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*Review*

# Apocrine secretory mechanism: Recent findings and unresolved problems

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**Summary.** Cell secretion is an important physiological process that ensures smooth metabolic activities, tissue repair and growth and immunological functions in the body. It occurs when the intracellular secretory materials are released to the exterior; these may be in the form of lipids, protein or mucous and may travel through a duct system or via blood to reach the target organ. To date three types of secretory mechanisms have been characterized, they include apocrine, holocrine and exocytosis. Apocrine secretion occurs when the release of secretory materials is accompanied with loss of part of cytoplasm. The secretory materials may be contained in the secretory vesicles or dissolved in the cytoplasm that is lost during secretion. In holocrine secretion, the entire cell is secreted into the glandular lumen, and it is presumed that the intended secretory materials are contained in the cell cytoplasm. Exocytosis is the most commonly occurring type of secretion; here the secretory materials are contained in the secretory vesicles and released without loss of cytoplasm. Apocrine secretory mechanisms have not been properly discussed; for example the biochemical and physiological pathways that regulate apocrine secretory process are not clearly known. Similarly, the plasma membrane dynamics during apocrine secretion has not been researched. In other glands morphological features during apocrine secretion have not been documented. The current paper reviews what is known about apocrine secretion, recent findings and highlights on the unresolved areas for future research.

**Key words:** Secretory mechanisms, Harderian gland, Myoepithelial cell, Intracellular calcium ions

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## Introduction

Apocrine secretion occurs when secretory process is accompanied with loss of part of the cell cytoplasm (Fig. 1). The secretory materials may be contained in the secretory vesicles or dissolved in the cytoplasm and during secretion they are released as cytoplasmic fragments into the glandular lumen or interstitial space (Roy et al., 1978; Agnew et al., 1980; Ream and Principato, 1981; Messelt, 1982; Eggli et al., 1991; Gesase et al. 1996). It has been described in glands of the genital tract (Nicander et al., 1974; Aumuller and Adler, 1979; Guggenheim et al., 1979; Hohbach and Ueberberg, 1982; Morales and Cavicchia, 1991; Van der putte, 1993; Steinhoff et al., 1994; Aumuller et al., 1997; Whilhelm et al., 1998; Groos et al., 1999), breast and other cutaneous glands (Charles, 1959; Hashimoto et al., 1966; Schaumburg-Lever and Lever, 1975; Kawabata and Kurosumi, 1976; Kurosumi et al., 1984; Atoji et al., 1998), goblet cells, olfactory mucosa, lungs and Harderian glands (Cohn, 1955; Etherton et al., 1979; Kurosumi et al., 1981; Jonhston et al., 1985; Lucheroni et al., 1986; Payne, 1994; Rogers, 1994; Gesase et al., 1995, 1996; Baccari et al., 2000), the choroid plexus, respiratory epithelium, parathyroid gland and the ciliary epithelial cells (Agnew et al., 1980; Pack et al., 1980; Ream and Principato, 1981; Gudeman et al., 1989; Eggli et al., 1991; Puchelle et al., 1991; Peao et al., 1993). The puzzling characteristic of most apocrine glands is that they also secrete via exocytosis (Schaumburg-Lever and Lever, 1975; Main and Lim, 1976; Stinson and Loosli, 1978; Smith and Hearn, 1979; Satoh et al., 1992; Lucheroni et al., 1986; 1990; Gesase et al., 1995; Atoji et al., 1998; Aumuller et al., 1999; Groos et al., 1999; Baccari et al., 2000; Zaviacic et al., 2000). In some glands exocytosis is predominant while in others apocrine secretion become the major pathway for secretion. In some glands apocrine secretion occurs at a low level as compared to exocytosis (Payne, 1994; Gesase et al., 1996), and in most cases it does not allow detailed morphological observations. Researches that have used the secretagogue to stimulate cell secretion

