Ultrastructure of Apis mellifera hypopharyngeal gland[+]

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The hypopharyngeal glands, begin to differentiate in very young pupae and are still undeveloped at the time the imago emerges. They are acinous glands in which each cell function as unicellular units. Each cell possesses a relatively long and unbranched duct that takes a sinuous course around the nucleus (Kratky, 1931; Beams and King, 1933; Beams and colls, 1959).

The glands are specially favorable for a study of morphology of secretion, for it has a unique secretory cycle and it is possible to obtain all stages of cellular differentiation. Newly emerged workers of summer bees, about 5 or 6 days old, begin to secrete the royal jelly and continue through a dozen days after which the hypopharyngeal glands degenerate. In the winter bees this cycle does not occur and the gland remains hypertrophied until the spring

The royal jelly has a very complex composition (Rembold, 1964), including lipids, vitamins, proteins. Besides the royal jelly the hypopharyngeal glands also secrete invertase (Kratky, 1931; Hitchcock, 1956; Simpson, 1960 and 1961; Simpson and colls., 1968; Maurizio, 1962) and one enzime that facilitates the oxidation of glucose to an acid, which ensures the invariable acidity of honey (Gauhe, 1940). The hypopharyngeal glands are, therefore, exceptional in the great variety of substances they secrete. In summer bees the royal jelly secretion reaches its peak in 10 to 12 day-old workers and the invertase increases as the bee gets older (Sympson and colls., 1968). The winter bees have glands rich in invertase and their pherograms do not change with age (Halberstadt, 1966). It is not certain that both, invertase and royal jelly, cannot be produced simultaneously, but invertase production seems to continue even after partial degeneration of the gland (Kosmin and Komarov, 1932; Butler and Simpson, 1951). The secretion masses visible in the glands by light microscopy are probably royal jelly. The inver-

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tase - containing secretion is not separately visible, even in glands where the pherograms show that it is abundant.

The scope of the presente work is the study of the morphological changes, occuring in the glandular cells during the secretion cycle and the possible differences in the secretion type. For this, honeybee workers were studied in three different stages of development: 1) newly emerged bees; 2) nurse bees; and 3) field bees.

The hypopharyngeal glands were dissected from the workers heads directly into the fixative 1% Osmium tetroxide (Palade, 1952), buffered at pH 7.4, and 3% Glutaraldehyde (Sabatini and al., 1963), with post-fixation in 1% Osmium tetroxide solution, buffered with phosphates at pH 7.4. The glands were dehydrated through a graded series of alcohols and embedded in Epon. This sections were cut on a Porter Blum MT2 ultramicrotome with glass knives. The sections were double-stained with uranyl acetate and lead hydroxide (Millonig, 1961) and observed in a **Siemens** electron microscope. Thick sections were cut for light microscopic studies and stained with 1% toluidine blue and PAS.

Results

The hypopharyngeal glands of the honeybee worker function as unicellular gland, although several cells are joined together to form an alveolus-like structure. Each cell possesses a relatively long and unbranched ductule that takes a sinuous course in the cytoplasm and finally leaves the cell. In leaving the cells, the ductules form a bundle that occupies the place where the lumen would be on a regular alveolus. This bundle connects the alveolus to the final glandular duct. The appearance of the alveolar secretory cells vary with the bee stage. The cellular organelles suffer modifications during the secretory cycle, mainly the nuclei the Golgi apparatus, the endoplasmic reticulum and the secretion.

Newly emerged bees - The glandular cells of newly emerged bees present their cytoplasm free from secretion. The nucleus has a slight irregular form and medium size. Several nucleoli are seen in this nucleus. The cytoplasm is strong basophylic.

The electron microscopy of these cells showed nuclei with several nucleoli of various size. Some of these nucleoli are attached to a chromosoma and some are free. These nucleoli present a very dense and granular periphe-

ry. Scattered in the nuclear sap small groupings of this granular material are also seen. The pores in the nuclear envelope are very conspicuous in this phase. Whether the particulate material in the nuclear sap passes through the pores into the cytoplasm is difficult to say but in a few cases, granules could be seen inside the pores.

During the development of this phase the endoplasmic reticulum encreases very much. In the begining there are a few membranes and numerous polyribosomes in the cytoplasm. The Golgi apparatus is absent by this time.

