

Original article

Ultrastructure of the wax gland complex and secretion of beeswax in the worker honey bee *Apis mellifera L.*

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Summary — The wax gland complex of the honey bee worker consists of 3 cell types, epithelial cells, oenocytes and adipocytes, which act synergistically to secrete wax, a complex mixture of hydrocarbons, fatty acids and proteins (lipophorins). The structure of the wax mirrors and the different types of cells were studied with scanning and transmission electron microscopy. The hydrocarbons coming from the oenocytes and the proteins from the haemolymph transit across the epithelium via the large SER cisternae and through the mirror plate along a well-developed extracellular and pore canal filamentous system connected to wax canal filaments of the epicuticle.

Apis mellifera ligustica / wax mirror / ultrastructure / wax gland / hydrocarbon / protein

INTRODUCTION

The secretion of wax by the honey bee worker is related to the wax gland complex located in the abdomen, at the anterior part of sterna IV to VII. In each sternum there are 2 polished surfaces, the wax plates or wax mirrors covering the wax glands, specialized glandular, epithelial areas of the epidermis, which are associated with fat body cells and oenocytes.

The height of the epithelial cells is age-dependent. In freshly emerged workers, the

epidermis is a flat epithelium. At the height of wax secretion the cells are greatly lengthened and have a longitudinally fibrillated appearance (Reimann, 1952). Sanford and Dietz (1976) and Hepburn *et al* (1991) reported that smooth endoplasmic reticulum (SER) is absent from the post-ecdysial life of honey bee workers and conclude that the epidermis has no role in the synthesis of beeswax. According to their view, the major role of the epidermis appears to be in the development of an elaborate system of small transport tubules (Reimann, 1952; Locke, 1961; Hepburn, 1986).

During the ageing of the worker, the fat body cells and the oenocytes show the volumetric variations similar to the epithelial cells (Rösch, 1927; Reiman, 1952; Boehm, 1965). The early mobilization of lipid from the adipocytes suggests that they might produce beeswax precursors. However, the fact that adipocyte lipid reserves are depleted prior to both the maximal development of oenocyte SER and wax production mitigate against this possibility (Hepburn *et al.*, 1991).

There are indications that the oenocytes synthesize the hydrocarbon fraction of the wax (Piek, 1961, 1964; Blomquist and Ries, 1979; Lambremont and Wykle, 1979). The fatty-acid and hydrocarbon composition of wax scales and oenocytes were similar (Hepburn *et al.*, 1991).

The cuticle of the wax plates has a stratified structure and the associated fibrous structures and pore canals certainly play a major function during the transit of the wax components from the wax glands to the exterior surface (Dreyling, 1903; Lewke, 1950; Reinamann, 1952; Locke, 1961; Locke and Huie, 1980; Hepburn, 1986). Reimann (1952) postulated that the wax components are transported in a protein medium through the gland cells to the outer surface of the mirrors, where the molecules condense to form scales. This hypothesis has recently been confirmed by Kurstjens *et al.* (1990), who electrophoretically detected proteins in the wax scales and in the comb wax.

The purpose of this study is to elucidate: (1) the contribution of the epithelial cells to the synthesis of the protein fractions of the wax; and (2) the transit modalities of the hydrocarbons from the oenocytes across the epithelial cells and the cuticle.

We have shown (SEM and TEM) the extrusion of wax droplets through the wax mirrors and, for the first time, large cisternae of SER in the epidermis. These cisternae are probably involved in the transit of wax from the oenocytes to the pore canal

system. They can also convey apolipoporphins from the haemolymph to the wax mirrors.

MATERIALS AND METHODS

We collected Italian honey bee workers (*Apis mellifera L ligustica* Spinola) at the Triwaks Bee Research Center from clusters of bees participating in the construction of a wax comb. The presence of wax scales was verified in the laboratory, where the workers were anaesthetized by chilling for 15 min at 4°C. The wax gland complexes were removed with iris scissors under a stereoscopic microscope. Transmission and scanning electron microscopy procedures have been described elsewhere (Cassier and Lensky, 1994).