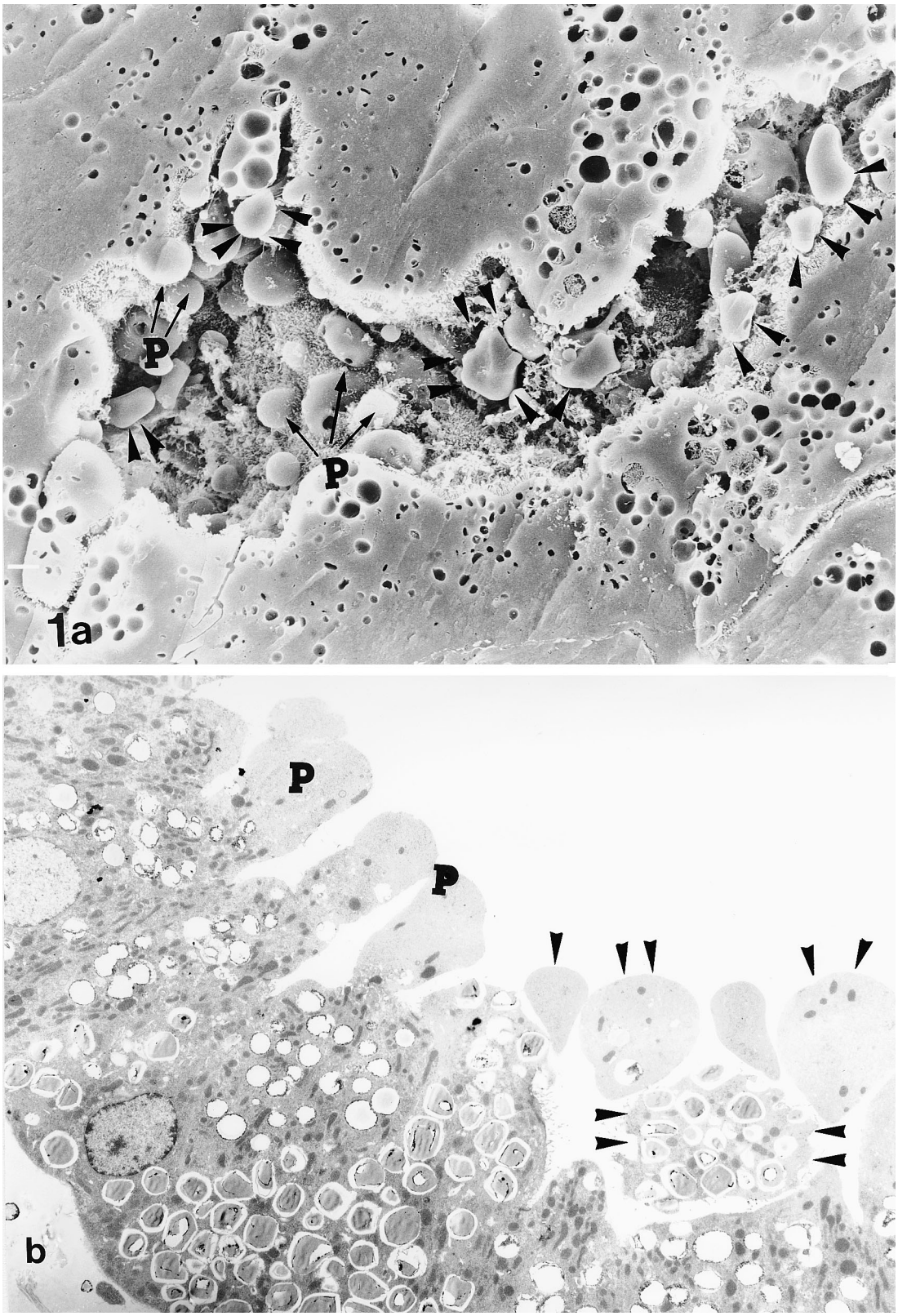
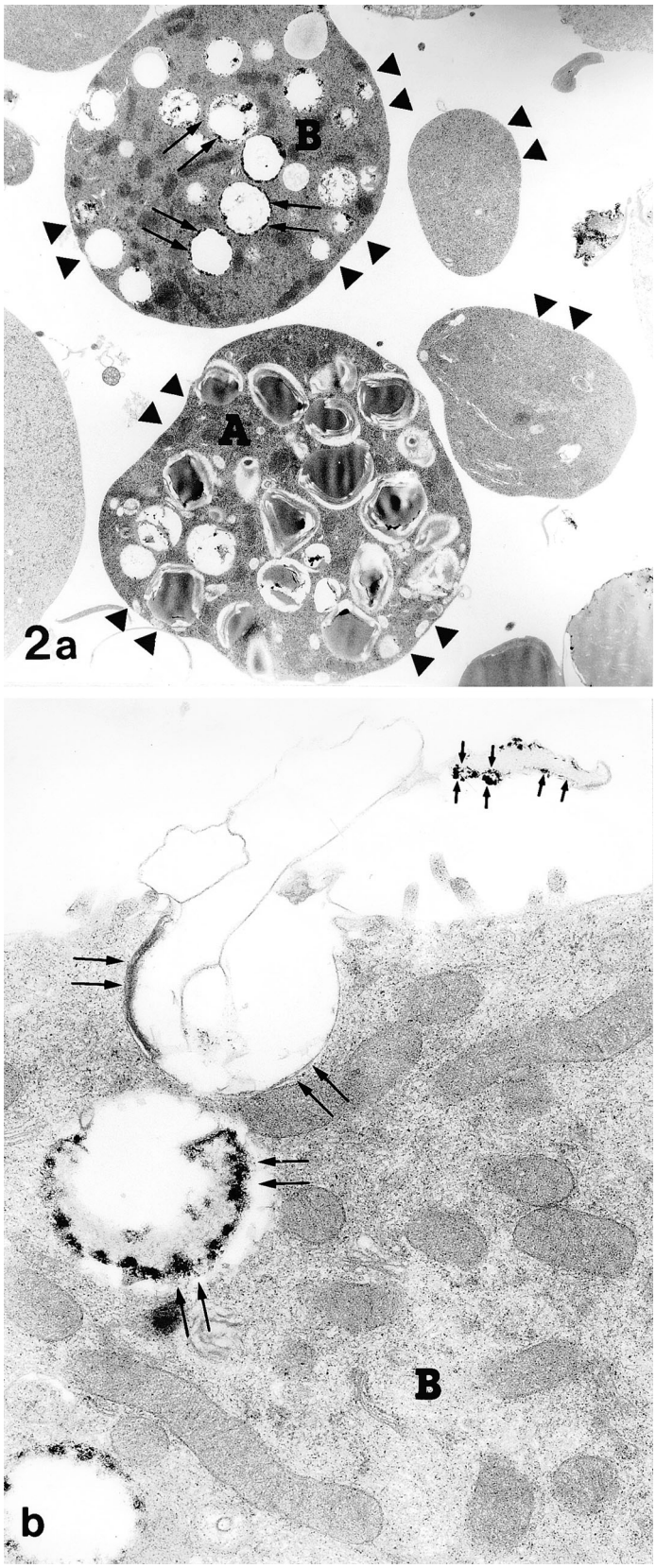



