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The Structure and Possible Function of the Calyx Region of the Female Reproductive System in the Granary Weevil, *Sitophilus granarius* (L.) (Col.)

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ABSTRACT. The structure of the calyx region of the female reproductive system in *Sitophilus granarius* (L.) has been described and its possible function suggested. The calyx region is composed of columnar epithelial cells, which are typical secretory cells that release large numbers of granules into the lumen. The results of the investigation indicate that the secretory droplets of the calyx may act as a lubricant during the passage of the egg through the egg canal. These droplets may also play a major role in the formation of the plug sealing the aperture of the hole where the eggs are laid within.

The anatomy and histology of the female reproductive system in *S. granarius* have been described by Krautwig (1930), Vernier (1970) and King and Al-Khalifa (1980). The typical female reproductive system consists of a number of ovarioles forming a pair of ovaries. Each of these ovaries opens into a separate lateral oviduct, the proximal part of which is narrower and is usually referred to as a calyx, through which an egg passes before it is finally deposited in the hole which the female makes within the wheat grains (Richards 1947). Very little information is available on the structure and function of the calyx and oviduct in Coleoptera. The present study investigates the structure and possible function of the calyx cells in *S. granarius*.

Material and Methods

Stocks of *S. granarius* were kept at 25°C on a 12 hr photoperiod. Newly emerged insects were placed in 1 lb honey jars and allowed to feed and oviposit in wheat grains.

For histological and histochemical investigations, females were dissected in insect saline, their ovaries removed, fixed in formol calcium-cadmium or Bouin's fixative depending upon the test to follow, dehydrated in ethanol, embedded in fibre-wax and sectioned at a thickness of 7-9 μm. Some fibre-wax and 1-2 μm. epoxy sections (see below) were histochemically stained for protein with the mercury-bromophenol blue (Bonhag 1955) and a 1:1 mixture of 1% methylene blue in 1% borax and 1% mallory's Azur II (Richardson *et al.* 1960). For carbohydrates, the periodic acid Schiff's (PAS) and Alcian blue techniques were used, while the Sudan Black B technique was used for the lipid test (All in Pearse 1960, Lillie 1965). For general histology, sections were stained with Mallory's triple stain.

For electron microscopy, females were dissected in ice-cold 5% gluteraldehyde (TAAB) fixative, the ovaries removed, placed in fresh fixative in 0.1 *M* sodium cacodylate and 0.17 *M* sucrose at pH 7.4 for 30 min and then washed for 24 hr in cacodylate buffer with 0.34 *M* sucrose at 0-4°C. Tissues were post-fixed in 1% osmium tetroxide in 0.1 *M* sodium cacodylate and 0.17 *M* sucrose at 0-4°C for 90 min, dehydrated in graded cold acetones and transferred to an acetone/TAAB resin mixture. Sections were cut on a Cambridge instruments Mark I Huxley microtome, mounted on untreated copper grids, stained in 30% uranyl acetate in methanol for 30 min and aqueous lead citrate for 8 min and viewed in a A.E.I. Corinth 275 electron microscope.

Results

Morphologically, all the cells of the calyx region appear to be of a single type. The basal surface of the calyx cells is characterized by numerous, deep folds which become even more discernible during the synthesis cycle of the cells (Fig. 1 and 2). It is covered on its outer side by a thin basement membrane and muscle sheath (Fig. 1 and 2). The basal two-thirds of the opposing lateral cell membrane of these cells forms a relatively straight profile, while the apical third is highly interdigitated and tightly bound by a separate junction (Fig. 1,3 and 4). Apically, where the lateral cell membrane meets with the lumen, there are terminal bars (Fig. 4).

Although numerous profiles of rough endoplasmic reticulum (RER) are found scattered throughout the cytoplasm of these cells, a high proportion of this membrane system is located in the basal region where much of the RER is assembled in parallel arrays (Fig. 2 and 3). The presence of abundant free ribosomes and numerous stalks of RER explains the strong basophila revealed histochemically by the pyronin/methyl green technique (Table 1).

Table. 1. The results of histochemical tests on the calyx region of *S. granarius* (−, negative; +, positive; ++, strongly positive).