Later the membranes of the endoplasmic reticulum proliferate and a typical rough reticulum is presented by the cells. The Golgi is now present and a great quantity os small dense vesicles (400-500Å in diameter) can be seen in its outer surface (Fig. 1).

In these sections the intercellular ductule wall appears constituted by three layers (Beams and colls, 1959). The inner layer is dense, incomplete and probably function as circular staves, keeping the duct open. The middle layer is continuous and granular and the outer layer is the cell membrane (Fig. 2). The secretion (or part of it) collects in large masses around the intercellular ductule, but in newly emerged bees the secretion is not present yet. The ductule, in this phase appears surrounded by microvilli-like structures (Fig. 2 and 3) firmly packed together. Small vesicles, very similar to the ones seen in the Golgi outer zone appear in the bottom of these microvilli. In some cases, morphological evidences of the fusion of these vesicles with the microvilli membrane are present in the micrographs. Besides the small vesicles, some others (800mμ in diameter) containing lower density material are also seen.

The mitochondria are not specially numerous or big, but have preferential distribution, near to the ductule or in the periphery of the cell, associated with the plasmalenma invaginations.

Prior to the appearance of secretion in the cell, the Golgi apparatus hypertrophies and two zones can be distinguished. The outer zone still presents many small vesicles, but the inner zone shows a few slightly irregular granules of great density.

Nurse bees - This phase is characterized by the presence of bug masses of secretion around the intracellular ductule (Fig. 2 and 3). These secretion masses are PAS negative and stain light blue with the toluidine. They have an outer membrane in which the microvilli appear as tubu-

lar invaginations. Inside the secretion masses, sections of the microvilli appear as very long tubules (400-500Å in diameter). The secretion masses are not continuous along all ductule, but form bubbles coinciding with the gaps in the inner layer of the ductule (Fig. 2). In these places the glandular cytoplasm comes close to the granular middle layer of the ductule and a dense, coarse granular material deposits below the cellular membrane. Some of the secretion masses show fibrilar or crystalized material in the interior.

The nuclei in this phase have a very irregular shape, spreading among the secretion masses. The nucleolar material appears fragmented, forming thread like masses that occupies almost all the nuclear sap.

The Golgi continues to produce dense granules in its inner zone different in size and density.

Later in this phase another type of secretion loads the cells. This appears as dense granules (700-800mμ in diameter) scattered in the cytoplasm among the secretion masses.

These granules are similar to the dense ones arising in the inner Golgi zone. They are present in the cells even after the descharging of the secretion around the ductules, and after the nurse stage is over. But they remain in the cell only for a short period at the end of which a new kind of structure appear in the cytoplasm. They are big granules (1 to 2μ in diameter) with an envolving membrane and a dense matrix partially crystalized. Some of them show zones of vesiculation.

Field bees - In field bees glands neither the secretion masses nor the granules are seen in the cytoplasm. The nuclei are again only slightly irregular and the nucleoli and chromatin condensed. The endoplasmic reticulum appear less rich in membranes and cytoplasmic basophylia has also decreased. The Golgi is absent.

In this stage some unusual organells show up in the cytoplasm. They are built as smooth membranes concentrically arranged and frequently surrounding lipoid droplets. Such structures are visible with light microscopy and are PAS positive.

Discussion

Much of the structure herein described for the hypopharyngeal glands has been previously reported by other investigators (Beams and colls., 1959; Painter and Biesele,

1966a and b) but some new details and discrepancies must be discussed.

Beams and colls. (1959) could not completely establish the origin of the membranes separating the secretion masses from the cytoplasm. In our micrographs it is clearly shown that these membranes are the glandular cell apical membrane, including forming microvilli. Beams and colls. (1959) show the microvilli in their micrographs but label it as endoplasmic reticulum inside of the secretion masses.

The points where the cellular membranes touch the granular layer of the intercellular ductule are points of adherence between the ductule and the glandular cell. We think that the dense granular material there acumulated functions as an atypical hemidesmosome.