**Fig. 1.** Electron micrograph of the golden hamster **(a)** and rat **(b)** Harderian gland showing the apical protrusions (P) and cytoplasmic fragments (arrowheads) in the lumina of secretory endpieces. Note that the plasma membrane covering the protrusions is smooth and devoid of the microvilli. x 2,000




have increased our understanding on apocrine secretory mechanism.

## Morphological features

Apocrine secretion entails loss of part of cytoplasm during cell secretion (Charles, 1959). Morphologically it is characterized by the presence of apical protrusions and/or the cytoplasmic fragments in the lumen (Fig. 1). The protrusions appear as a result of gradual accumulation of the intended secretory materials beneath the cell membrane and, eventually they form balloon-like swellings that protrude into the lumina (Nicander et al., 1974). Observation made in the cilliary body of the rat eye indicated that accumulation of apocrine secreted materials occur between the basal plasma membrane and a cortical cytoskeleton (Eggli et al., 1991). It has been suggested that apocrine secreted molecules are synthesized within the cytoplasm then transported and concentrated at the apical protrusion-forming region of the plasma membrane (Eggli et al., 1991; Steinhoff et al., 1994; Aumuller et al., 1997; Wilhelm et al., 1998). In this case apical protrusions will be homogenous in appearance (Nicander et al., 1974; Schaumburg-Lever and Lever, 1975; Atoji et al., 1993). However, the intracellular events that regulate the formation of the apical protrusions are yet to be determined. In another category the protrusions are filled by secretory vesicles that contain apocrine-secreted materials and are released as a group into the lumen where they disintegrate to release the secretory materials (Fig. 2a). Observations made in the rat Harderian gland have revealed that, similarly looking secretory vesicles are concomitantly released via exocytosis (Brouwnscheidle and Niewenhuis, 1978; Satoh et al., 1992; Gesase et al., 1995) (Fig. 2b). The physiological significance of the occurrence of both exocytosis and apocrine secretory mechanisms in a single cell is not clearly known, but it may indicate that the glandular cells synthesize both apocrine secreted substances and those released via exocytosis (Cristofoletti et al., 2001). Similarly, the intracellular pathways that determine when and what secretory materials are released by which secretory mechanisms are not known. The significance of this phenomenon in cell secretion and the factors that determine the secretory mode via which the vesicles are released are subjects for future research. Although the vesicles appear to be morphologically identical, it is possible that they posses some molecular differences that

**Fig. 2.** Transmission electron micrographs of the rat Harderian gland. **a.** Shows the cytoplasmic fragments (arrowheads) in the lumen of the secretory endpieces released by type A (A) and B (B) cells. Some cytoplasmic fragments contain the secretory vesicles and others have a homogenous appearance (arrowheads). x 5,000. **b.** Similarly looking secretory vesicles (arrows) in type B cell are also released via exocytosis. x 20,000

allow vesicular sorting in accordance to the mode of secretion. In the Harderian gland both type of apical protrusion appears to occur; the ones that contain the secretory vesicles and those with homogenous appearance (Gesase et al., 1995, 1996). The Harderian gland is mainly a lipid-secreting gland; other substances include the porphyrins and melatonin (Cohn, 1955; Bubenik et al., 1976; Brownscheidle and Niewenhuis, 1978). It has been shown that lipids are concentrated in the secretory vesicles, while porphyrins are mainly found in the cytoplasm (Brownscheidle and Niewenhuis, 1978; Payne, 1994).

The release of secretory materials in apocrine secretion is carried out in three distinct processes: decapitation, pinching-off and pore formation on the plasma membrane that covers the apical protrusions (Charles, 1959; Hashimoto et al., 1966; Kurosumi et al., 1968, 1984; Nicander et al., 1974, Schaumburg-Lever and Lever, 1975; Smith and Hearn, 1979; Gesase et al., 1996). Recently we observed the occurrence of apocrine release in the Harderian gland that does not fall under the documented categories.