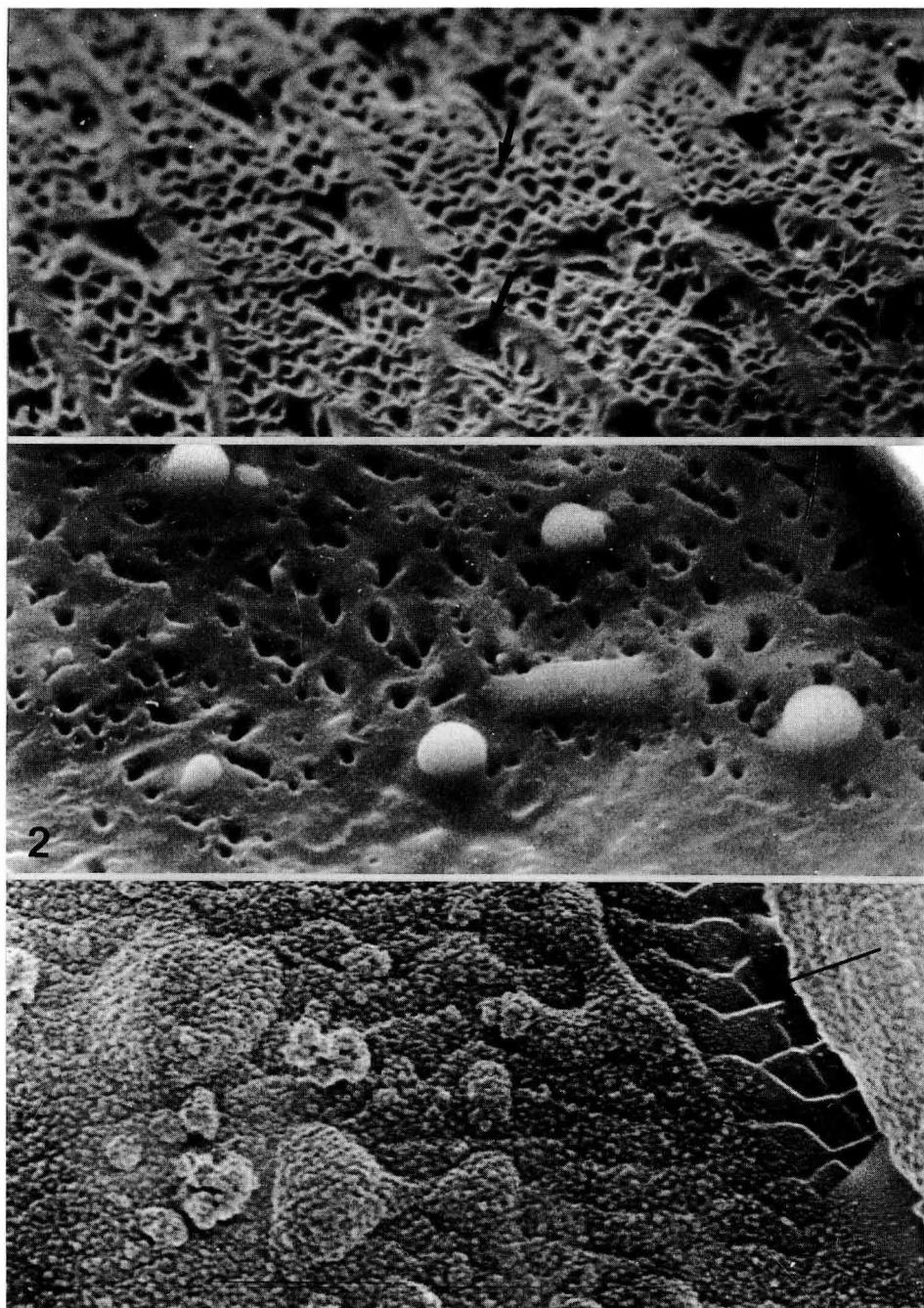
RESULTS

Above the wax mirror, each wax gland complex has an epithelial glandular layer surrounded by fat body cells and oenocytes. In the vicinity of the mirror plate the flattened epithelium shows no similar associations with adipocytes and oenocytes.

