Apidologie (1996) 27, 145-156

Original article

Cornual gland of the honeybee drone (*Apis mellifera* L): structure and secretion

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(Received 18 December 1995; accepted 12 May 1996)

Summary — The cornual gland of the drone's endophallus is an epithelial gland, whose cells extend from the tip of the cornual tube, along its lateral parts to the basic hump (dorsal cornua). The gland forms a stripe with a central region of dense and homogeneous secretory cells. To the sides the gland cells become sparse. The secretory cells contain mainly rough endoplasmatic reticulum and numerous mitochondria. The nuclei have several nucleoli and are situated at the apical part. Microvilli border the microfibrillar cuticula into which cell projections often intrude. At the basal membrane the cells are often subtended by a layer of fatty tissue, and oenocytes can also be attached. The secretion in a 3-day-old drone appears either as an orange-coloured layer (deep frozen sections) or as osmiophilic granula (EM) mainly near the lumen of the cornual tube. The cuticula of the cornual tube is untanned and non-sclerotized. Along the lumen of the cornua it is extremely folded and papillated, forming in situ small cavities which contain osmiophilic drops of the secretion, often accumulated in groups. No pores were found, although small granules of secretion occur within the cuticular layers, with the highest concentration near the lumen. During eversion of the cornua the secretion merges on the surface of the cuticula. The cuticula consists of two differently structured layers. Near the lumen of the cornual tube the microfibers extend regular and parallel to the folds; above the gland cells the microfibers are less dense and more irregular.

***Apis mellifera* / drone / sexual organ / endophallus / cornual gland**

INTRODUCTION

The endophallus of the honeybee drone is a highly specialized structure, consisting of an eversible cuticular membrane with different functional parts and appendices. It develops ontogenetically from the ectoderm (Zander, 1922). Thus in the adult drone the wall of the endophallus has basically the

Abbreviations: Ch = channels within the dense cuticular membrane of the cornua; CM1 = cuticular membrane, less dense for electrons; CM2 = cuticular membrane, electron dense; Co = cornua (dCo = dorsal cornua), vCo = ventral cornua; CP = cell projection; E = inactive epithelial cell; F = fat tissue; G = cornual gland cells; L = lumen of cornu in situ; Mv = microvilli; N = nucleus; Oe = oenocyte; S = orange-coloured secretion.

structure of the integument: basal membrane, epidermis and cuticula, which is not sclerotised. Since the synthesis of the cuticula is completed in the emerged drone, the epidermal cells do not secrete additional cuticular substances and generally are reduced to a thin epithelium.

The uneverted endophallus lies in the ventral part of the abdomen. From the basal part, the vestibulum, the pair of cornua and their orange-coloured secretions project from both sides. In newly emerged drones the cornua have no pigmentation, about 1.7 days after emergence the first pigment can be measured spectrophotometrically at 300 nm. The amount of pigments increases in a sigmoidal curve, with the main increase between the 2nd and 6th day of age (Koeniger et al, 1990).

Though the orange-coloured secretion on the cornua of sexually mature drones is conspicuous, it was often neglected in classical descriptions of the anatomy of the endophallus (Zander, 1922; Snodgrass, 1956). However, it was described by Fyg (1952) as ranging from reddish-yellow to brownish-red. On the everted cornua it was described as being split on the dorsal side, then covering only the dorsal and outer lateral wall as a viscid layer of the everted cornua (Woyke, 1955). During the mating process it becomes part of the mating sign (Woyke, 1958 a, b, Woyke and Ruttner, 1958).

The biological function of the cornual secretion is not fully understood. It is connected to processes of mating, as it forms the outer layer of the mating sign, which fills the sting chamber after each mating. In a two-choice test, drones preferably grasped and mated queens with a mating sign. The white mucus and the thin orange layer – besides other possible functions – may serve as an optical cue for the drones, which are known to have good vision (van Praagh et al, 1980; Vallet and Coles, 1993). Thus the mating sign facilitates and possibly accelerates the mating frequency on the nuptial flight of the polyandrous honeybee queen (Koeniger, 1984, 1986, 1990).

There is no evidence of the origin of the orange secretion. A preliminary short note reported that special gland cells occur at the cornua (Koeniger et al, 1990). The aim of our study was to learn more about the cornual gland structure.

MATERIAL AND METHODS

Sealed drone brood was put into an incubator (34.5 °C, 75% humidity). Every 12 h the comb was inspected. The emerged drones were marked individually and introduced into a beehive. They were recaptured at ages between 1 and 6 days. The cornua were dissected either in situ or in the everted stage and fixed with different methods.

For light microscopy they were fixed in 10% formaldehyde and later embedded either in paraffin or, for frozen sections, in gelatin (Romeis, 1989). In paraffin sections the secretion was diluted in the last step of dehydration in xylene. The deep frozen sections were stained by hematoxyline (nucleus) and fat red 7B by Merck (fat tissue). With this treatment the secretion did not dissolve nor loose its orange colour.