### *Decapitation*

Decapitation mechanism has been reported in apocrine glands of human axilla, anal scent glands of woodchurk and the Harderian glands of rat and golden hamster (Schaumburg-Lever and Lever, 1975; Smith and Hearn, 1979; Gesase et al., 1996). In this mechanism, there is formation of protrusions on the plasma membrane, which are released following changes that take place in their basal part. Two morphological changes have so far been described: In one category formation of apical protrusion is followed by the appearance of a row of demarcating vesicles at the base of the protrusion, forming a boundary with the remaining glandular cell (Fig. 3). Fusion of the demarcating vesicles results into decapitation with subsequent release of the cytoplasmic fragments into the lumen (Smith and Hearn, 1979; Gesase et al., 1996). Observation made in anal scent glands of the Woodchuck indicated that the demarcating vesicles were morphologically different from the secretory vesicles (Smith and Hearn, 1979). However, In the Harderian gland of the rat, the demarcating vesicles appeared to be morphologically similar to the lipid secretory vesicles. The second category of decapitation is seen in glands in which the protrusion forms over the entire apical surface of the cell (Schaumburg-Lever and Lever, 1975). It is characterized by the formation of the dividing plasma membranes; one beneath the protrusion and the other on the apical part of the remaining cell, both terminating at the lateral cell margin. Observation made in human

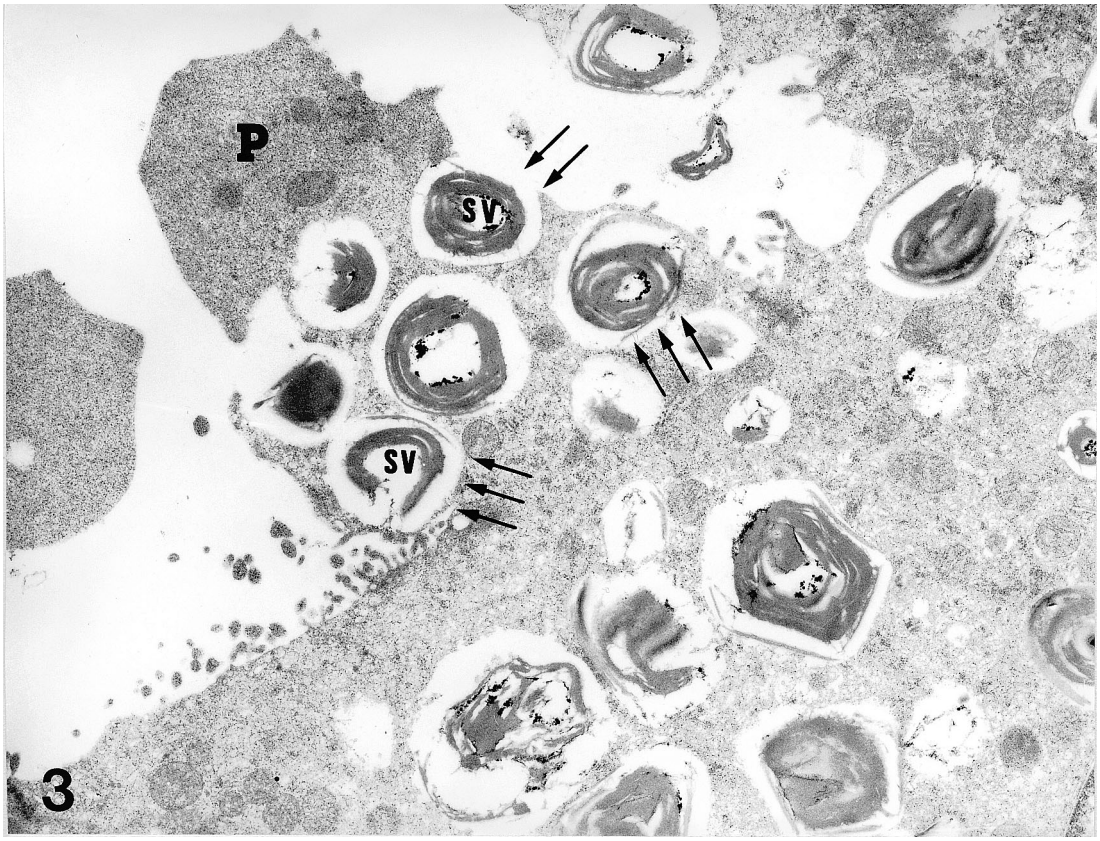



**Fig. 3.** Transmission electron micrographs of the rat Harderian gland illustrating the release of apical protrusion (P) via decapitation process. The secretory vesicles (SV) are seen at the base of the protrusion; fusion of the secretory vesicles with each other and with the apical membrane (arrows) results into decapitation and subsequent release of the protrusion (P) into the lumen. x 1,0000

apocrine glands indicated that formation of the dividing membrane was followed by the appearance of tubules above and parallel to the dividing membranes and fusion of the tubules caused detachment of the protrusion (Schaumburg-Lever and Lever, 1975). It appears that decapitation during apocrine secretion is regulated differently in different glands.