The cuticular plate or wax mirror

The outer face of the cuticular plate shows a sub-regular, hexagonal pattern, each unit corresponding to an underlying epithelial cell. On perfectly clean samples the covering epicuticle shows numerous holes or depressed areas (fig 1) from which the new biosynthesized wax masses exude (fig 2), fuse and form irregular puddles (fig 3). In fixed samples, the wax mirrors are covered by irregular lamellar structures (fig 4) corresponding to insoluble wastes (ethanol, propylene oxide, acetone) from the wax secretions.

The sternal cuticle is particularly thin at the level of the mirror plates (2–4 µm). It is composed of an outer trilaminar epicuticle



Figs 1–3. Scanning electron microscopy of the wax mirrors. 1. Outer surface of a cleaned wax mirror showing the cuticular pattern. Each unit shows numerous holes and pits (arrows). G x 1 800. 2. Extrusion of globular droplets of wax through the holes of the wax mirrors. G x 2 500. 3. Droplets of wax fuse and form irregular puddles which mask the cuticular pattern (arrow) G x 750.

(15–20 nm), a homogeneous inner epicuticle (120–150 nm) and a 2- or 4-layered procuticle (figs 4–6). The main characteristic of the cuticular plate is the presence of numerous pore canals containing microfilamentous structures (20 nm). From the base to the apex of the cuticular plate (fig 6) the initially reticular disposition of the pore canals evolves to constitute twisted bundles connected to epicuticular filaments (wax canal filaments; Locke, 1961).

The epithelium

The elongate epithelial cells (about 40 x 10 µm) form a palisade layer (figs 4–5). Intercellular spaces and infoldings of the apical plasma membrane delimit large spaces where twisted filamentous structures (20 nm in diameter) run and connect to those of the pore canals (fig 6). The lateral plasma membranes of adjacent cells are connected in the supranuclear portion by *maculae adherens*, septate desmosomes and gap junctions (fig 7).

The ellipsoid nucleus (10–12 x 5–6 µm) is parallel to the major axis of the epithelial cells (fig 4). It contains 2 or 3 nucleoli and small clumps of heterochromatin dispersed into an electron-lucent nucleoplasm.

The striped appearance of the epithelial cells is enhanced by the disposition of long cisternae of rough endoplasmic reticulum (RER) and mitochondria (fig 4). These cisternae are generally associated with cisternae of smooth endoplasmic reticulum (SER) containing patches of an amorphous substance with a medium electron density. These seem to be connected to extracellular spaces (fig 8).

The cytoplasm also contains lytic structures, small Golgi apparatus, polyribosomes and microtubules. They belong to the type I glandular cells described by Noirot and Quennedey (1974).