For electron microscopy, the cornua were fixed for 2 h in 2% glutaraldehyde, in 0.1 M phosphate buffer, pH 7.2. After washing four times in buffer, they were post-fixed in 2% osmium tetroxide dissolved in the same buffer for 2 h. Further processing for EM was as described by Herth et al (1981).

RESULTS

Cornua in situ

The cornua in situ are flat and the dorsal cornua are folded three times: while the basic part extends from the vestibulum to the side, the middle part is bent to the vestibulum and the tip points to the side again (fig 1, right dorsal cornu). The cor-

nual secretion occurs only in drones older than 3 days. It extends from the tip of the ventral cornua (vC), along the lateral sides and ends at the basic hump (dorsal cornua). This band of secretion is bordered by regions with less concentrated secretion (fig 1). In situ the secretion occurs in the lumen of the cornual tube in a length of about 5 mm. The thinner parts of the layer form a netlike structure (fig 2), supported by papillae in the cuticular membrane (figs 3–5). After the eversion of the endophallus the secretion covers the surface of the tube. Then the netlike formation of the secretion is even more conspicuous because the cuticula is extended and the netlike structure becomes wider. The papillate external cuticular membrane containing the secretion (fig 2) separates easily from the less dense part and can be pulled off completely without damaging the remaining part of the cornu. Because of the viscid character of the orange secretion, the removed membrane sticks to everything (forceps, etc) and also tends to stick together immediately.

Epithelium

The light photomicrographs show that the epidermis contains special regions of gland cells. In figure 4 normal epidermal cells (E) form a flat epithelium, while the gland cells (G) are elongated to 20–30 μm. In the frozen section they appear partially separated by intercellular spaces. The round nuclei are situated at the apical part of the cells. In many sections the gland cells are subtended by a layer of fatty tissue (fig 4). Sometimes oenocytes are closely appressed to the gland epithelium (fig 5).

The electron microscopical studies reveal that the gland cells have a functional and morphological polarity (fig 6). The apical cell membrane ends with microvilli (figs 6 and 8), often there are long projections which

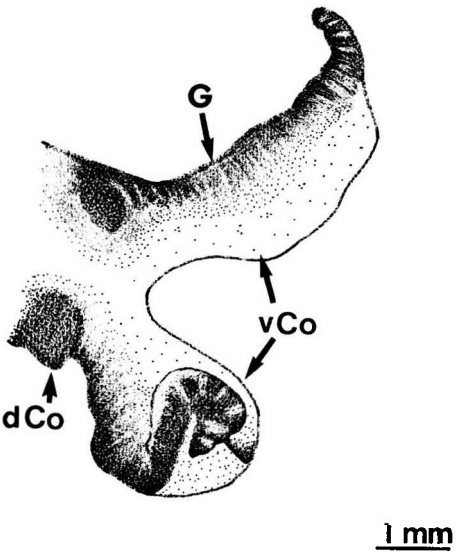

Fig 1. Extension of the cornual gland. One cornu is drawn in situ, the other is stretched (scale 1 mm).

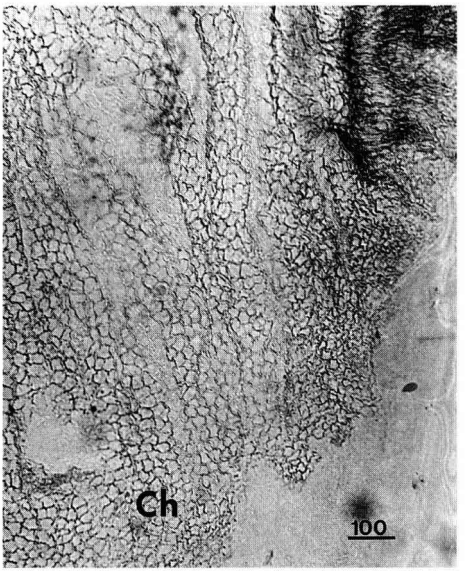

Fig 2. Detached dense cuticular membrane with the secretion in a network of tiny channels (Ch) (scale 100 μm).