### *Pinching-off*

Pinching-off mechanism has been described in several glands (Kurosumi et al., 1968, 1981; Nicander et al., 1974; Kachar et al., 1980; Eggli et al., 1991; Morales and Cavicchia, 1991; Gesase et al., 1996; Atoji et al., 1998). In this case the apical protrusions have the connecting stalk attached to the cell and progressive thinning of the stalk causes pinching off at the base of the stalk and the release of the protrusion (Fig. 4). Although the exact mechanisms that cause progressive thinning of the connecting stalk are not known, a few researchers have suggested the possible role of actin filaments (Metzler et al., 1992; Aumuller et al., 1999). Recent immunohistochemical study using anti-actin demonstrated the immunopositive actin in the apical protrusion; an indication that actin may be involved in the formation of a constriction ring during pinching off process (Stoecklhuber et al., 2000). Previous observation in human skin also detected actin microfilament in apical protrusion (Metzler et al., 1992). Actin belongs to the cytoskeleton and has been shown to play a major role in the formation of the contractile ring during cytokinesis (Alberts et al., 1994). Strong immunoreactivity of actin filament in the apical protrusions indicates that it may be involved during pinching off process.

### *Pore formation*

The other documented mechanism of apocrine secretion occurs differently from decapitation and pinching-off and the difference is seen on how the secretory materials are released. In pore formation mechanism, the appearance of apical protrusion is followed by the formation of small defective areas (sieve-like pores), through which cytoplasmic secretory materials escape onto the lumen. The process has been reported in human axillary apocrine glands (Charles, 1959; Hashimoto et al., 1966; Kurosumi et al., 1981), and it is thought that the cytoplasm containing apocrine secreted materials pass through the pores to reach the exterior. Often, glands that show this type of apocrine release process have been wrongly labeled as not apocrine in nature because the formed cytoplasmic fragments are not seen in the lumen. However, loss of part of the cytoplasm during secretion is indicative of apocrine secretory process; and absence or presence of the cytoplasmic fragments in the lumen entails different secretory process during apocrine release. Molecular pathways that lead to pore formation on the apical protrusion are not known, previous observations suggested that decapitation of the microvilli may leave minute holes through which the cytoplasm containing secretory materials pass (Charles, 1959; Hashimoto et

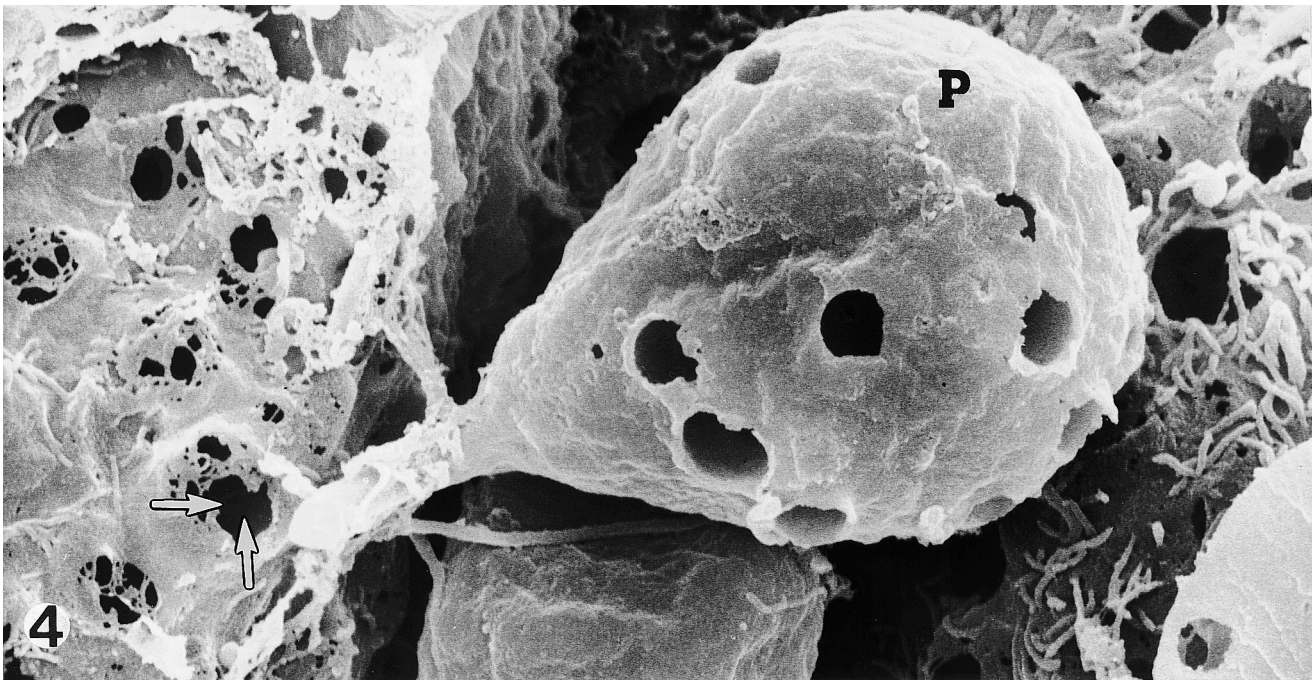



**Fig. 4.** Scanning electron micrographs of the rat Harderian gland illustrating the release of apical protrusion (P) via "pinching-off process". Thinning of the connecting stalk results into pinching-off of the apical protrusion (arrows). x 6,500

