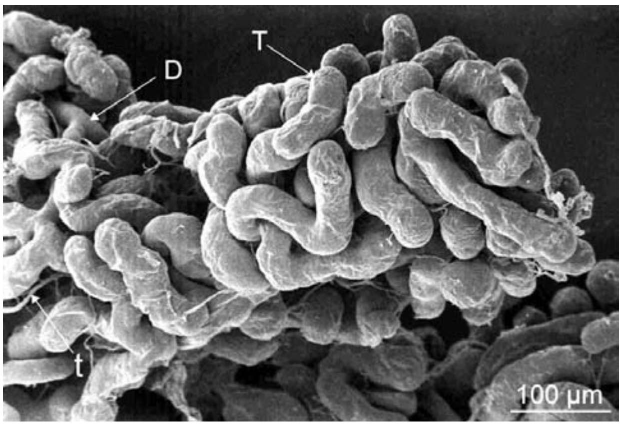

Fig. 1. – Scanning micrograph of the thoracic labial gland in a *A. m. carnica* worker. D : duct; T : secretory tube; t : trachea.

RESULTS

The thoracic labial gland of the honeybee is situated in the ventral anterior part of the thorax. The gland is paired, with two units in each part : a big median and internal lobe and a smaller external lobe. Each lobe consists of a dense cluster of secretory tubes and channels that are sometimes branched (Fig. 1). In this mass we distinguished secretory tubes and collecting tubes with a gradual change between them. Cross sections of secretory tubes show three to five epithelial cells surrounding a central lumen, which is lined with a cuticle with taenidiae (Fig. 2). The region between the gland epithelium and the cuticle is called the subcuticular space (SCHÖNITZER & SEIFERT, 1990). The apical part of the epithelium is very irregular. The cell membrane displays microvilli, which create a surface enlargement to enable a higher secretory capacity. A subcuticular space is also seen between cells. The epithelium contains two types of cells : secretory and parietal cells (SCHÖNITZER & SEIFERT, 1990). The parietal cells are only seen at the periphery (Fig. 3F). They are smaller and more elongated than secretory cells, and have clear vesicles and a high number of mitochondria. The apical cell membranes of the parietal cells appear as a microvillar border. The cytoplasm is not as electron-dense as in the secretory cells. We observed conspicuous basal invaginations in the gland epithelium.

The ducts are clearly distinguishable from the secretory tubes, the cuticle also has taenidiae and is surrounded by epithelial cells, but there is no subcuticular space. These cells have large basal invaginations and apical microvilli with electron-dense zones, which appear to be hemidesmosomes. The basal invaginations become bigger when workers get older. The cytoskeleton is well-developed and microtubules are clearly visible. There is an abundance of mitochondria in the cytoplasm.