The hypopharyngeal glands present at least two kinds of secretion: 1) The masses around the intracellular ductule; and 2) The granules with 800mμ in diameter. The masses appear first in the cell, preceding the nurse duties of the worker in the hive, continuing during the entire period of this function. The granules start to appear in the final third of this period and are seen in the cell until the workers became forages. Among the granules at least two kinds can be differentiated by the content density. It is difficult to accompany the secretion from the Golgi apparatus to the masses, but there are some evidences that the small vesicles on the Golgi outer zone contribute to its formation. Painter and Biesele (1966a) show a semidiagrammatic representation of the cellular organelles during the secretory cell cycle in which the endoplasmic reticulum tubules are continuous with the secretion on the ductules. They also mention the presence of fibrous substances in the reticulum cistarnae. In this work those features were not seen.

The secretion on the masses is delivered by the cells, into the ductule lumen, through the porous granular layer in the gaps of the inner layer. It is believed to be the royal jelly because its appearance is concomitant with the nurse duties of workers. The granules arising in the inner Golgi zone might contain the zymogenic secretion. How they are eliminated from the cell were not seen, but They are absent from field bees. In this phase some different larger granules can be seen in the glandular cytoplasm. They are provided with an envolving membrane and frequently present crystal-like structures and vesiculation. We think that they have some morphological features of lysosomes.

The organelles constituted by smooth lemellae concentrically arrayed are typical of degenerating gland cells (Cruz-Landim, 1968) and are similar to the parasomes of Gabe and Arvy (1961). The presence of lipidic droplets in degenerating cells is also frequent in bees (Cruz-Landim and Puga, 1966).

The nuclei pass through modifications in their nucleoli behavior that must be correlated with the liberation of the ribosomes during the secretion cycle. Painter and Biesele (1966a) believe that endomitosis precendes the secretory stage. The encrease in glandular cell ploidy was verified in many insects including bees (Cruz-Landim and Mello, 1969), and its occurence is quite possible in the hypopharyngeal glands.

Summary

The hypopharyngeal gland cells have a well defined secretory cycle. The cells from young bees show an abundance of rough endoplasmic reticulum which often is arranged as parallel membranes. In this phase, secretion is not seen in the cell. In a more advanced phase the cells still have a large amount of endoplasmic reticulum and many Golgi zones also appear. The Golgi complex has many small vesicles (400-500Å in diameter) in its outer zone and granules 800Å in diameter) in its inner zone. Later, the cells appear loaded with secretions which are at least of two types: secretion masses that collect around the ductule and probably diffuse into its lumen, and dense granules. The dense granules appear a little later. In the old worker, therefore, in the late stage of their secretory cycle, only granules remain and the endoplasmic reticulum is reduced. In this phase (in most of the cells), some unusual structures appear. They are built as smooth membranes, concentrically arranged, and they surround a dense lipid-like drop.

How the secretion passes from the Golgi to the masses surrounding the ductule or how the granules desappear from the cell is uncertain; nevertheless, microvillus-like structures are always present. These structures are packed in the initial stage, before the secretion appears, and also at the end of the cycle when the secretion is no longer present. In the initial stage of the formation of the masses, many small vesicles appear in the bottom of these microvilli. They are very similar to the ones which are found in the outer Golgi zone.

The honeybee hypopharyngeal gland has a double function, i.e, first it acts as a nurse gland to produce the

royal jelly, and later it acts as digestive gland which produces enzimes. It appears that the big masses which surround the ductules are the first type of secretion (royal jelly) because their appearance coincides with the function of nurse by the worker. Perhaps the granules constitute the zymogenic secretion.

