MORPHOLOGY OF MYOEPITHELIUM AND BASAL SURFACE OF THE GLANDULAR CELLS IN THE HARDERIAN GLAND OF THE GOLDEN HAMSTER

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Abstract

Background: Observations made in golden hamster Harderian gland have shown that the female glands contained a higher numerical density of the myoepithelial cells compared to those in the male glands. The findings were based on a single report and did not indicate clearly the part of the gland that was examined.

Broad Objective: To study the morphology of the myoepithelial cells and the basal surfaces of the glandular cells in the small and large lobes of the Harderian gland.

Study Setting and Methodology: The study used the scanning electron and lesser confocal microscopes to observe the Harderian gland of the golden hamster.

Results: The morphology of the myoepithelial cells of the large lobes both in male and female glands appeared to be similar. The myoepithelial cells of the small lobes from both male and female glands also appeared to be similar but unlike those in the large lobes contained broader cytoplasmic processes

Conclusions: The morphology of the myoepithelial cells in the male and female glands does not show sexual differences an indication that these cells may be not sexually regulated. The findings open way for future research towards the understanding of the myoepithelial cell physiology in the Harderian gland of the golden hamster.

Key Words: Harderian gland, Golden hamster, Basal surface, Myoepithelium, Glandular cells

Introduction

The Harderian gland was discovered in the fallow deer over 300 years ago and since then it has been described in many animal species including birds, reptiles, amphibians and mammals [1, 2, 3, 4]. In the golden hamster the gland is located in the orbital cavity and has attracted much attention because it shows clear sexual dimorphism in morphology, secretion, lipid metabolism and expression of some compounds such as porphyrins [5, 6, 7, 8, 9]. Like in many other exocrine glands, the secretory endpieces of the Harderian gland of the golden hamster consists of the glandular and myoepithelial cells [10]. The myoepithelial cells are contractile elements that are found within the same basal lamina that surrounds the glandular cells [11].

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They are generally stellate shaped and form a basket-like network around the acini [12, 13, 14, 15, 16, 17, 18]. In the golden hamster the observations made by the scanning electron microscope indicated that the female glands contained a higher numerical density of the myoepithelial cells compared to those in the male glands [19]. The study also reported that the size of the cells is the same both in male and female glands. The findings were based on a single report and did not indicate clearly the part of the gland that was examined. Recent observations have shown that the

Harderian gland of the male and female golden hamster is divided into small and large lobes [9]. In females, there is aclear distinction between the small and large lobes. The large lobe is characterized by black spots; the small lobe resembles the male gland in that it is paler and has very few dark spots [9]. The large lobe appears with black spots and it secretes mainly via exocytosis; apocrine features are rarely seen [2, 9]. The male gland contains also both large and small lobes that appear similar; they are pale and do not have the black spots [9]. It is unknown if the myoepithelial cells are morphologically different in these two lobes. There are no studies that have addressed the appearance of the basal surface of the glandular cells in the secretory endpieces of the Harderian gland of the golden hamster.

This study used the lesser confocal microscope and scanning electron microscope (SEM) to investigate morphological features of the myoepithelial cells in the small and large lobes of the male and female Harderian glands of the golden hamster. Observations were also made on the basal surface of the glandular cells in the small and large lobes.

Materials and Methods

Thirty-five male and female adult golden hamsters (age 10 weeks) were used in the present study. They received commercial food pellets and water ad libitum, and were kept under constant laboratory conditions. The animals were anaesthetized with ether, thoracotomized, and then perfused via the left cardiac ventricle with oxygenated HEPES-buffered Ringer solution (HR) (37°C, pH 7.4, adjusted with NaOH) at hydrostatic pressure (ca. 1m H_2O) for 10 min. The standard HR contained 150 mM NaCl,

4.7 mM KCl, 1.3 mM $CaCl_2$, 1.13 mM $MgCl_2$, 1.0 mM Na_2HPO_4, 5.5 mM glucose, and 10 mM HEPES, 0.2% bovine serum albumin (Sigma, St. Louis, MO, USA), minimal Eagle's medium essential amino acids (Flow Laboratories, Irvine, UK), and 2.0mM L-glutamate.