The oenocytes

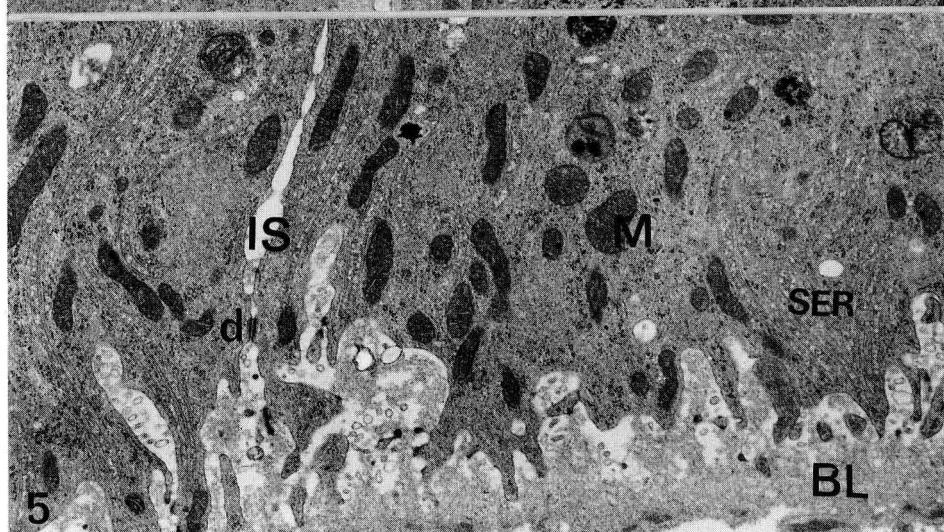
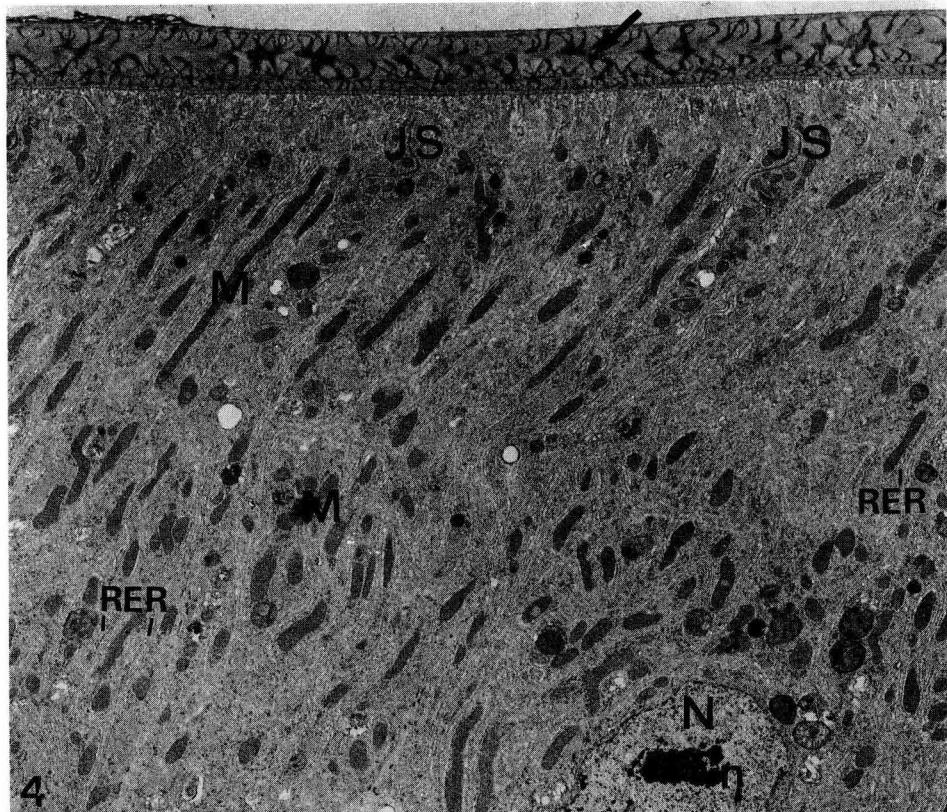
The oenocytes (fig 9) are associated with the peripheral part of the wax gland complex and provided with their own basal lamina. They show the same general features as the oenocytes associated with epidermis or fat body (Dean *et al.*, 1985). These spherical (mean diameter 25 µm) or ovoid cells contain a large nucleus (mean diameter 12 µm) with a sinuous envelope, numerous nucleoli (mean diameter 2 µm) and a clear nucleoplasm. The cytoplasm is mainly occupied by rod-like mitochondria and extensively developed tubular SER. They also contain ribosomes, short cisternae of RER, lytic lamellar or dense bodies. The plasma membrane is associated with a thick basal lamina (0.22–0.25 µm).

Fat body cells or adipocytes (data not shown)