Test	Calyx cells	Contents of calyx lumen
Mercury-bromophenol-blue	+	++
Methylene blue	++	++
Periodic acid Schiff (PAS)	+	+
Alcian blue	+	+
Sudan Black B	+	−

A number of golgi complexes are present and these contain secretory granules in various stages of condensation. The characteristic configuration of these organelles consists of several parallel arrays of smooth paired membranes with dilated ends (Fig. 2, 5 and 8). Associated with these are vesicles of various sizes and a continuity was often noted between a vesicle and a membrane pair. All this suggested that there is an active secretion taking place. The electron-dense secretion is passed to the lumen of the calyx, in one of two conditions; either the small vesicles containing the adielectronic material fuse to form larger droplets which are then passed to the lumen of the calyx, or the small vesicles together with their contents are passed directly to the lumen without fusion taking place (Fig. 6, 7 and 9). The method of passage of these inclusions into the lumen is similar to that reported by Palade (1959) for zymogen granules in the pancreas of the guinea pig. The membrane surrounding the secretory granules fuses with the apical plasma membrane of the calyx periphery cells and after membrane rupture, the contents of the vesicles are released into the calyx lumen. A high proportion of these droplets is distributed throughout the cytoplasm and mainly in the apical portion prior to passing between the microvilli into the lumen (Fig. 7 and 9). Microtubules have various orientations and are abundant throughout the cytoplasm. They presumably assist in the transport of material through the cytoplasm (Fig. 4 and 9). Adjoining the calyx lumen, the cells have a microvillous border (Fig. 6). Droplets similar to those in the cells of the calyx can be seen in the lumen (Fig. 10). These have been released from the calyx cells and have filled the calyx lumen.

Discussion

The histochemistry, structure and organelle content of the calyx columnar epithelial cells indicate that these cells are fairly typical of secretory epithelia. This interpretation is sup-ported by the presence of well developed RER, numerous free ribosomes and Golgi complexes suggesting a general hypothesis of protein synthesis (Caro and Palade 1964). This was also reported in other insect secretory

epithelia. (Atkins *et al.* 1965, Rafai and King 1972, Szopa 1981 and Hamon *et al.* 1982).

Microtubules have been found in a number of insect cell types: in the sternal gland of *Zootermopsis nevadensis* (Hagen) (Satir and Stuart 1965); in the accessory glands of the male *Schistocerca gregaria* (Odhiambo 1969) and in the cement gland of *Rhodnius prolixus* ((Lococo and Huebner 1980). It is generally believed that microtubules function as a microcirculatory and transport system for small molecules and cell secretory products and are important for the maintenance of the cytoarchitecture of certain specialised cells (Satir and Stuart 1965, Odhiambo 1969).

The numerous deep invaginations of the basal surface of the calyx epithelial cells increase the absorptive area of the cells, thus permitting an increased intake of metabolites from the hemocoele. This type of highly folded basal surface is also present in other secretory insect cells (Hamon *et al.* 1982, Meola 1982). The septate junction has been recorded in other insect epithelial tissues (Farquhar and Palade 1963, Meola 1982). They suggested that the functional significance of the septate junction is cell to cell adhesion.

Sitophilus granarius has been observed in our laboratory (unpublished data) to form a plug that covers the aperture of the hole made in the wheat grain in which the egg is laid. Similar observations were also made in *Schistocerca gregaria* by Szopa (1981). The materials for the formation of such a plug might have come from the secretions of calyx cells due to the absence of accessory glands in the reproductive system of this insect as well as in other Coleoptera (Williams 1945, Waloff and Richards 1958, Gupta and Riley 1967, Hamon *et al.* 1982). Moreover, such secretions being sufficiently copious might also act as a lubricant for the passage of the egg down the oviduct as have been reported in *Dahlobominus fuscipennis* (Zett) and *Nasonia viterripennis* (Walker) (Wilkes 1965, Rafai and King 1972).