Fluorescence

Following perfusion with HR the animals (*n=8*) were perfused with phosphate-buffered saline (PBS; 0.1 M, pH 7.4) fixative containing 4% paraformaldehyde at 4° C for 5 min. The Harderian glands were removed from the eye orbit, separated into small and large lobes and stored in the same fixative at 4°C. The samples were cut into small pieces ($1mm^3$) and then placed on glass slides coated with Poly-L-Lysine (Sigma, St. Louis, Mo., USA) and allowed to air-dry. Before staining the dried glandular tissues were re-hydrated for 10 minutes with PBS. The samples were stained by bodipy phallacidin using the protocol by Satoh et al. 1994 [18].

Electron microscopy

The animals (*n=27*) were perfused with HR followed by phosphate-buffered saline (PBS; 0.1 M, pH 7.4) fixative containing 1.25% glutaraldehyde and 4% paraformaldehyde at 4° C for 5 min. For observation of the myoepithelial cells (*n=12*) the large and small lobes of the Harderian gland were separated, then cut into small pieces and treated in 5M potassium hydroxide (KOH) for 30 minutes at 37° C. For observation of the basal surface of the glandular cells (*n=15*), the Harderian glands were removed from the orbit and separated into large and small lobes and then immersed in HR containing 200U/ml collagenase (Worthington Biochemical Corporation USA) for 3-4 hours, followed by treatment in 8N HCl for 40 minutes. The specimens from both groups (for observing the basal surface of the glandular cells and the myoepithelial cells) were treated in iso-amyl acetate for 1 h, followed by critical point drying using liquid CO_2. They were then mounted on aluminum stubs and coated with platinum by the ion-sputter method, and then observed by an S-4100 scanning electron microscope (Hitachi, Japan).

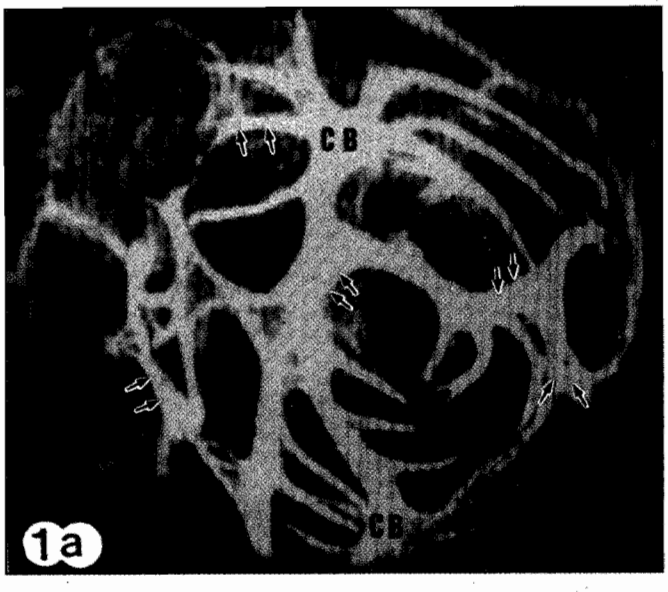
Results

Myoepithelial cells

The configuration of myoepithelial cells were revealed in the small and large lobes of the Harderian gland following staining of the secretory endpieces with bodipy phalacidin and by scanning electron microscope after treatment with KOH. Generally the myoepithelial cells were stellate in shape and gave rise to cytoplasmic processes that formed basket-like network around the endpieces. Morphological differences were observed between the myoepithelial cells of the small and large lobes as outlined below.

The myoepithelial cells of the large lobe in both male and female animals consisted of the cell body and cytoplasmic processes (Fig. 1a,b). Observation by the SEM revealed the cell bodies to be oval or triangular in shape and each gave rise into 3-8 cytoplasmic processes (Fig. 1a,b). The processes were of uniform size both in male and female large glands and they measured between 0.3-1.5μm wide. They gave rise to branches that united with those from the neighbouring processes. At their distal ends the processes split into 3-6 tracts that joined with the processes from the neighbouring cells. The processes did not cover the entire basal surface of the glandular cells; uncovered glandular regions (measuring between 1.2-2.7μm wide) appeared to protrude in a space between the myoepithelial cell processes. The number of cytoplasmic processes and the area that they occupied on the basal surface of the glandular cells appeared to be the same in both male and female glands. There were no differences between the male and female gland; the morphology and configuration of the myoepithelial cells remained similar in the large lobes of both male and female Harderian glands.