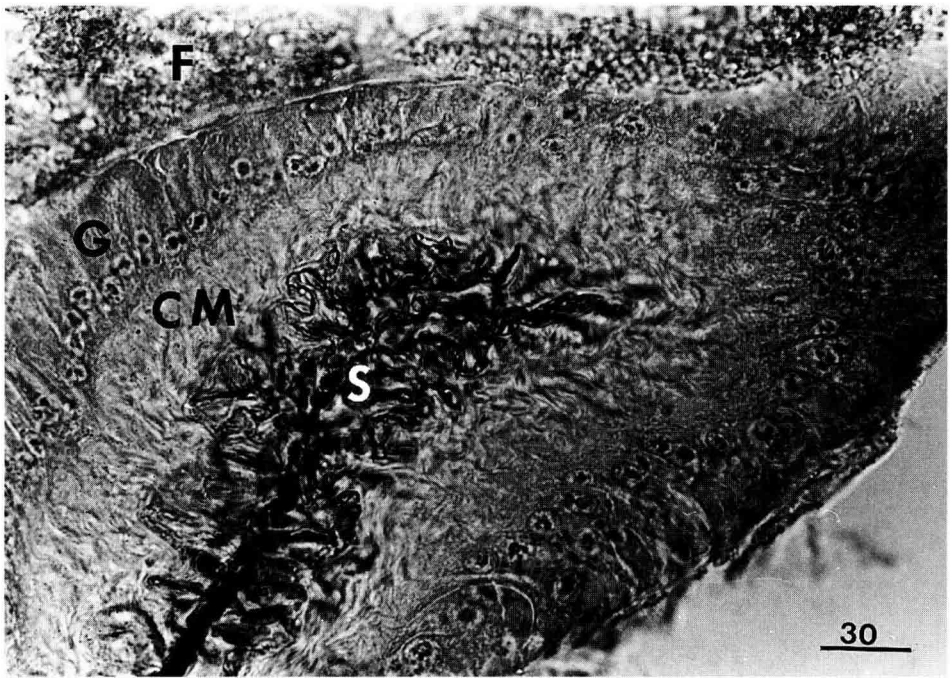

Fig 3. Frozen section: cornual gland cells (G) border the cuticular membrane (CM). The secretion (S) is concentrated in the inner tube (scale 30 μm).

intrude and sometimes even branch in the cuticula (fig 7). The intercellular spaces between the gland cells occur at the basal and middle part (figs 3 and 6) while there are junctions between the cells in the apical part. Small plasma membrane infoldings are winding between the cells and finally end blind within the cells (figs 6 and 8). As already seen in the light photomicrograph the nuclei appear round to ellipsoid and are positioned in the apical part (figs 3 and 6). Their diameter is between 3 and 6 μm. They contain nucleoli, which are often enlarged. The cytoplasma of the cell contains numerous mitochondria, a rough endoplasmatic reticulum, ribosomes and some microtubuli. A few secretory droplets are found in the cells (arrows). However, a Golgi apparatus could not be observed and smooth endoplasmatic reticulum is scarce.

Cuticular membrane

The thickness of the cuticular membrane ranges between 30 and 60 μm. It has two zones of different density (figs 6 and 8). Above the gland cells the fibers are less compact and follow the pattern of the cell layer. The arrangement of the fibrils near the lumen of the cornual tube are such that in situ the layer is dense, regular and equidistant to the tubules (fig 3). Between both parts of the membrane there is a thin electron-dense layer. No pores could be found. Small granules of secretion can be recognized within the cuticular layers (figs 8 and 9), either single or as an aggregation of up to 50 granules of different size. The highest aggregation and the biggest granules occur in the dense cuticular layer near the lumen of the cornua in situ (fig 9). The

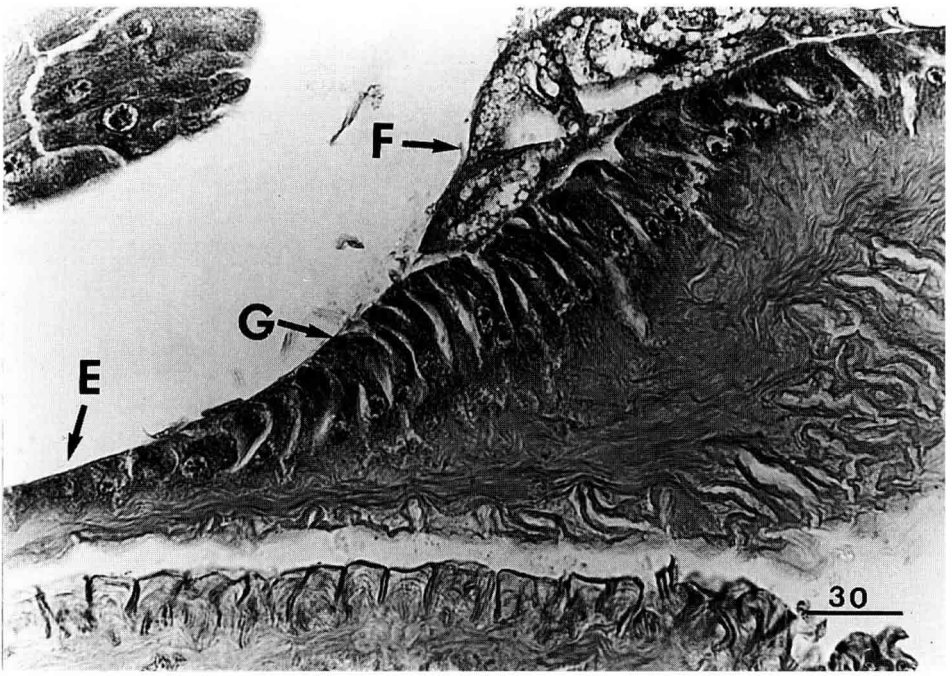

Fig 4. Paraffin section: glandular (G) and inactive epithel cells (E) alternate. Fat tissue (F) is attached to the gland cells (scale 30 μm).

fine system of papillae and tubules in the inner tube enclose the secretion. In the everted stage the cuticular membrane is extended and the secretion fuses on the surface (fig 6).