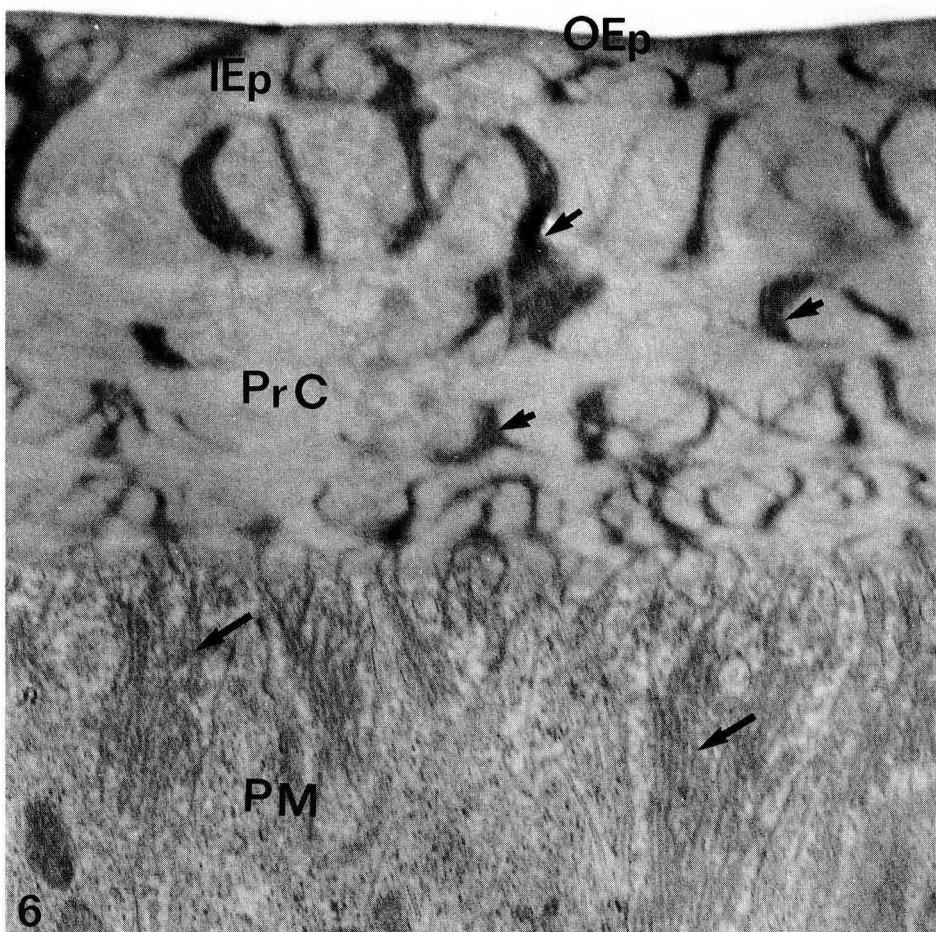
The fat body cells represent a significant part of the wax gland complex of the honeybee workers. They are intimately associated with oenocytes from which they are separated by a thick glycocalyx. The ramified or star-like nucleus contains numerous nucleoli (mean diameter 0.7 µm) and clumps of heterochromatin. At the periphery numerous tubular invaginations form a characteristic labyrinth. The cytoplasm contains numerous rod-like mitochondria, lipid droplets, peroxisomes, small Golgi areas, stalks of RER cisternae, dense bodies and small Golgi apparatus (see Cassier and Lensky, 1994; fig 9).