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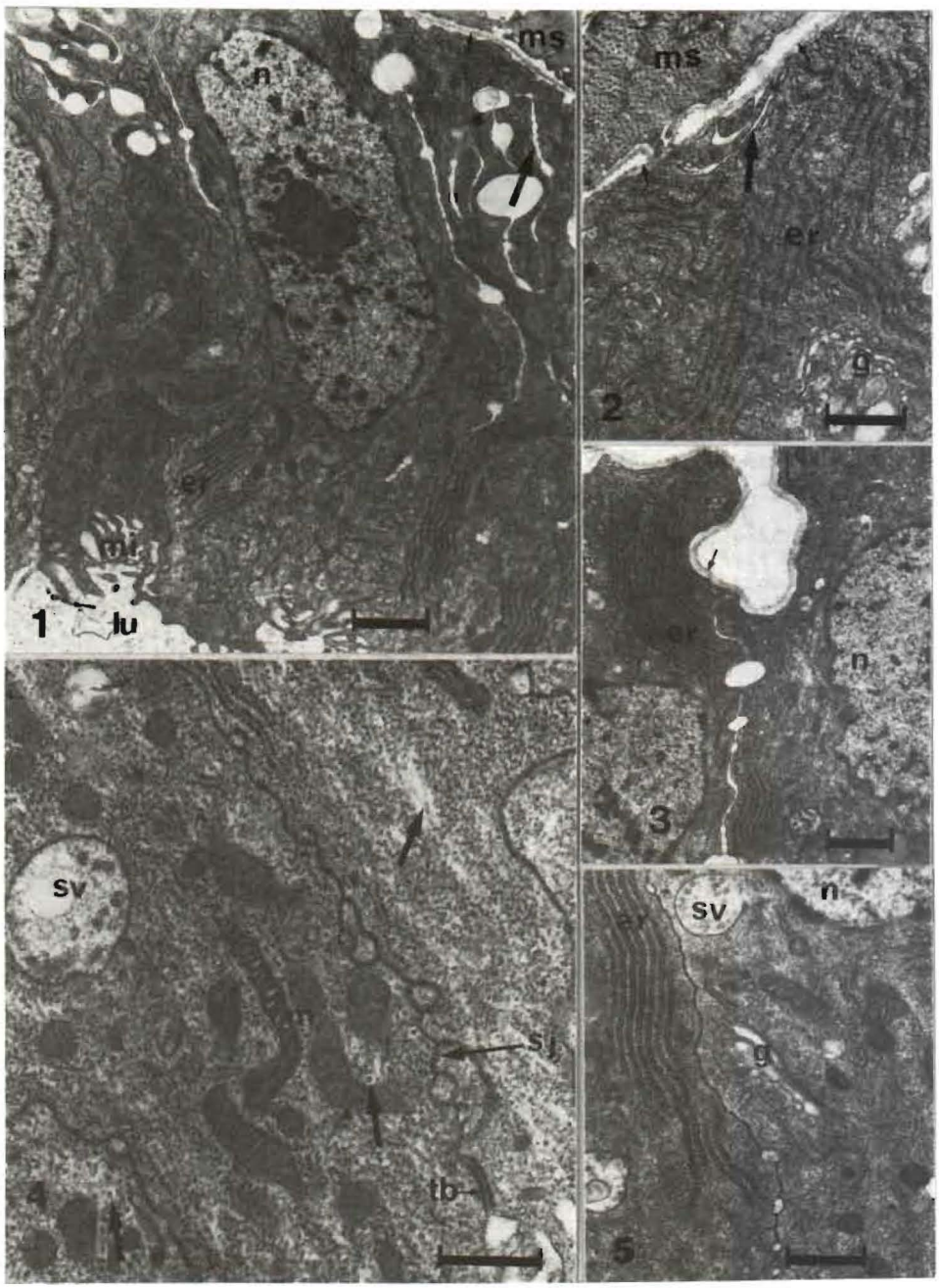
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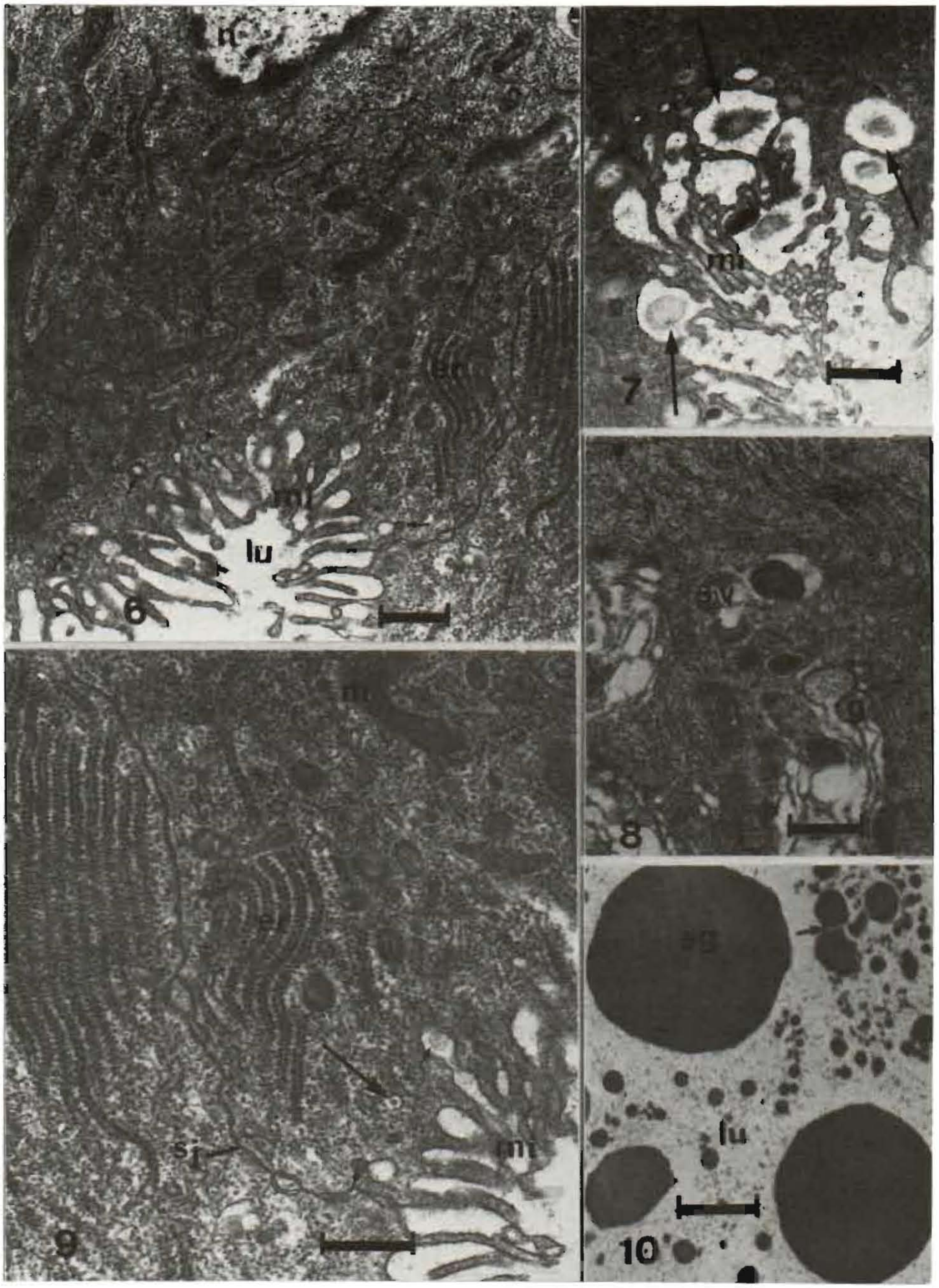
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Fig. 1. A low power micrograph showing a general view of calyx columnar cells with central nucleus (n) and folded basal surface (large arrow). Basement membrane (small arrow), lumen (lu), muscle sheath (ms), microvilli (mi) and rough endoplasmic reticulum (er).
scale bar = 1 μm.