DISCUSSION

According to the classification of Noirot and Quennedey (1974), the cornual gland belongs to a type 1 of epidermal glands: the overlying cuticle has been secreted by the gland cells which are in direct contact with the cuticle. The cuticular surface above the gland cells has no pores but shows special structures; it is folded and papillated. The glandular area is very large: it extends from the cornual tip along the lateral region ending only at the tiny dorsal hump of the cornua, thus having a length of about 5 mm. The width is not regular: there is only a slender line with a continuous layer of gland cells; to the sides they form a netlike tissue with inactive cells in between. Most described epidermal glands cover a small well-defined area and often the overlaying cuticula shows special structures. For example the four different epithelial sternal glands of *Pachycondyla tridentata* Smith (Formicidae) cover areas between 510 x 390 μm to 270 x 180 μm, the cuticula has no pores (Jessen and Maschwitz, 1983). The activated wax glands of worker bees (*Apis mellifera)* are larger, they cover an area of roughly 2 000 x 1 200 μm of the 7th to 10th sternites and the overlaying cuticle is perforated (see review of Hepburn, 1986). The epithelial ring glands of several Lepidoptera are large too. They often extend over the

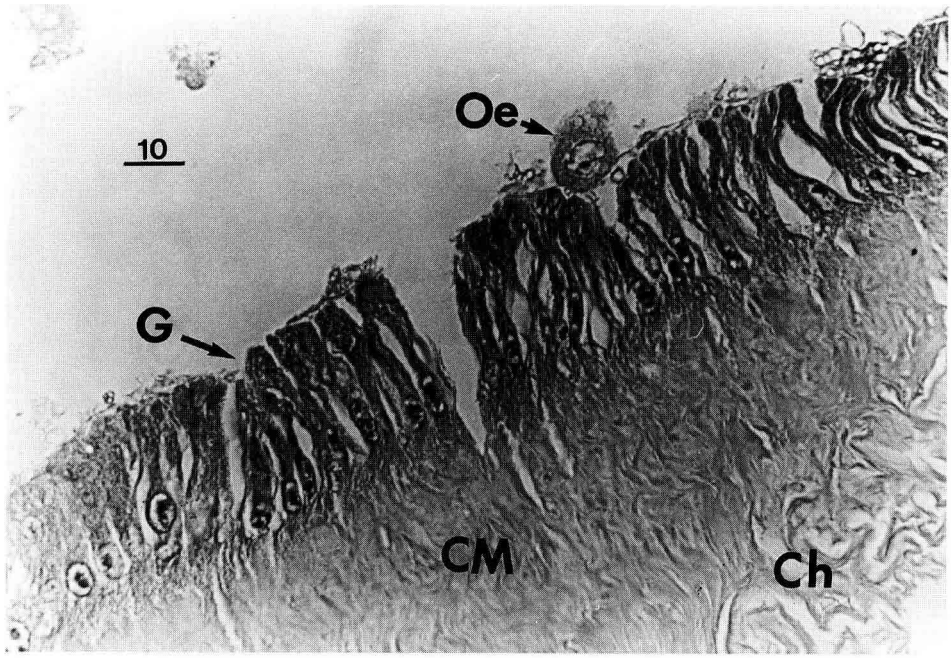

Fig 5. Paraffin section: an oenocyte (Oe) is attached to the gland cells. The channels within the cuticular membrane are visible. The secretion has been dissolved in xylene from the channels (Ch) (scale 10 μm).

whole intersegmental membranes of the sternite and tergite interrupted only laterally at the apophyses (Percy-Cunningham and MacDonald, 1987).

The glandular cells of the cornua show some typical secretory characteristics: they are elongated and show a functional polarity; cells often project into the cuticle; and the apically positioned nuclei have several enlarged nucleoli. Also the density of mitochondria, microtubules and microvilli are indicative of biosynthetic activity. However, the high portion of rough ER and the absence of a Golgi apparatus or vesicles are very atypical for gland cells. This phenomenon has also been described for honeybee wax glands (Hepburn et al, 1991). For these cells Cassier and Lensky (1995) hypothesized that the few cisternae of the smooth ER, running parallel to the major cell axis, are preferential sites of transit for hydrocarbons biosynthesized by oenocytes.

The secretion of the cornual gland consists of substances which are soluble in nonpolar organic solutions like xylene or dichloromethane. The conspicuous orange-coloured pigments and other substances are insoluble in methanol. Besides the pigments, steroids, cholesterol, lipids, wax, fatty acids and hydrocarbons were also found (Reder and Veith, unpublished data). Adjacent fat cells and oenocytes were also observed in sections of the cornual gland. Thus the cornual gland may possess a mechanism of synthesis similar to the wax gland complex, where synergistic functions of glandular cells, oenocytes and adipocytes are found. More detailed investigations are needed to confirm these ideas.