The myoepithelial cells of the small lobe appeared to be different from those of the large lobe. In the small lobes it was difficult to locate the cell bodies both under the scanning electron and lesser confocal microscopy (Fig. 1c). The processes were broader (measuring between 1.3-2.6μm wide) than those of the myoepithelial cells in the large lobes. They appeared to run for a long distance around the endpieces giving out short branches that united with those from the adjacent cells. In some cases the processes were very short and gave rise to 4-12 thin branches that united with those from the adjacent cells.



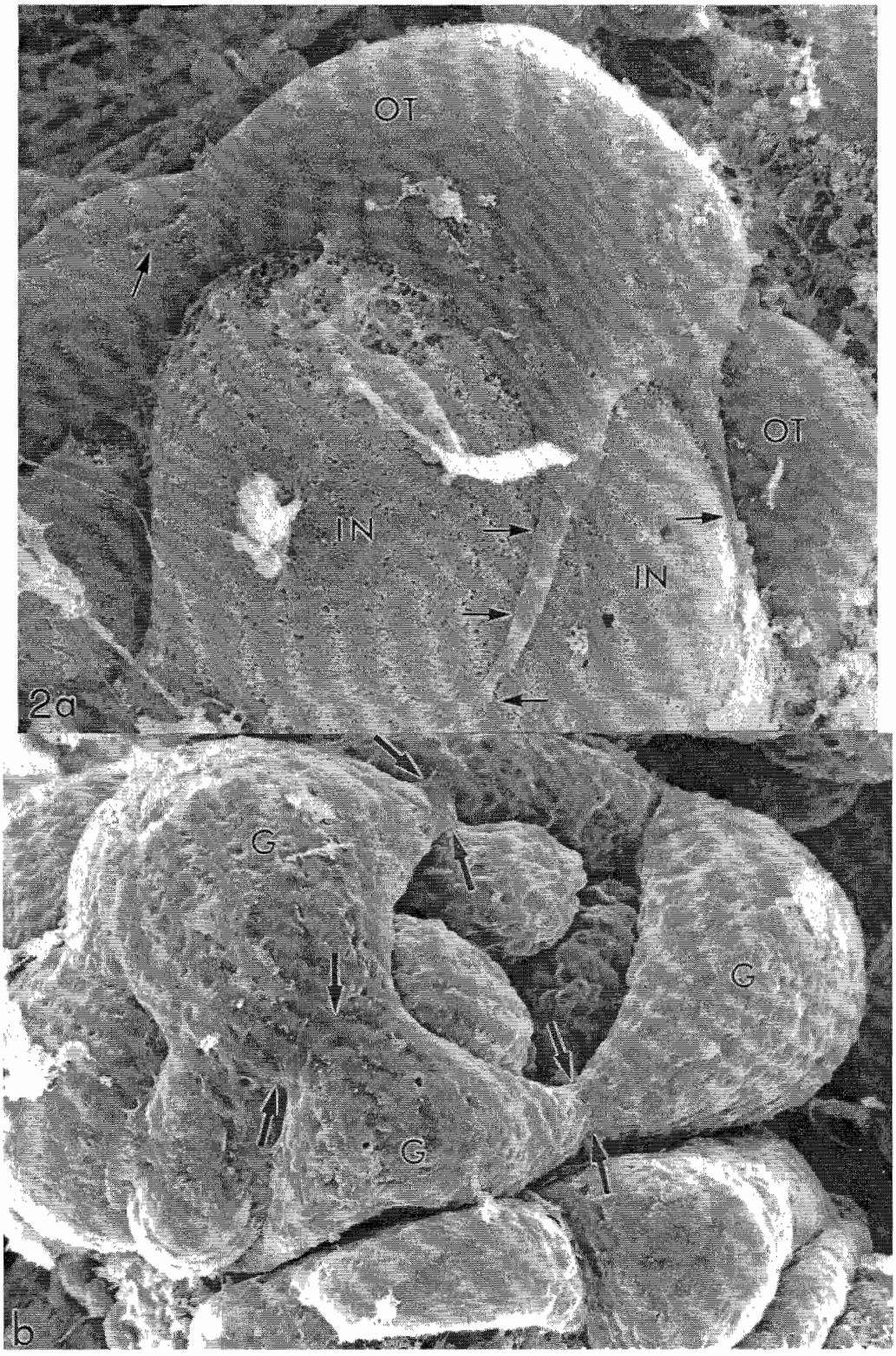
Observation on the basal surfaces of glandular cells

Prolonged collagenous digestion followed by treatment with HCl, resulted into removal of the basement membrane and myoepithelial cells permitting visualization of the basal surfaces of the glandular cells. The female large lobe displayed different arrangement from that of the male glands and female small lobes. The male and female small lobes and the large lobe in males appeared to have a similar morphological arrangement.