DISCUSSION

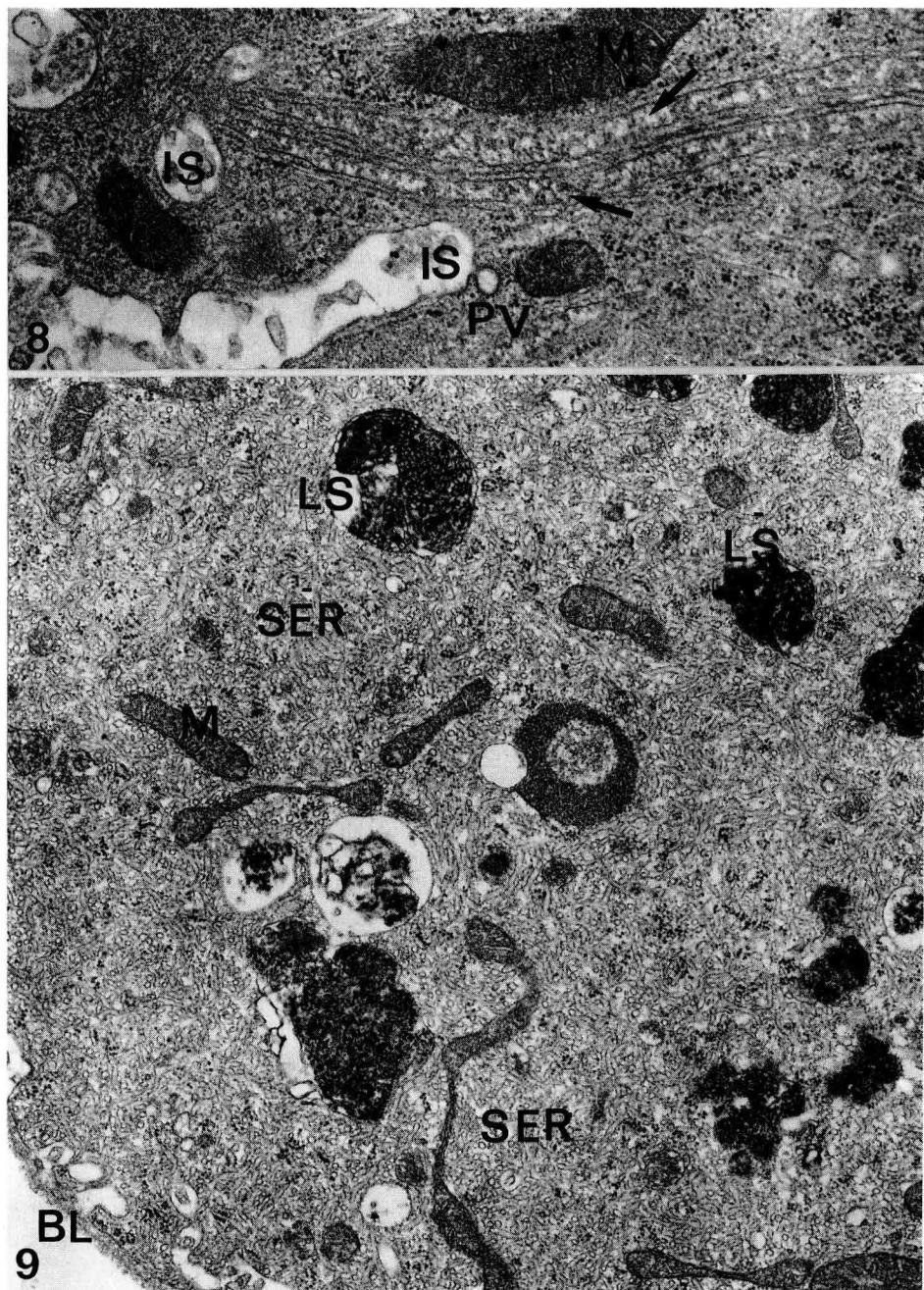
The ultrastructure of the wax gland complex (epithelial cells, oenocytes and adipocytes) in honey bee workers has been studied in relation to the synthesis, the



Figs 4 and 5. Transmission electron microscopy. The epithelial cells. 4. Apical part of a cell under the wax mirrors (arrow). Note the development of the pore canal system, the striped aspect of the epithelial cells, and the presence of insoluble material on the outer surface of the wax plate. JS = junctional system, M = rod-like mitochondria, N = nucleus. G x 14 000. 5. Basal part of a cell. BL = basal lamina, d = desmosome, IS = intercellular space, M = mitochondria. G x 9 000.



Figs 6 and 7. Transmission electron microscopy. Detail of the apical part of an epithelial cell.
6. Microfilaments (arrows) linked to the apical plasma membrane infoldings formed the pore-canal system (arrow heads) of the wax plate. IEp = inner epicuticle, OEp = outer epicuticle, PM = plasma membrane, PrC = procuticle. G x 30 000. 7. Detail of a junctional system between 2 epithelial cells. d = desmosome, GJ = gap junction, SD = septate desmosome. G x 40 000.



Figs 8 and 9. Transmission electron microscopy. Epithelial cells and oenocytes. 8. Basal part of an epithelial cell showing large SER cisternae (arrow) connected to intercellular spaces (IS). Note the presence of an amorphous, electron-dense material. M = mitochondria, PV = pinocytotic vesicle. G x 30 000. 9. Part of an oenocyte showing the well-developed tubular SER, the mitochondrial (M) system and numerous lytic structures (LS). BL = basal lamina. G x 20 000.

transport and the secretion of beeswax, a composite mixture of hydrocarbons, fatty acids (Hepburn *et al.*, 1984, 1991) and proteins (Kurstjens *et al.*, 1990). Because beeswax is hydrophobic it is probably transported from the wax glands to the mirrors by haemolymph lipophorins *via* the SER cisternae; this is a means of carbohydrate transport known in other insects. The wax scales and comb wax contain 11 and 13 protein bands, respectively. Seven of the bands were common to both scale and comb wax (19–100 kDa; Kurstjens *et al.*, 1990).