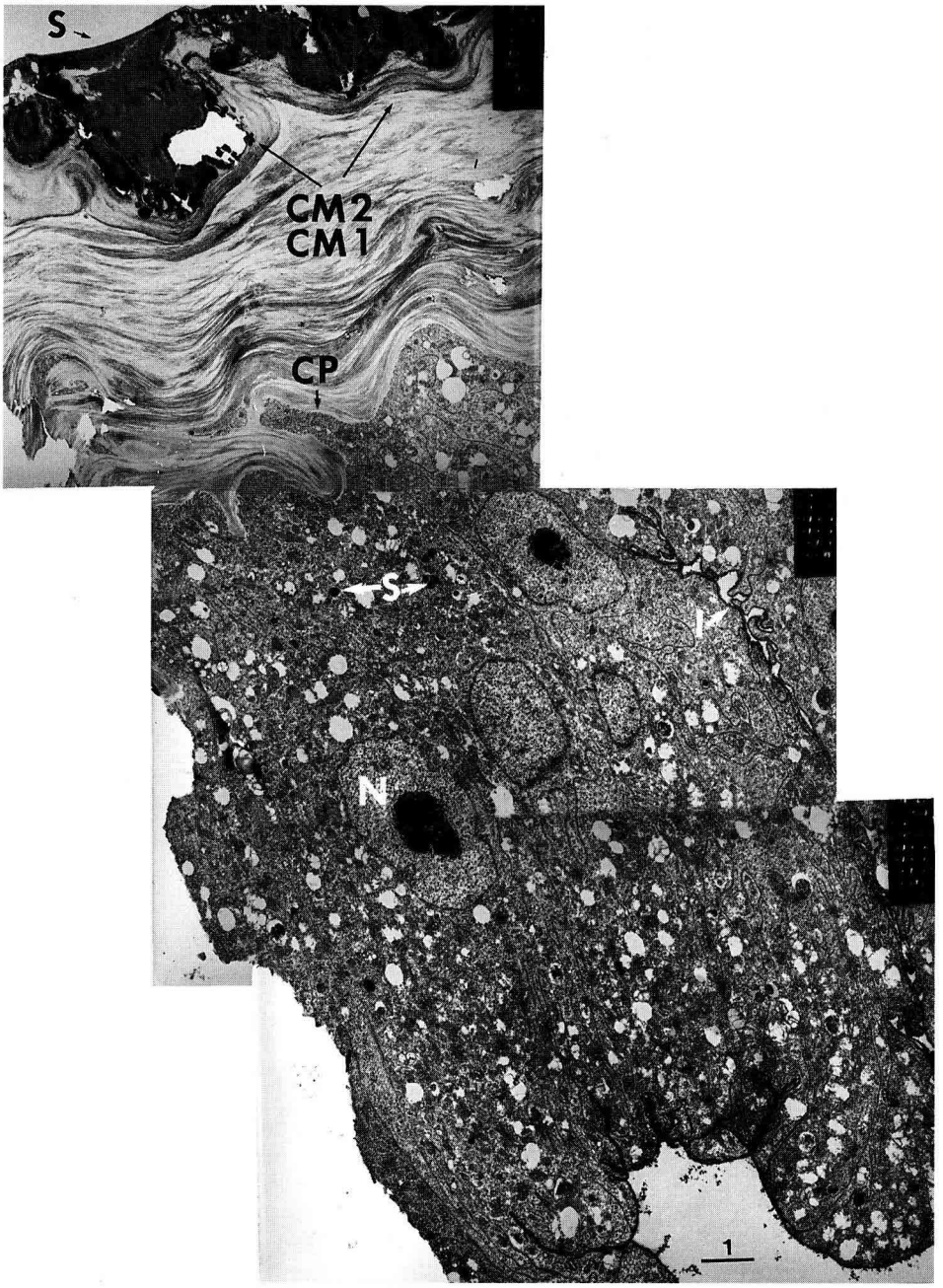

Fig 6. EM of an everted cornua: the secretion has merged on the surface of the cuticular membrane. The elongated gland cells show a morphological and functional polarity. In the cells the nucleus, mitochondria, plasma membrane infoldings (I) and some droplets of secretion (S) can be recognized. The different density of the cuticular membrane (CM1 CM2) is clearly visible (scale 1 μm).

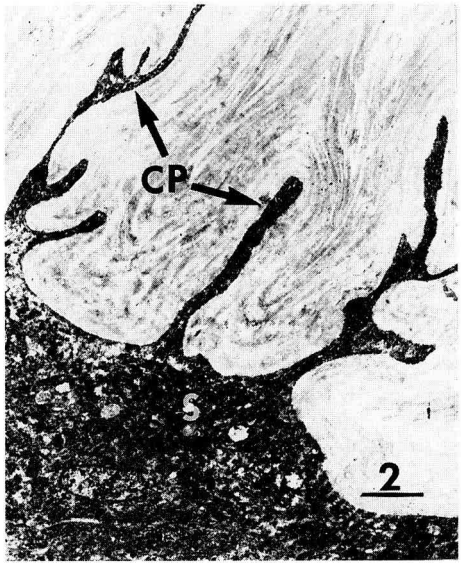

Fig 7. EM: long cell projections (CP) intrude into the cuticular membrane (scale 2 μm).