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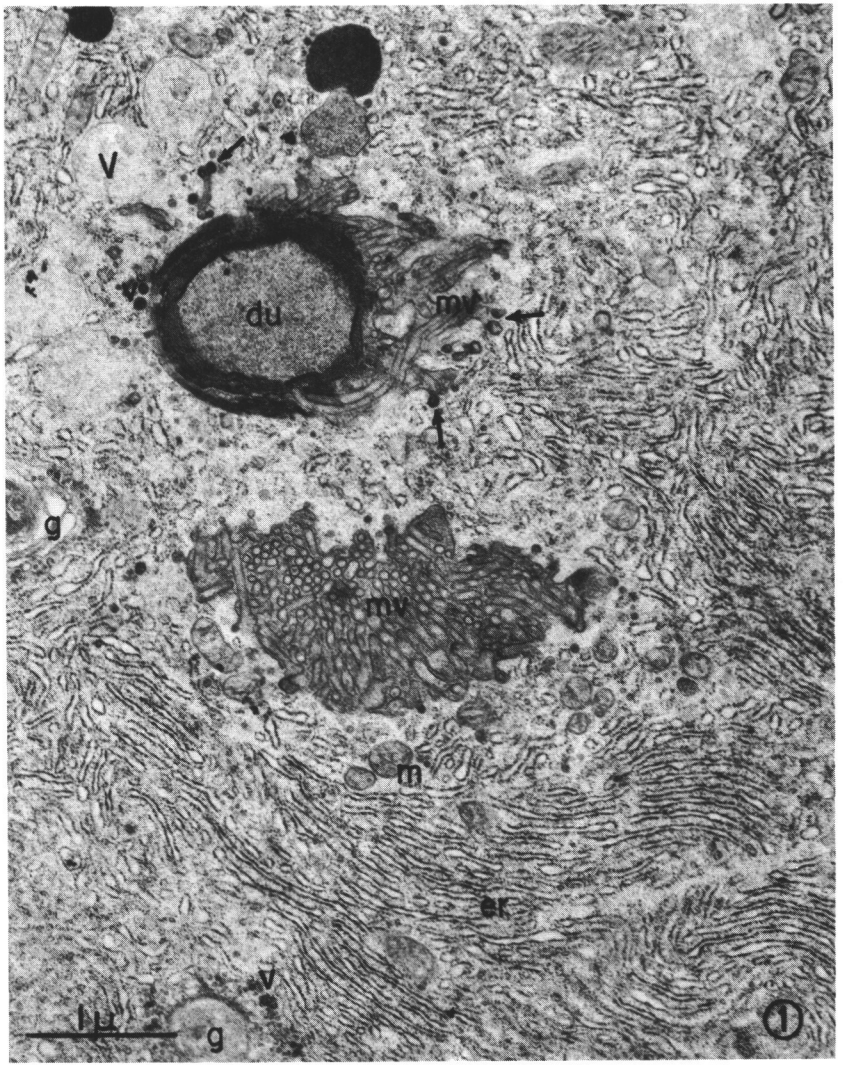
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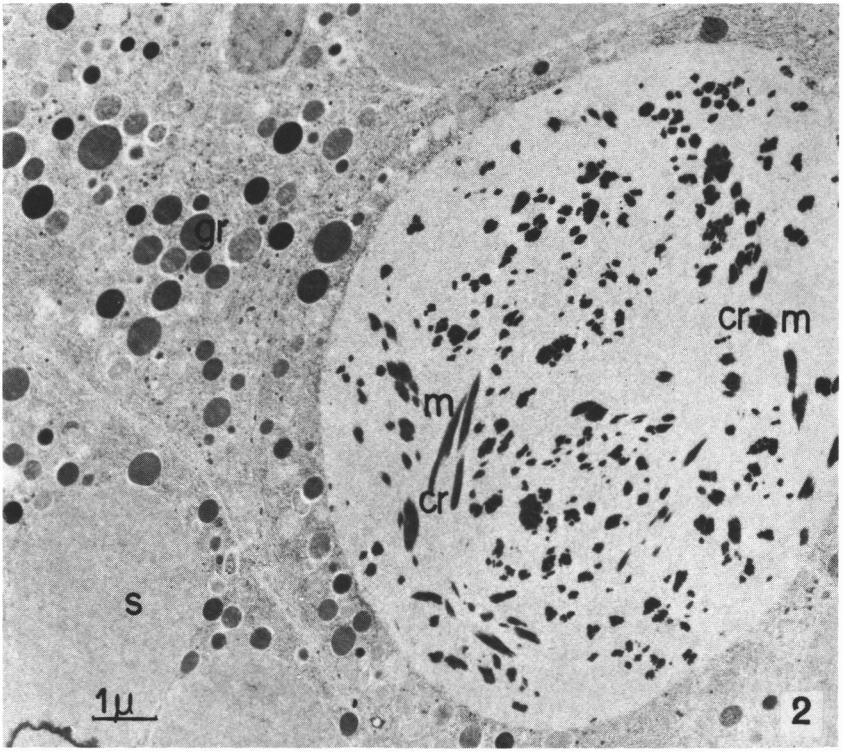
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Figures