al., 1966). This conclusion was reached because the authors also observed a concomitant disappearance of the microvilli during apocrine release. However, recent studies have showed that the dissapearance of microvilli is not specific to apocrine secretion, it occurs also during other non-apocrine secretory process (Satoh et al., 1992; Gesase et al., 1995, 1996; Segawa et al., 1998). Similarly, during pinching-off and decapitation mechanisms the plasma membrane covering the apical protrusion lacks the microvilli (Figs. 1, 3, 4). Microvilli may have a different role during secretory process that need to be determined. In exocytosis pore formation is said to occur following fusion of the exocytotic vesicles with the plasma membrane (Segawa et al., 1998). Studies done in neurones, exocrine and endocrine cells have produced sufficient knowledge on molecules that regulate exocytosis. We now know that membrane associated proteins such as N-ethylmaleimidine sensitive factor (NSF), soluble NSF attachment proteins (SNAPs), synaptophysin, synaptotagmin, and synaptobrevin trigger exocytosis and pore formation (Baumert et al., 1989; Petrenko et al., 1991; Söllner et al., 1993; Zhang et al., 1994; Gratz 1995; Morganet et al., 1995; Ravachandran et al., 1996). Whether such molecules have a role in apocrine pore formation is open for future research.

### *Undocumented apocrine secretory processes*

Other types of apocrine secretory process have not been documented. Studies done in the rat Harderian gland following stimulation with CCh, revealed that in the endpieces that did not contain apical protrusions secretion was accompanied with loss of part of the cytoplasm. This secretory process was interpreted as "Massive Exocytosis" (Satoh et al., 1992; Gesase et al.,

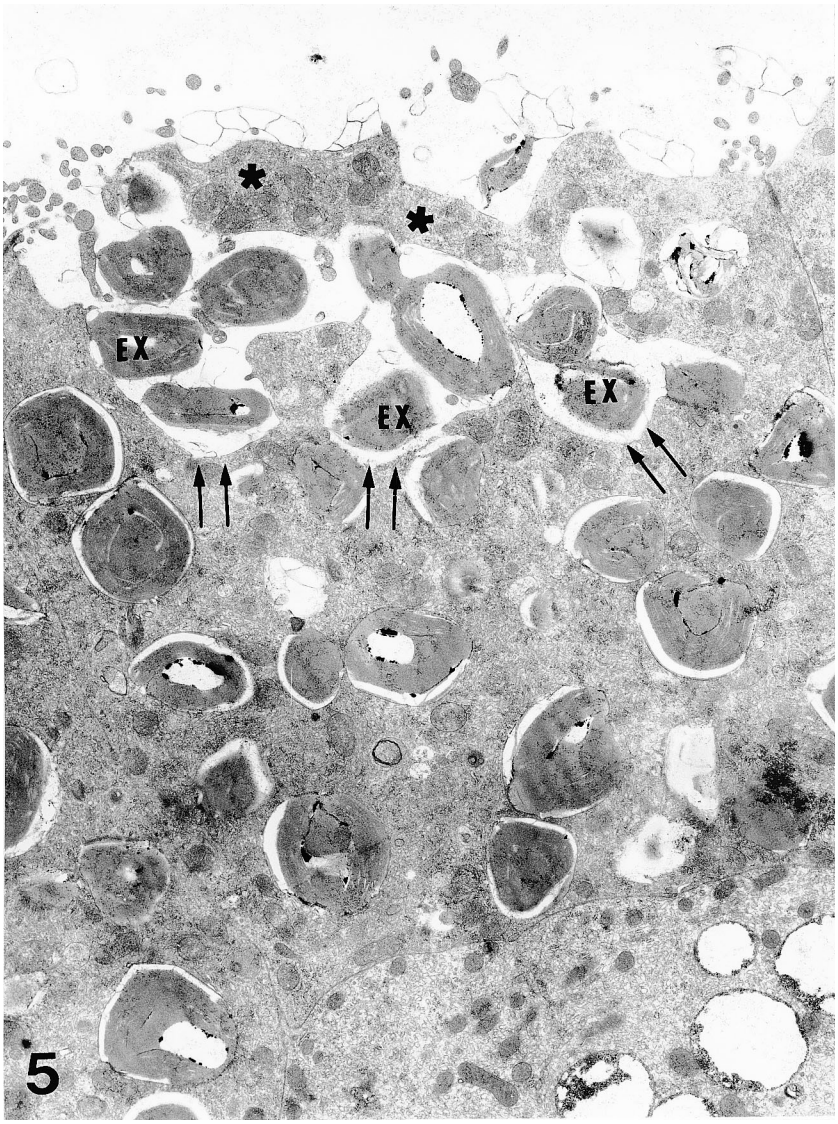



**Fig. 5.** Transmission electron micrographs of the rat Harderian gland illustrating the release of cytoplasmic fragments (asterisks) into the lumen. The cytoplasm is trapped and released together with exocytotic vesicles. x 5,000