The secretory activity starts only after the drone has emerged from the brood cell. About 24 h later the first granules could be recognized. This is in accordance with the spectrophotometric measurements of the orange-coloured pigments (Koeniger et al, 1990). The secretion migrates as small droplets through the different cuticular layers. This was also described for the secretion of the Dufour's gland of *Apis mellifera ligustica* workers (Ikenga and Chapman, 1989). The question of how the granules reach the lumen of the cornua remains unanswered. After migration through the chitinous layer of the cuticle, the secretion accumulates near the folded surface. After eversion of the endophallus, the folds are distended. In addition the cuticle forms irregular protuberances, which were described by Woyke (1958). Further they were well illustrated in the scanning electron microscope atlas of the honeybee (Erickson et al, 1986,

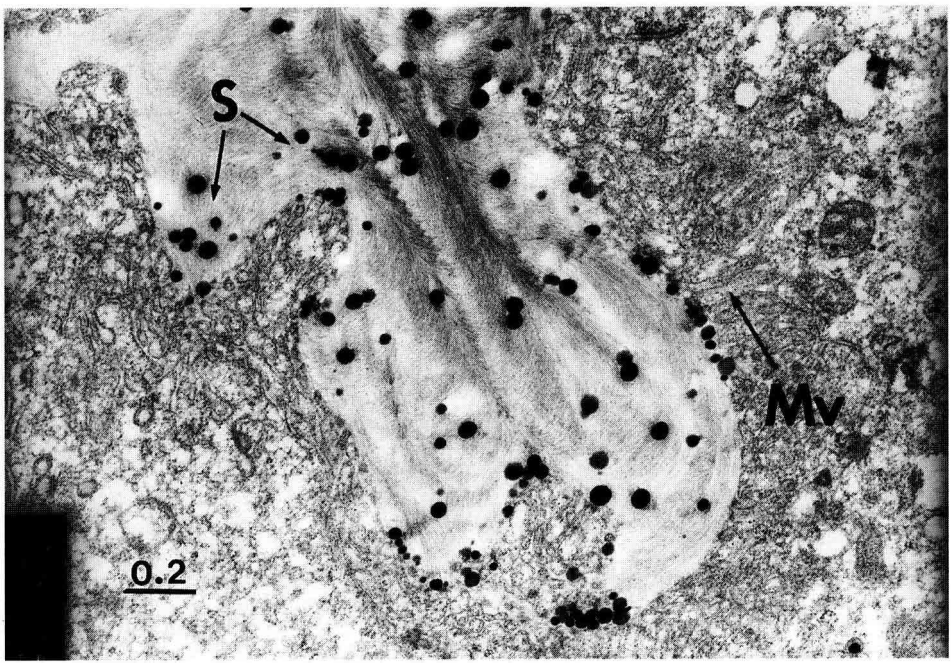

Fig 8. EM (cornua in situ): microvilli (Mv) and extrusion of secretion (S) (scale 0.2 μm).