The biosynthesis of wax implies the synergistic function of the 3 cell types, although no direct relationships were observed. Similar associations have also been observed for tergal gland (Renner glands) of the queen bee (unpublished results) and of the Nasanov gland of the workers (Cassier and Lensky, 1994). The pheromonal products of these abdominal glands are also associated with protein components (Zupko *et al.*, 1993).

The constant associations between epithelial cells, adipocytes and oenocytes, suggest a synergistic contribution to the biosynthesis of the secretory product. Based on biochemical and structural data, it is possible to suggest the contributions of each cell type to the wax gland complex. Oenocytes are involved in the secretion of the hydrocarbon fraction of the wax (Piek, 1961, 1964; Blomquist and Ries, 1979; Lambremont and Wykle, 1979; Hepburn *et al.*, 1991). The epithelial cell provided with ribosomes, polysomes, RER cisternae and electron-dense granules probably synthesizes a part of the protein fraction of the wax product, the other part coming directly from haemolymph through SER cisternae. Fat body cells provided plastic and energetic products. Thus, according to Hepburn *et al.* (1991), massive lipid droplets occupy about 60% of the cell's cytoplasm in the young worker, but they decrease substantially over the next few days. During wax synthesis glycogen stores are notably large and the plasma membrane reticular system is well

developed. As synthesis wanes, the lipid droplets increase in size while the other organelles either remain unchanged or show a small decrease in size.

The problem of wax transit across the epithelial cells and then the cuticle deserves a comment. In contrast to the observations by previous workers (Sanford and Dietz, 1976; Hepburn *et al.*, 1991), we found that the SER is well developed both in oenocytes and epidermal cells. In the latter, SER forms long cisternae parallel to the major axis, from the basal to the distal pole of the epithelial cells. They seem to be connected to extracellular spaces. Moreover, the lumen of the cisternae contains clumps of amorphous and insoluble material (due to the solvents used during the electron microscope procedures), which have the same aspect and the same electron density as the deposit covering the epicuticle. These data suggest that SER cisternae are preferential sites of transit for hydrocarbons biosynthesized by oenocytes. The epidermal cell provided with SER can also be involved in the secretion of some wax components. The transit through the extracellular spaces (intercellular cavities, subcuticular spaces) and the cuticle of wax associated with proteins is certainly favoured by extracellular and pore-canal filaments (Locke, 1961; Locke and Huie, 1980), as are the hydrocarbons of the wax layer after ecdysis. The pore canal filaments have the same characteristics (size and electron density) as the wax canal filaments. Moreover, the transit through the cuticle is easier owing to the absence of the cement layer (Locke, 1961) and the lipophilic characteristics of the outer epicuticle, which is made of oriented lipids.

The production of wax, according to classical observations, begins in workers that are slightly less than 1 week old, peaks at the age of about 2 weeks and then wanes (Rösch, 1927; Freudenstein, 1960; Boehm, 1965; Darchen, 1968; Hepburn *et al.*, 1984). In fact, the secretion of wax is a constant process even in overwintering workers (unpublished data; cf Hepburn, 1986). This

process seems essentially to be triggered by feeding with honey or sugar, which affects the level of sugars in the haemolymph and the qualities of the queen (Darchen, 1968; Whiffler and Hepburn, 1991).

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Résumé — Ultrastructure des glandes cirières et sécrétion de la cire chez l'ouvrière de l'abeille (*Apis mellifera* L.). La sécrétion de la cire par les ouvrières de l'abeille italienne (*A m var ligustica*) est le fait de complexes glandulaires, localisés à la partie antérieure des sternites abdominaux IV à VII, recouverts par des zones cuticulaires amincies ou miroirs. Chaque complexe glandulaire comporte 3 types cellulaires (cellules épithéliales, œnocytes et adipocytes), dont l'activité synchrone est fonction de l'âge de l'abeille. La cire est un mélange d'hydrocarbures, d'acides gras et de protéines. La structure des miroirs et des différents types cellulaires a été étudiée à l'échelle de la microscopie électronique à balayage et à transmission afin de préciser les relations entre structures cuticulaires et cellulaires, et d'envisager le transit des constituants de la cire. Au niveau des miroirs (figs 1-4) la cuticule amincie (2-4 µm) est remarquable par le grand développement du système fibrillaire (20 nm) des canaux poraires connectés d'une part aux canaux cirières épicuticulaires et d'autre part aux fibrilles extracellulaires localisées dans des invaginations de la membrane plasmique apicale des cellules épithéliales. Les cellules épithéliales (figs 4-6) réunies par un système jonctionnel complexe (fig 7) (desmosome, desmosome cloisonné, jonction serrée) possèdent de longues citernes de