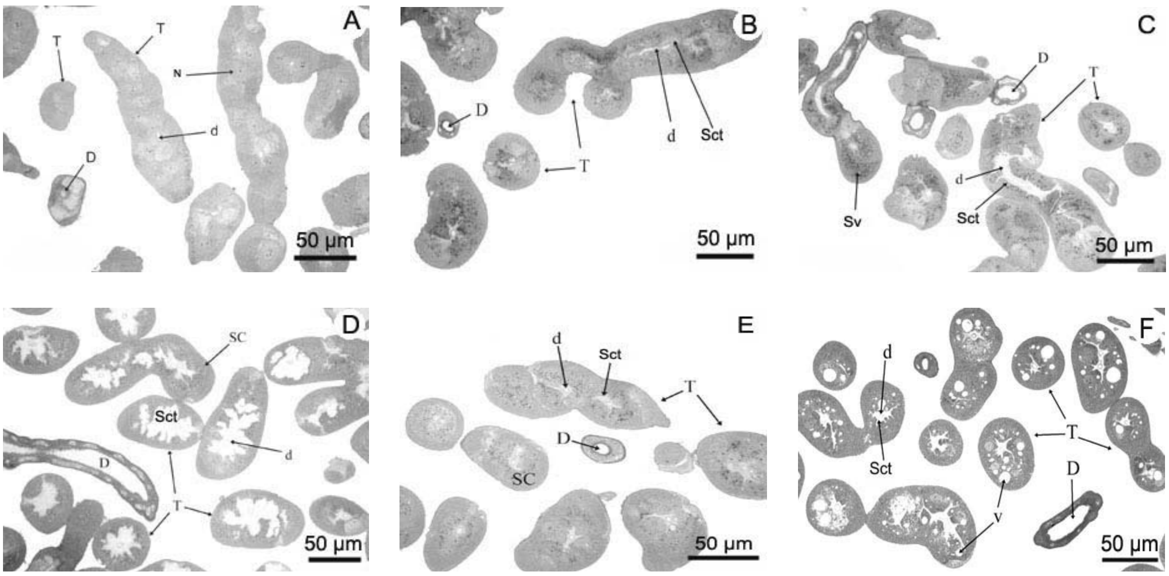

Fig. 2. – Semithin light micrographs of the thoracic labial gland in *A. m. carnica* workers. (A) newly emerged; (B) 3 days old; (C) 9 days old; (D) 15 days old; (E) 24 days old; (F) winter bee. D : duct; d : central duct; N : nucleus; SC : secretory cell; Sct : subcuticular space; Sv : secretory vesicle; T : secretory tube; V : vesicle.

Age-dependent observations

In newly emerged worker bees, we did not see secretory vesicles in the cytoplasm nor secretion in the ducts. The subcuticular space is very small. The nucleus contains several nucleoli. The cytoplasm of the gland cell contains an abundance of mitochondria, mainly between the basal invaginations. Mitochondria are also seen in the apical cytoplasm. Tubular granular endoplasmic reticulum (RER) and free ribosomes are mainly found in the apical region, as well as a well-developed Golgi-apparatus (Figs. 2A – 3A).

At an age of three days we observed secretory vesicles in the apical part of the cytoplasm. The subcuticular space has not grown. Again we see mitochondria at the basal part of the cytoplasm, but larger in size (approx. 0.6 µm). There are electron-dense vesicles visible apically in the cytoplasm. No vesicles are seen in the basal part of the cell. The RER is mainly vesicular and is observed throughout the cytoplasm. A thick (approx. 0.25 µm) basement membrane borders the gland (Figs. 2B-3B).

At an age of six days, the apical side of the gland epithelium is more irregular and the subcuticular space bigger, probably because of accumulation of secretion within it.

At an age of nine days, secretory vesicles are not only seen in the apical part but throughout the cytoplasm, with an increasing density towards the apical side of the gland cell. Two types of gland cells can be distinguished according to the electron density of their cytoplasm. In both types we observed approximately the same number of secretory vesicles in the apical part. Clear vesicles are also seen in both types of gland cells (Figs. 2C-3C). At an age of 12 days the RER is vesicular. The mitochondria on the basal side are directed in a basal-apical direction. Large and clear vesicles are grouped.