Observation of the basal surface of the female large lobe revealed the presence of outer and inner cells (Fig. 2a). The outer cells were oval-round in shape and appeared to sit on top of the inner electron lucent cells. Many outer cells contained 2-3 short rounded cytoplasmic processes that attached to the surface of the inner cells. At the beginning the processes appeared thicker, but they gradually became thinner as they approached their terminal points (Fig. 2a). The outer cells appeared to unite with each other via short stumps. The inner cells were named so because they appeared to be partially covered by the outer cell. Close observations revealed that in the majority of the secretory endpieces the inner cells were more electron-lucent and generally they appeared paler than the outer cells, which were more electron-dense. In a few secretory endpieces 3-4 glandular cells appeared to unite with each other via short stumps to form a circle around the protruding glandular cells (Fig. 2b). These endpieces appeared to have a rough surface and did not show differences in the electron density.

The male large lobe and the small lobes from the male and female glands displayed a common arrangement at the basal surface that was different from that of the female lobes. The glandular cells from these lobes showed short cytoplasmic processes that made extensive interlocking and connection (Fig. 2c). Observation with SEM revealed extensive interlocking among the glandular cells in the male glands and the female small lobes and therefore it was difficult to follow the entire basal surface of most glandular cells. The glandular cells appeared to have many processes of various size and shapes. Some were short and broad; others were long and thin or long and broad. Similarly the processes displayed different course; some appeared to pass beneath those from other adjacent cells and emerge after a short distance to terminate onto the surface of the neighboring cell. Other processes took a straight course to terminate on the surface of the adjacent cells (Fig. 2c). In a few occasions the glandular cells gave rise into broad and long processes that run on top of other cells for a long distance giving out short branches that terminated on the surfaces of the adjacent cells. The long processes could easily be mistaken for myoepithelial cell processes. Despite the wide variation in the size and course of the cytoplasmic processes the cells showed a uniform electron density and it was not possible to identify the inner and outer cell pattern similar to the one observed in the cells of the female large lobes.

Figure 1a-c. The secretory endpieces from the Harderian glands golden hamster showing the profiles of the myoepithelial cells after staining the actin filaments with Bodipy- phallacidin **a,c** and after treatment with 5M potassium hydroxide **(b,c)**. **a)** The lesser-scanning confocal micrograph of the male large lobe showing the location of the cell bodies (*CB*) and the arrangements of the myoepithelial cell processes around the endpieces (*small arrows*). **b)** Scanning electron micrograph of the large lobe from a female gland showing the cell body (*CB*) and the myoepithelial cell processes (*arrows*) on the basal surface of the glandular cells. Note that it is not easy to locate the position of the cell bodies of the myoepithelial cells in this section. x1000 **(a,c)**, x3000 **(b,c)**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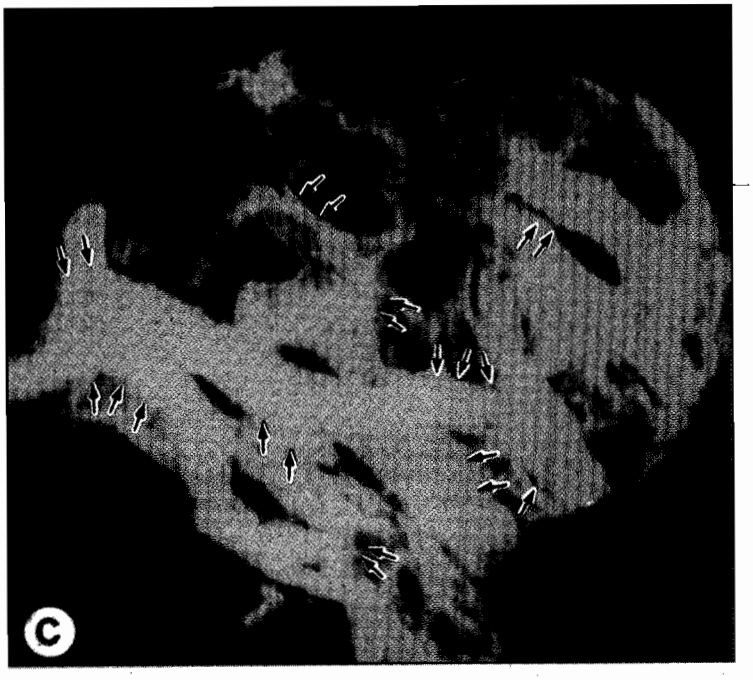