réticulum endoplasmique agranulaire (fig 8) contenant le même matériel que celui qui subsiste à la surface des miroirs à l'issue des traitements de la microscopie électronique. Les citernes semblent en relation avec les espaces extracellulaires (fig 8). Les œnocytes (fig 9) à l'origine des hydrocarbures de la cire possèdent un abondant réticulum endoplasmique agranulaire, tubulaire et de nombreuses structures lytiques traduisant un intense métabolisme. Les adipocytes riches en réticulum granulaire, en lipides et en glycogène doivent couvrir les besoins énergétiques des complexes glandulaires. Lipophorines hémolymphatiques, hydrocarbures synthétisés par les œnocytes traversent les cellules épithéliales par l'intermédiaire des citernes de réticulum agranulaire et la cuticule le long des structures fibrillaires (canaux poraires et canaux cirières).

Apis mellifera / glande cirière / plage cirière / ultrastructure / hydrocarbure / protéine

Zusammenfassung — Ultrastruktur der Wachsdrüsen und Wachssektion bei den Arbeiterinnen der Honigbiene (*Apis mellifera* L.). Die Sekretion von Wachs durch Arbeiterinnen der italienischen Biene (*A m ligustica*) erfolgt in drüsigen Komplexen, die am vorderen Ende der Abdominalsternite IV bis VII liegen und die durch eine dünne, glänzende cuticuläre Schicht, dem Wachsspiegel, abgedeckt sind. Jeder Drüsengangkomplex besteht aus 3 Zelltypen, den Epithelzellen, den Oenocytten und den Adipozyten, deren synchrone Aktivität vom Alter der Biene abhängt. Das Wachs ist ein Gemisch aus Kohlenwasserstoffen, Fettsäuren und Proteinen. Der Bau der Wachsspiegel und der verschiedenen Zelltypen wurden nacheinander durch Raster- (REM) und Transmissionselektronenmikroskopie (TEM) untersucht, um die Verbindungen zwischen den cuticulären und zellulären Strukturen zu präzisieren, und um den Transport

der Wachskomponenten zu beobachten. Bemerkenswert ist die starke Ausbildung des fibrillären Systems (20 nm) der Porenkanäle in der dünnen Cuticula (2–4 µm) in Höhe des Wachsspiegels (Abb 1–4). Diese stehen zum Teil mit den epicuticulären Wachskanälchen und zum anderen Teil mit extrazellulären, durch Einstülpung der apicalen Plasmamembran der Epithelzellen entstandenen Fibrillen in Verbindung. Die Epithelzellen (Abb 4–6), die durch ein komplexes Verbindungssystem miteinander vereinigt sind (Abb 7) (Desmosomen), besitzen lange Zisternen aus glattem endoplasmatischen Reticulum (SER) (Abb 8). Diese enthalten das gleiche durch die Behandlung für die Elektronenmikroskopie entstandene Material, das auch als Ablagerung auf der Epicuticula des Wachsspiegels vorhanden ist. Die Oenocyten (Abb 9), von denen die Kohlenwasserstoffe für das Wachs stammen, besitzen reichlich tubuläres SER und zahlreiche lytische Strukturen, die einen intensiven Metabolismus anzeigen. Die Adipocyten sind reich an rauhem endoplasmatischen Reticulum (RER), Fetten und Glykogen, die den energetischen Bedarf des Drüsengewebes abdecken. Lipophorine der Hämolymphe und Kohlenwasserstoffe, die in den Oenocyten synthetisiert werden, werden zu den Epithelzellen durch intermediaire Zisternen des SER und in der Cuticula entlang der fibrillären Strukturen (Poren- und Wachskanäle) übertragen.

Apis mellifera / Wachsdrüse / Wachsspiegel / Ultrastruktur / Kohlenwasserstoff / Protein

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