1995), mainly due to the absence of apical protrusion and the presence of exocytotic vesicles at the site of secretion (Fig. 5). However, further observation on the secretory endpieces revealed the following features: A closely applied group of exocytotic vesicles were seen to fuse with the apical membrane, thereafter, other secretory vesicles moved to associate with the exocytotic vesicles and in the process the cytoplasm was trapped in between the vesicles. During secretion both exocytotic and associated vesicles together with the intervening cytoplasm were released into the lumen leaving wide "cup-like" concavities on the luminal membrane. In interpreting these findings there are two observations which do not favor exocytosis; first the release of cytoplasmic fragments and secondly the extrusion of intact secretory vesicles into the lumen. During exocytosis it is the vesicular content that are released and not the intact vesicles; exocytotic vesicles fuse with the plasma membrane resulting into release of their contents and the vesicular membrane remains on the cell membrane (Wooding, 1980; Kelly, 1985; Burgoyne and Morgan, 1993). Massive exocytosis is a misleading and ambiguous term; what occurs here is non-protrusion forming apocrine secretion. In exocytosis the mechanism for release of secretory materials is the same, the reason for having variations during apocrine secretory process is not clear. It will be interesting to know whether the variations on how secretory materials are released reflect the nature or function of secreted materials or if it simply indicates the presence of tissue/cell differences.

## Physiology of apocrine secretion

How apocrine secretion occurs is still debatable. Molecular pathways that bring about apical protrusions and plasma membrane dynamics during apocrine release are not clearly understood. Some facts have emerged recently indicating the possible role of sex hormones in the appearance of apocrine features in sex-related glands. Androgens have been linked with the appearance of apocrine features in some glandular cells (Selim and Wells, 1999). Studies done in breast tumors have showed higher levels of androgens receptors in cells with apocrine metaplasia (Offideni and Campanati, 1999; Selim and Wells, 1999; Sapino et al., 2001), an indication that androgens may have a role in the expression of apocrine features. A study done in the Harderian gland of the golden hamster revealed apocrine secretory features in large lobes of male glands and that castration abolished apocrine secretion. In female glands there was no apocrine secretion but ovariectomy restored apocrine secretory activities (Gesase et al., 2001) (Fig. 6). These observations shows that androgens may favor expression of apocrine secretion in the large lobe of the Harderian gland while estrogen suppress apocrine activities. Although the exact pathway through which these hormones influences apocrine features is still an enigma, there are strong indications that apocrine secretion is physiologically regulated by intracellular messengers.

Some researchers have linked vigorous contraction of the myoepithelial cells with the appearance of apical protrusions and cytoplasmic fragments in the lumen (Tandler et al., 1970), however, it is unlikely that apocrine secretory process is mechanically regulated. Myoepithelial cells are contractile cells and possess ultrastructural features similar to those in the smooth muscle cells (Garret and Emmelin, 1975; Satoh et al., 1994). They are found in exocrine glands such as mammary, sweat, lacrimal, salivary and Harderian gland where they lie on the basal surface between the glandular cells and the basal lamina (Linzel, 1955; Moore, et al., 1987; Satoh et al., 1994) (Fig. 7). The role of Myoepithelial cell during cell secretion has been widely investigated and strong myoepithelial cell contraction has been linked to appearance of apocrine-like features in mucous salivary glands in the human lip (Tandler et al., 1970). This observation was further supported by the experiment done in the rat Harderian gland by Satoh et al. (1992). On stimulating the Harderian gland with 10$\mu$M carbamyl choline, they observed concomitant the apical protrusion, cytoplasmic fragments and basal protrusions. This observation created confusions as to whether the apocrine features are caused purely by glandular cell stimulation or are a result of extrinsic force caused by myoepithelial cells contraction. The findings necessitated additional experiments in which the glandular cells were stimulated to secrete in the absence of myoepithelial cell contraction (Gesase et al., 1996). In order to paralyze the myoepithelial cells the perfusion solution contained Papaverine, a smooth muscle relaxant (Martin et al., 1993). In this experiment the glandular endpieces did not show evidence for myoepithelial cell contraction; as characterized by basal protrusions (Satoh et al., 1993; Gesase et al., 1995). However, the secretory endpieces showed the occurrence of apocrine secretion; the lumina were jammed with apical protrusion and cytoplasmic fragments, an indication that apocrine secretion occurred in the absence of myoepithelial cell contraction (Fig. 8). The study made the observations that myoepithelial cell contraction does not cause apical protrusions or the appearance of cytoplasmic fragments in the lumina. This conclusion is further supported by experiment which have showed that glandular cells that are not associated with myoepithelial cells such as goblet secrete via apocrine (Freeman, 1966; Kurosumi et al., 1981; Radwan et al. 1990; Kessler et al., 1995), and similarly, the secretory endpieces which have myoepithelial cells, such as the lacrimal gland do not secrete via apocrine (Ruskell et al., 1975; Satoh et al., 1997). Myoepithelial cells may play a role of supporting the glandular endpieces during secretory process.