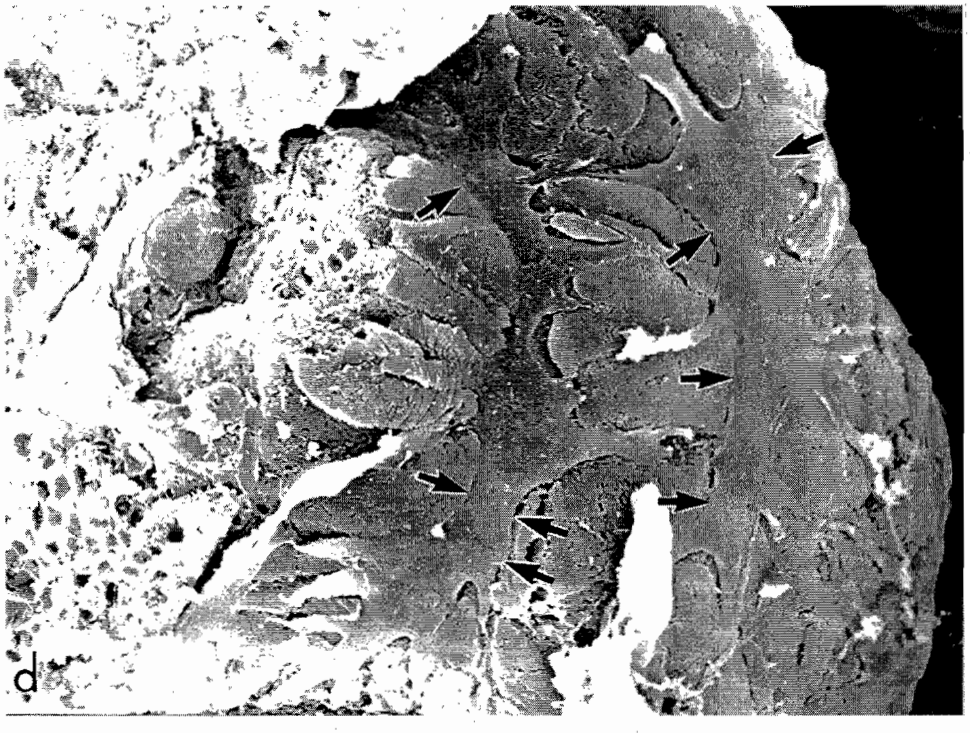

Figure 2a-c. The scanning electron micrographs of the secretory endpieces of the Harderian gland of the golden hamster showing the basal surface of the glandular cells after prolonged collagenase digestion followed by treatment with HCl. **a**) The basal surface of the glandular cells of the large lobe from a female gland showing the outer (*OT*) and inner (*IN*) cells. The outer cells contained rounded cytoplasmic processes (*arrows*) that terminated on the surface of the inner cells (*IN*). **b**) In another observation the glandular cell (*G*) united via short cytoplasmic processes (*arrows*). **c**) The basal surface of the glandular cells from a male large lobe shows interlocking among the cytoplasmic processes (*open arrows*). Note a broad glandular cell process (*LP*) running on top of the glandular cells and sending out cytoplasmic processes (*open arrows*). The appearance can be confused with a myoepithelial cell processes. x6000

Discussion

The current study has shown the presence of intraglandular differences in the morphology of the myoepithelial cells of the golden hamster. The myoepithelial cells of the small lobes from both male and female glands appeared to have broader cytoplasmic processes compared to those of the large lobes. Despite the intraglandular differences they did not show sexual differences; the results indicated that there were similarities in the myoepithelial cell morphology between the corresponding lobes in the male and female glands. Observations on the basal surface of the glandular cells revealed the presence of sexual differences between the male and female large lobes in terms of appearance and arrangement of glandular cells in the basal region.