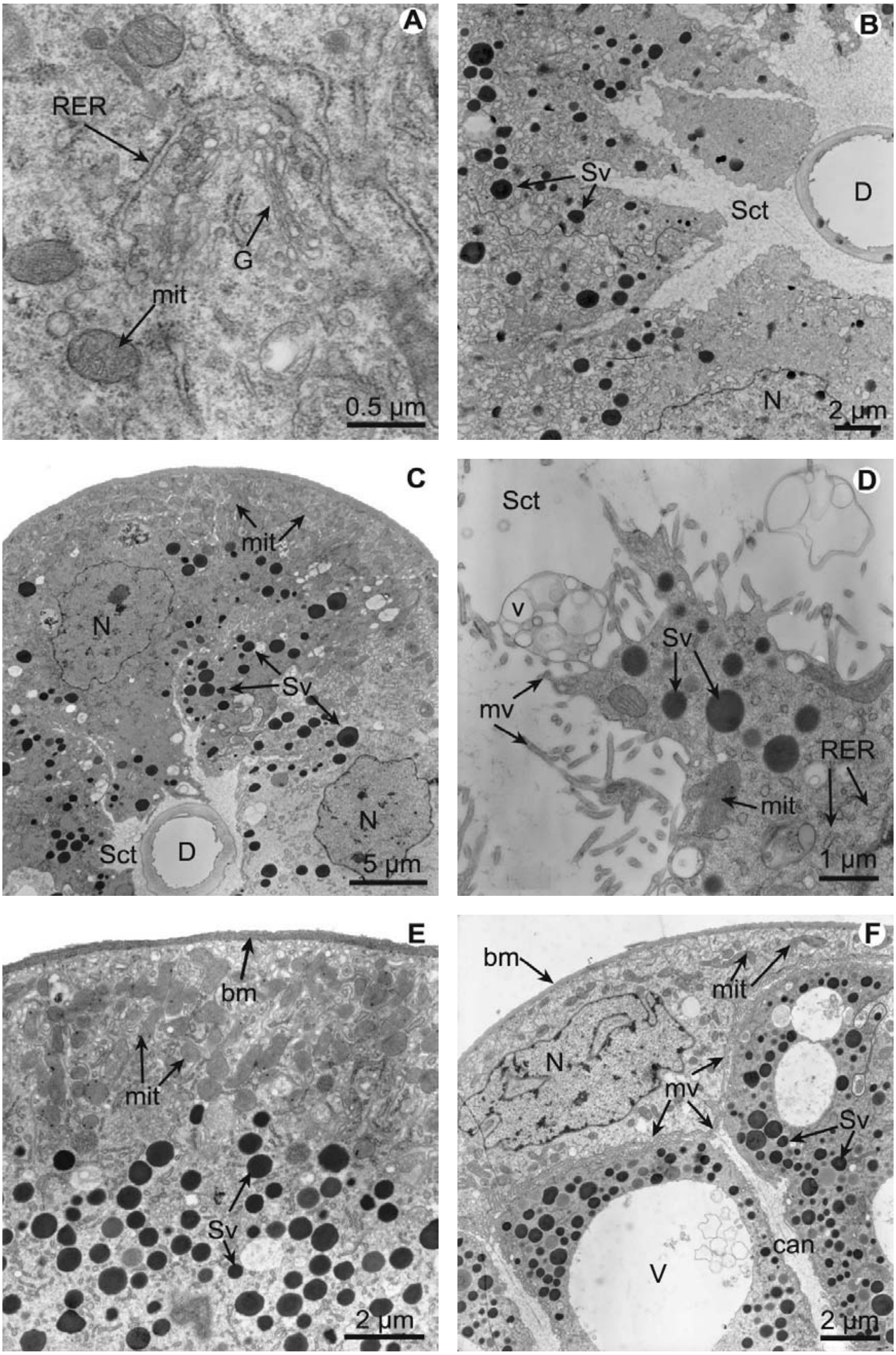

Fig. 3. – Transmission electron micrographs of the thoracic labial gland in *A. m. carnica* workers. (A) newly emerged, detail of cytoplasm; (B) 3 days old, apical side of epithelium; (C) 9 days old, 2 types of secretory cells; (D) 15 days old, apical side of epithelium; (E) 33 days old, basal side of epithelium; (F) winter bee, parietal cell and basal side of epithelium. bm : basement membrane; can : canalicular system; D : duct; G : Golgi apparatus; mit : mitochondria; mv : microvilli; N : nucleus; RER : granular endoplasmic reticulum; Sct : subcuticular space; Sv : secretory vesicle; V : vesicle.

At an age of 15 days, a decrease is seen in the thickness of the gland epithelium. A consequence is an increasing subcuticular space, probably filled with secretion. The canalicular system between the cells is now part of the subcuticular system. The microvilli on the apical side of the gland epithelium are well-developed. In the subcuticular space there are groups of vesicles near the microvilli of the apical part of the gland cell. These probably originate from the cytoplasm. Mitochondria are more electron-dense than before and have a changed internal structure; the cristae are no longer parallel (Figs. 2D-3D). At an age of 18 days the subcuticular space has become smaller. The RER in the cytoplasm of the secretory cells is mainly vesicular. At an age of 21 days the RER becomes more reticular again and at an age of 24 days the RER is tubular. We observed fewer secretion vesicles. No change occurs after this age (Figs. 2E-3E).

In winter bees we saw electron-dense secretory vesicles in the apical part of the secretory cells, as in summer bees. We observed clear spherical inclusions of a different size, scattered over the cytoplasm. Sometimes these inclusions can occupy half of the gland cell. Differences occur between cells in their electron density. The nuclei have a spherical form (Figs. 2F-3F).

No changes were observed in diameter of the secretory tubes or in thickness of the epithelium (Figs. 4-5).

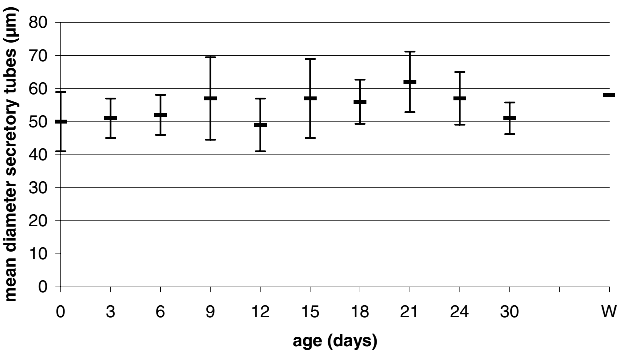

Fig. 4. – Mean diameter of secretory tubes of thoracic labial gland with respect to age (n=3). W : winter bees.