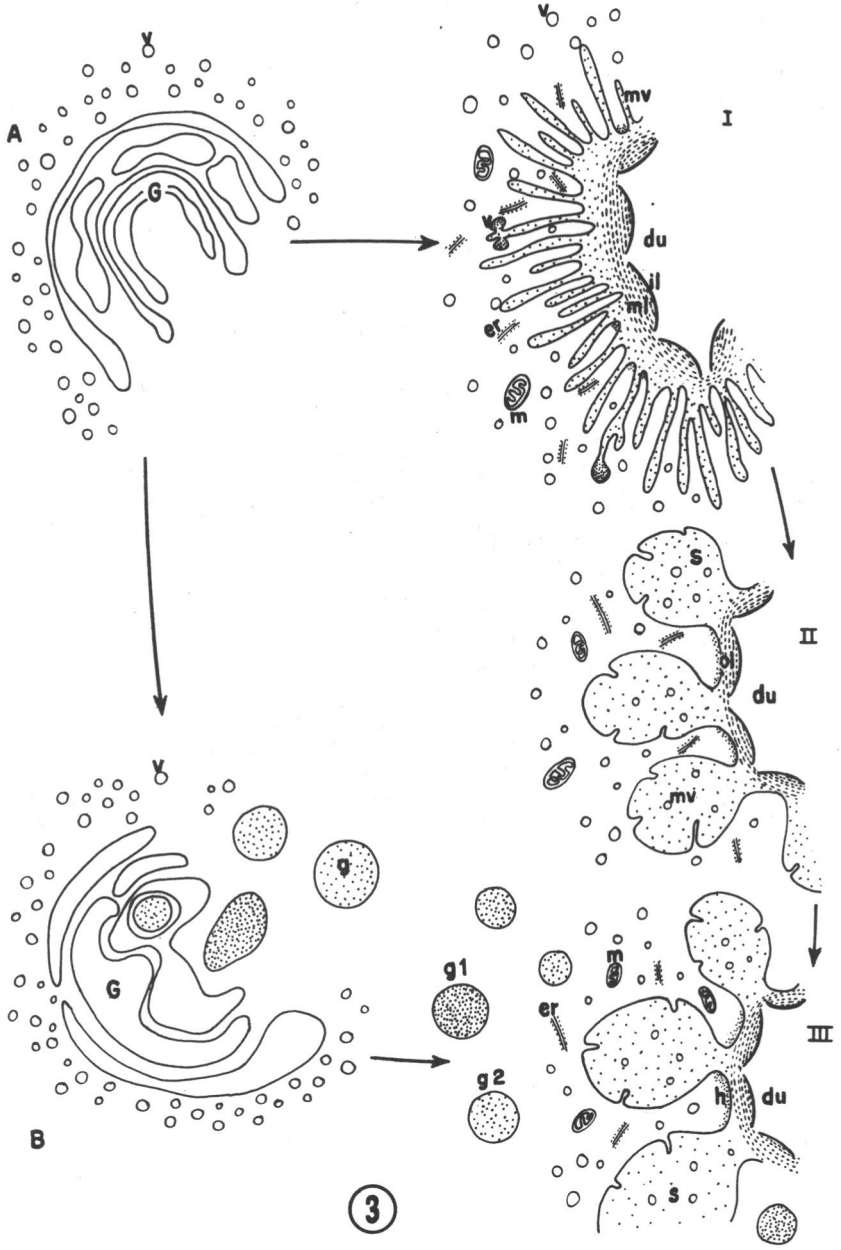
Fig. 1 - Glandular cell from a young worker showing microvilli (mv) around the intracellular ductule (du). Note the small vesicles (v) in the Golgi outer zone and microvilli bottom. In some places (arrows) suggestions of fusion between microvilli and vesicles are seen.

er = endoplasmic reticulum;
m = mitochondrion;
V = large vesicles.

Fig. 2 - Secretion masses (s) and granules (g) in the secretory cell.

cr = crystal-like structures inside the secretion masses

Fig. 3 - Schematic representation of the secretory cycle. On a newly emerged bee the Golgi appear as in a and the secretion masses are absent (I). In the nurse phase the Golgi is as in a and the secretion masses appear around the ductule (II). Later in this same stage the Golgi would desappear and granules would be seen among the masses (III).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