Fig. 2. Basal portion of calyx columnar cell showing large active golgi complex (g). Note the folded basal surface (large arrow). Basement membrane (small arrow), muscle sheath (ms) and rough endoplasmic reticulum (er).
scale bar = 0.5 μm.

Fig. 3. Basal portion of calyx columnar cells showing well developed rough endoplasmic reticulum (er). Basement membrane (arrow) and nucleus (n).
scale bar = 0.5 μm.

Fig. 4. Electron micrograph showing septate junctions (sj) at the apical end of adjacent cells. Note the microtubules with various orientations (arrows). Mitochondria (m), secretory vesicle (sv), and terminal bar (tb).
scale bar = 0.5 μm.

Fig. 5. Apical portion of calyx columnar cell, showing well developed rough endoplasmic reticulum (er) and golgi complex (g). Nucleus (n) and secretory vesicle (sv).
scale bar = 0.5 μm.

Fig. 6. Electron micrograph showing the passage of secretory veiscles (arrows) between microvilli (mi). Lumen (lu), nucleus (n) and rough endoplasmic reticulum (er).
scale bar = 0.5 μm.

Fig. 7. Electron micrograph of the apical portion of a columnar cell showing the accumulation and passage of secretory vesicles (arrows) into the calyx lumen. Microvilli (mi).
scale bar = 0.5 μm.

Fig. 8. Micrograph showing well developed golgi complex (g) and associated secretory vesicles (sv) in the calyx columnar cells. Rough endoplasmic reticulum (er).
scale bar = 0.5 μm.

Fig. 9. An enlarged portion of Fig. 6 showing the septate junction (sj) between adjacent cell membranes. Note the passage of secretory vesicles (small arrows) between microvilli (mi). Microtubules (large arrow), mitochondrion (m) and rough endoplasmic reticulum (er).
scale bar = 0.5 μm.

Fig. 10. Low power micrograph of the calyx lumen (lu) showing different sized secretory globules (sg).
scale bar = 2 μm.

التركيب الدقيق والوظيفة المحتملة لمنطقة الكأس في الجهاز التناسلي الأنثوي في حشرة سوسة الحبوب

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درس التركيب الدقيق لخلايا منطقة الكأس في حشرة سوسة الحبوب *(Sitophilus granarius)*، ووجد أن منطقة الكأس في الجهاز التناسلي لأنثى هذه السوسة تتكون من طبقة واحدة من الخلايا الطلائية العمادية المهدبة. ولقد لوحظ أن هذه الطبقة تقوم بتكوين عدد كبير من الحويصلات الإفرازية تتجه نحو تجويف منطقة الكأس.

تعمل هذه الحويصلات الإفرازية كبطانة تزليق تسهل عملية إنزلاق البيضة من قناة البيض كما تقوم هذه الحويصلات الإفرازية بعمل أغطية للفتحات التي تثقبها الأنثى في حبوب القمح بعد أن تضع بيضها في هذه الثقوب.