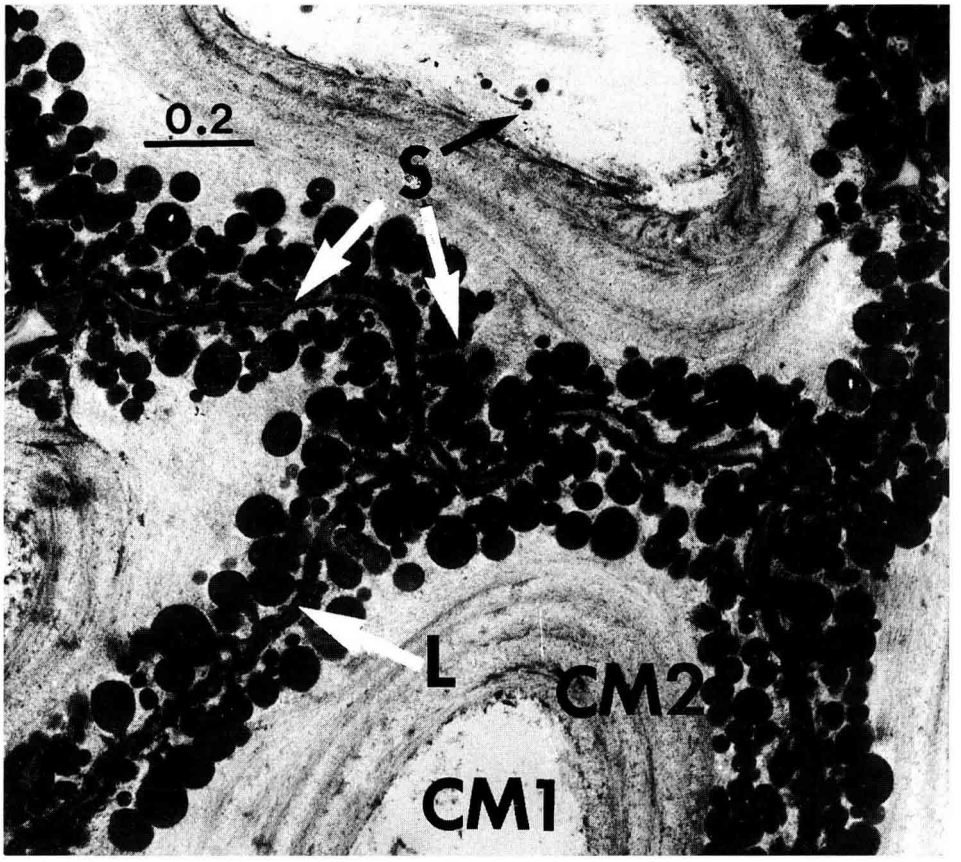

Fig 9. EM (cornua in situ): aggregation of secretory droplets (S) near the lumen (L) of the cornual tube (scale 0.2 μm).

page 265): "The dorsal and lateral surfaces of the cornua are papillated while the ventral surface is relatively smooth with longitudinal folds". The secretion is distributed within these dorsal and lateral projections.

The papillated thin membrane of the cornua containing the orange-coloured secretion is part of the mating sign which is left in the sting chamber of the queen. It is spread as a thin layer from the chitin plates over the bow of the bulb until it fuses opposite to the chitin plates (Woyke and Ruttner, 1958). This regular form is guaranteed because the apical part of the membrane totally separates from the rest of the cornual tubes. Probably in the first step it may stick to parts of the sting chamber, but afterwards it gets attached to the mucus of the mating sign.

Summarising, the gland cells seem not to form a homogeneous layer around the cornua but are concentrated at some special regions from the tip of the cornua along the lateral and ventral part and finally end at the vestibulum. The activity of the cells is not synchronized, it is dependent on age and on the position on the cornua. Thus, it is difficult to follow the activity cycle of the whole gland with histological methods.

Résumé — La glande des cornules du mâle d'abeille (*Apis mellifera* L) : structure et sécrétion. L'endophallus des mâles d'*Apis* est un organe hautement spécialisé. Au cours de l'ontogenèse il se développe à partir de l'ectoderme. Il comprend une membrane cuticulaire éversible et des parties et appendices fonctionnels. Avant l'accouplement il est situé dans l'abdomen en position ventrale. De part et d'autre du vestibulum, qui débouche dans l'orifice génital, sont situées les cornules, appendices étroitement repliés, et leur sécrétion de couleur orange. Jusqu'à présent on sait seulement qu'il existe des cellules glandulaires sur les cornules et qu'elles commencent à sécréter à peine deux jours après l'émergence. Nous avons dans ce travail étudié la structure des glandes. Pour cela nous avons effectué des coupes de cornules chez des mâles âgés de 1 à 6 jours pour les étudier en microscopie optique (montage à la paraffine ou à la congélation) et en microscopie électronique. Chaque cornule comprend une partie oblongue ventrale, la cornule ventrale (vCo), et une petite protubérance dorsale, la cornule dorsale (dCo) (fig 1). Les cellules glandulaires s'étendent de la pointe de la cornule ventrale jusqu'à la cornule dorsale. Elles entourent entièrement la pointe, ne sont denses et concentrées que le long des bords de la cornule et présentes sporadiquement dans la partie médiane, où elles forment une structure réticulée (fig 2). La cornule dorsale est presque totalement garnie de cellules glandulaires. La glande forme au total une bande d'environ 5 mm de long. Les coupes à la paraffine et les coupes à la congélation (figs 3–5) montrent qu'il s'agit d'une glande épithéliale monocouche qui se forme dans des régions particulières de l'épiderme. La membrane basale est souvent sous-tendue d'une couche de tissu adipeux (fig 4). Parfois des œnocytes sont étroitement appliqués contre l'épithélium glandulaire (fig 5). Ces cellules glandulaires sont oblongues et renferment principalement du reticulum endoplasmique rugueux et de nombreuses mitochondries (figs 6–8). Les noyaux sont situés en position apicale près de la cuticule et renferment plusieurs nucléoles. On n'a pas trouvé d'appareil de Golgi. De nombreuses microvillosités se forment à la limite de la cuticule. Des projections cellulaires, souvent ramifiées, pénètrent dans la cuticule (fig 7). Celle-ci est constituée de microfibrilles qui délimitent deux zones de densité différente : in situ les fibrilles au-dessus des cellules glandulaires sont en couche lâche ; près du lumen de la cornule elles forment une couche dense avec des vacuoles et des replis. On n'a pas trouvé de pores. La sécrétion est présente sous forme de granules, isolés ou groupés, entre les fibrilles (figs 8 et 9). Les plus gros granules et les amas les plus denses sont situés près du lumen. Le fin système de papilles et de canicules à la surface de la cuticule renferme aussi la sécrétion. Outre les pigments oranges spectaculaires, la sécrétion de la glande des cornules contient aussi des stéroïdes, du cholestérol, des lipides, des cires, des acides gras et des hydrocarbures (Reder et Veith, comm pers). On suppose que la sécrétion est synthétisée, comme dans le complexe des glandes cirières de l'ouvrière (Cassier et Lensky, 1995), par une fonction synergétique des cellules glandulaires, des œnocytes et des cellules adipeuses. Au cours de l'accouplement la couche la plus externe de la cuticule et la sécrétion se détachent des cornules et constituent la couche externe du signe de fécondation. La fonction biologique de ce processus reste à ce jour inconnue.