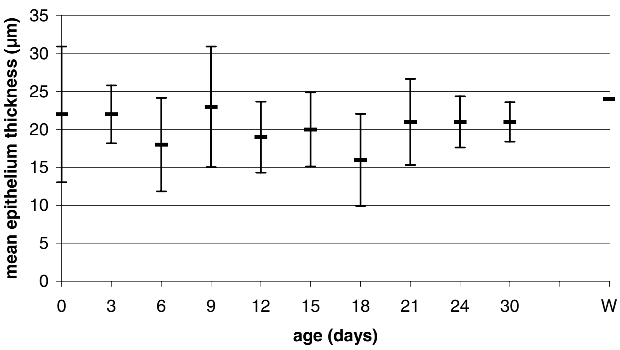

Fig. 5. – Mean epithelium thickness of thoracic labial gland with respect to age (n=3). W : winter bees.

DISCUSSION

The cytoplasm of the duct epithelium contains numerous mitochondria, probably because of an active transport function. Changes in the size of the basal invaginations with age are probably linked to this transepithelial transport. In the secretory tubes, the parietal cells contain many mitochondria, which show great activity. The fact that these cells contain many vesicles and apical microvilli, which are linked with the canalicular system, suggests a secretory function. It may also be that they have a special function regulating the concentration of ions in the saliva, or that secretory cells and parietal cells produce different components of the secretion (SCHÖNITZER & SEIFERT, 1990).

In the secretory cells we see little variation in the amount of RER, mitochondria and secretory vesicles with respect to age, but in correspondence with the observations of SCHÖNITZER & SEIFERT (1990), we see a different electron density in the cytoplasm of the secretory cells in the same section. Probably the secretory cycle is not synchronous in all cells. Two types of secretory vesicles with different electron density are found. The production of these two types is independent of age and physiological condition of workers. This corresponds with functions of the gland that are needed over the whole life span, such as production of metabolic enzymes. The secretion is a proteinaceous solution and has invertase activity (CRUZ-LANDIM, 1973). This enzyme catalyses the conversion of nectar to honey. Also other enzymes are found in the secretion, e.g. acid phosphatase, naphtol-AS-BI-phosphohydrolase catalysing protein synthesis, leucine arylamidase for hydrolysis of proteins, α-glucosidase for conversion of nectar into honey and β-galactosidase in younger bees for energy supply (COSTA & CRUZ-LANDIM, 2001). The secretion also serves as a wax softener, and is also used for dissolving food (SCHÖNITZER & SEIFERT, 1990). The presence of RER suggests the secretion is a protein-rich fluid. Only the size of the subcuticular space changes with age, possibly because of filling with secretion. It seems that it has a reservoir-like function. This corresponds with the studies of REIMANN (1953). Other studies also suggest that the size of the tubes is variable according to the age and secretory cycle (COSTA & CRUZ-LANDIM, 2001), with a peak period between eight and 17 days. This corresponds with certain tasks : cleaning the queen, receiving nectar, pollen storing and nursing. Our quantitative estimates do not show any visible trend (Figs. 4, 5). The subcuticular space is biggest at 15 days. It is possible that at this age there is the largest amount of secretion, as we then see grouped clear vesicles near the microvilli in the subcuticular space, but no electron-dense secretion. The secretion is discharged via the medial duct into the secretory tube. This duct is surrounded with a porous cuticle on which taenidiae-like reinforcements are found (SCHÖNITZER & SEIFERT, 1990).

In winter bees we see many electron-dense vesicles. These are also apical as in summer bees. We could also observe large clear vesicles with internal lamellar structures. Probably this is an accumulation of the clear secretory vesicles we see in summer bees. The gland in winter bees produces secretory vesicles, but probably does not release the secretion. By such accumulation, this gland is loaded and when circumstances change in spring, they can directly discharge secretion.

We conclude that the production and function of secretion of this gland probably does not change in the life of a honeybee worker, there is only an accumulation of secretion. However, different functions are not excluded and further investigation is required to relate this morphology to function and task regulation.

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