Myoepithelial cells are contractile cells that are associated with the secretory endpieces of many exocrine glands. Three-dimensional studies done in many glands have revealed a uniform characteristic that they are stellate shaped cells and form a basket-like network around the secretory endpieces [11, 12, 13, 14, 15, 16, 17, 18]. The results of the current study are in agreement with the previous findings with regards to the arrangement of the myoepithelial cell processes around the secretory endpieces [17, 18]. However this study has revealed the presence of intraglandular morphological differences between the myoepithelial cells of the large and small lobes. The myoepithelial cells of the small lobes in both male and female glands contained broader cytoplasmic processes than those of the large lobes. In the large lobes of the male and female glands the myoepthelial cells contained relatively thinner cytoplasmic processes. The reasons for the differences are not known, but could be due to regional differences that exist in the Harderian gland of the golden hamster. Past observations have showed that the Harderian gland of the golden hamster contains the large and small lobes [9]. The two lobes are different in terms of location and secretory mechanisms, such features could account for the differences in the size of the myoepithelial cells process observed in the current study between the large and small lobes. The large lobes are located deep in the orbital cavity around the posterior surface of the eyeball and its glandular cells secrete via exocytosis and apocrine mechanisms. The small lobe is more superficial, situated between the eyeball and the upper orbital rim of the orbital bone. In terms of secretion the small lobes have more of apocrine secretory mechanisms than those of the large lobe [9]. It is possible that such factors can explain the differences in morphology between the myoepithelial cells in the small and large lobes of the Harderian gland. Myoepithelial cells develop in close association with the glandular cells and it is likely that morphology of the glandular cells can influence the size of myoepithelial cell processes [20, 21].

Previous observation on the myoepithelial cells of the golden hamster reported the presence of sexual differences in terms of the number of myoepithelial cells between the male and female glands [19]. The study indicated that the secretory endpieces in the female glands contained a large number of myoepithelial cells than those in the male glands. These data are different from the current findings, which have showed similarities in the myoepithelial cells morphology between the corresponding lobes in the male and female glands. The reasons for this are not known but could be due to technical differences; for example the study did not recognize the presence of large and small lobes. The present results indicated that the myoepithelial cells of the small lobes contained broader cytoplasmic processes compared to those in the large lobe, if these lobes are not properly separated during preparation of specimens misinterpretation of the data may occur. The current findings points to the possibility that the myoepithelial cells in the Harderian gland of the golden hamster may not be sexually regulated. This is an interesting observation in a gland that is regulated by sex hormones and has opened way for future research towards the understanding of the myoepithelial cell physiology.

Scanning electron microscopy has revealed the basal appearance of the glandular cells and how the cells are related to each other in the male and female glands. The results indicated the presence of sexual differences between the large lobes of male and female glands. In the female large lobes most of the glandular endpieces contained the inner and outer cells that appeared to sit on top of the inner cells. The cytoplasmic processes observed in the outer cells did not show interlocking; they were attached on the surface of the inner cells. In some secretory endpieces the inner cells appeared electron lucent and the outer cells were electron dense. The reason for having cells with different electron density is not clear but it may indicate the presence of two cells in different physiological state. Past studies on the Harderian gland of the golden hamster have revealed some important observations that can be correlated with the current findings [22, 23]. First the female glands produce and accumulate large amount of porphyrins that become toxic to cells leading to cell death [22, 23, 24, 25]. Secondly cells that accumulate porphyrins change to become less electron-dense and were named "clear cells". Transmission electron microscopy observations indicated that the clear cells undergoes death and become released into the lumina. It is possible that the electron lucent inner cells observed in the current study are the clear cells that have accumulated large amount of porphyrins and are about to be shed into the lumina. However, future work is needed to verify this suggestion. It is also important in future to investigate the role of the outer electron dense cells and see whether they develop primarily in order to replace the inner electron lucent cells when they become lost into the lumen.

In the male glands and female small lobes the basal surfaces of the glandular cells appeared to have the same electron density and the relation was that of extensive interlocking among the cells and their processes. It appeared that glandular cells were closely and tightly held together at the basal surface than in the female large lobes. Why these three lobes (small male, large male and small female) display a similar morphological pattern in the basal surfaces

of the glandular cells that is different from that of the female large lobe cannot be answered in this study. However, the unifying feature between the three lobes is that they all secrete via exocytosis and apocrine mechanisms [9]. Apocrine features occur more frequently in these lobes compared to the large lobes of the female glands. The female large lobes secrete mainly via exocytosis and the lumina of most endpieces contain the accumulated porphyrin materials (2, 9). Future work is needed because there are no studies that have addressed the morphology of the basal surface of the glandular cells in the Harderian gland.

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