***Apis mellifera* / organe reproducteur / mâle / endophallus / glande cornule**

Zusammenfassung — Die Cornualdrüse der Drohnen (*Apis mellifera* L): Bau und Sekretion. Der Endophallus der *Apis*-Drohnen ist hoch spezialisiert. Er entwickelt sich ontogenetisch aus dem Ektoderm und besteht aus einer ausstülpbaren kutikulären

Membran mit verschiedenen funktionellen Teilen und Anhängen. Vor der Paarung liegt er ventral im Abdomen. An der Genitalöffnung beginnt das Vestibulum, von dem paarige, eng zusammengefaltete Anhänge mit einem orangefarbenen Sekret abzweigen: die Cornua (Hörnchen). Bisher ist nur bekannt, daß Drüsenzellen an den Cornua vorkommen, die knapp 2 Tage nach dem Schlupf der Drohnen beginnen, das Sekret zu sezernieren. In dieser Arbeit soll der Bau der Drüse untersucht werden. Dazu wurden ein- bis 6-tägige Drohnen präpariert, es wurden Paraffin- und Gefrierschnitte für Lichtmikroskopie und Schnitte für das Elektronenmikroskop angefertigt. Die paarigen Cornua sind jeweils in einen ventralen langestreckten Schlauch, das ventrale Cornu (vCo) und einen dorsalen kleinen Höcker, das dorsale Cornu (dCo) gegliedert (Abb 1). Die Drüsenzellen erstrecken sich von der Spitze des ventralen Cornus bis zum dorsalen Cornus. Während sie die Spitze ganz umschließen, liegen sie im weiteren Verlauf nur an den Seiten des Cornus dicht und konzentriert, zur Mitte hin kommen sie nur vereinzelt vor, wo sie netzartig miteinander verbunden scheinen (Abb 2). Das dorsale Cornu ist fast vollständig mit Drüsenzellen versehen. Insgesamt ergibt sich für die Drüse eine bandartige Form in einer Länge von etwa 5mm. Die Paraffin- und Gefrierschnitte (Abb 3–5) zeigen, daß es sich um eine einschichtige Epitheldrüse handelt, die in speziellen Regionen der Epidermis ausgebildet ist. An die Basalmembran ist oft Fettgewebe angelagert, manchmal wurden auch Oenozyten an den Zellen beobachtet. Die Drüsenzellen sind langgestreckt, sie enthalten vor allem rauhes endoplasmatisches Reticulum und zahlreiche Mitochondrien (Abb 6 und 8). Die Kerne liegen apical nahe der Kutikula und haben mehrere Nucleoli. Es wurden keine Golgi Apparate gefunden. An der Grenze zur Kutikula sind meist Mikrovilli ausgebildet. Zellfortsätze, oft verzweigt, dringen in die Kutikula vor (Abb 7). Die Kutikula besteht aus Mikrofibrillen, die durch verschieden dichte Lagerungen zwei Zonen bilden: in situ sind die Fibrillen über den Drüsenzellen locker gelagert, nahe dem Lumen der Cornua bilden sie eine dichte Schicht mit Hohlräumen und Gängen. Es wurden keine Poren gefunden, das Sekret findet sich in Form von Granula zwischen den Fibrillen, entweder einzeln oder in Gruppen (Abb 8 und 9). Die größten Sekretgranula und dichtesten Ansammlungen liegen nahe dem Lumen der Cornua. Bei der Eversion des Endophallus wird die Kutikula gedehnt, die Hohlräume platzen auf und die Sekrettropfen verschmelzen zu einer Schicht. Da die Kutikula an der Oberfläche trotz der Dehnung Noppen und Kanälchen formt, wird das Sekret dort weiter festgehalten. Das Sekret der Cornualdrüse enthält neben den auffälligen orangefarbenen Pigmenten noch Steroide, Cholesterol, Lipide, Wachse, Fettsäuren und Kohlenwasserstoffe (Reder und Veith, pers. Kommunikation). Es wird vermutet, daß es ähnlich wie bei dem Wachsdrüsenkomplex der Arbeiterinnen (Cassier und Lensky, 1995) durch eine synergistische Funktion von Drüsenzellen, Oenozyten und Fettzellen synthetisiert wird. Während der Paarung löst sich die äußerste Schicht der Kutikula mitsamt dem Sekret von den Cornua und bildet an den Seiten die äußere Schicht des Begattungszeichens. Die biologische Funktion bei diesem Vorgang ist noch nicht geklärt.

***Apis mellifera* / Drohn / Begattungsorgan / Endophallus / Cornualdrüse**

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