### *Constitutive and regulated secretion in apocrine secretion*

In exocytosis, the terms constitutive and regulated

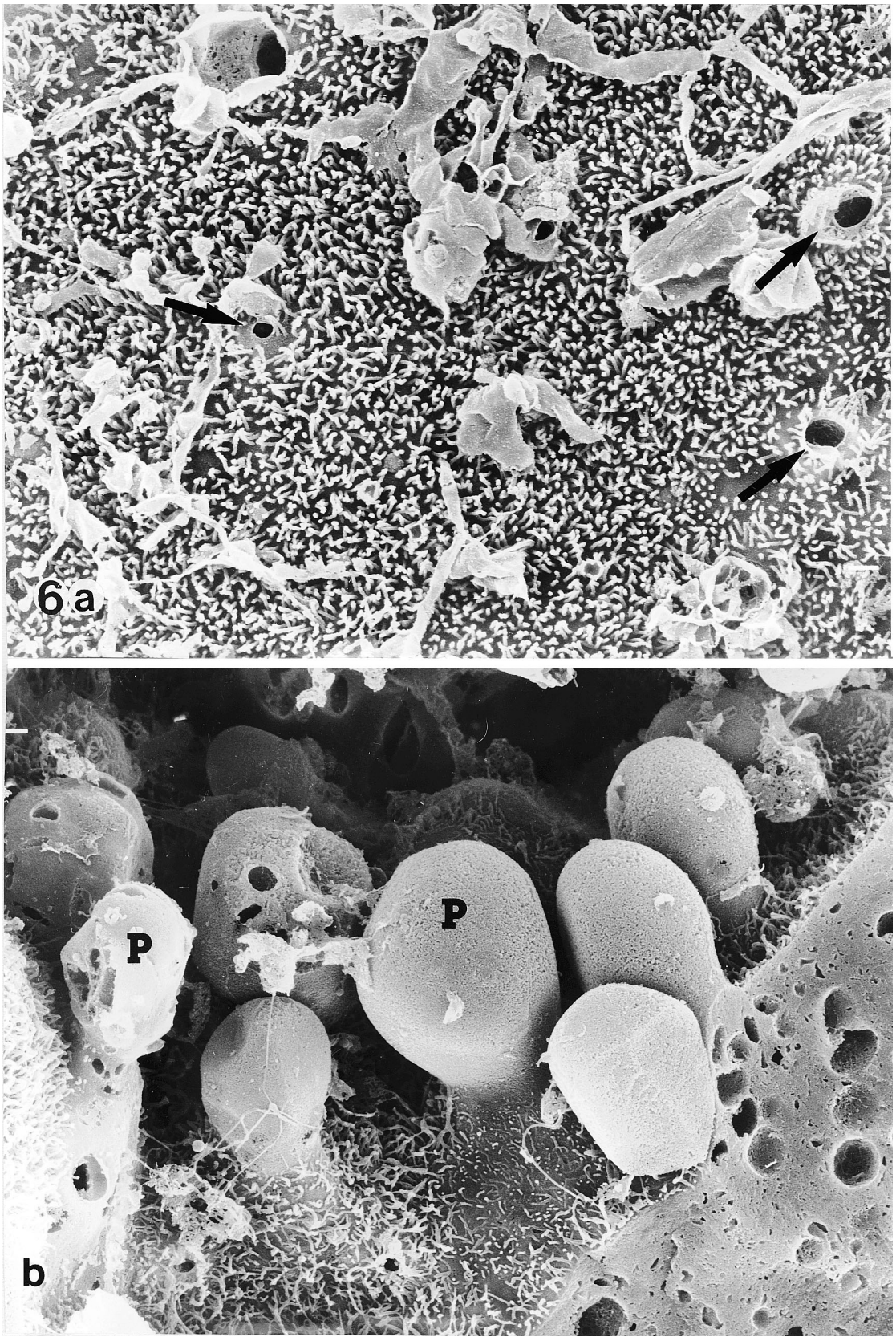



**Fig. 6.** Scanning electron micrographs of the Harderian gland of the female golden hamster showing the secretion profile in the glandular endpieces before **(a)** and after **(b)** ovariectomy. **a.** In the intact female the exocytotic figures (arrows) are seen on the apical membrane. x 1,500. **b.** Following castration apical protrusions (P) are seen on the apical membrane. x 7,000

secretion are used, depending on the presence of a specific factor that stimulate secretion (Kelly, 1985; Beaudoin and Grondin, 1991; Burgoyne and Morgan 1993). Regulated secretion occurs when the glandular cell is stimulated while constitutive secretion proceeds spontaneously without external stimuli (Kelly, 1985, Burgoyne and Morgan, 1993). It has also been suggested that constitutive exocytosis is unimpaired even if the concentration of intracellular $Ca^{2+}$ ($[Ca^{2+}]_c$) is reduced below resting levels (Helms et al., 1990; Edwardson and Daniels-Holgate, 1992; Turner et al., 1992; Bourgoyne and Morgan, 1993). Glands such as the exocrine pancrease posses a regulated secretion; observation of unstimulated endpieces shows an empty glandular lumen with no exocytotic figures. However, following stimulation exocytotic figures are commonly observed and the lumina contains secretory materials. The concept of regulated and constitutive secretion have not been applied to apocrine glands, this is partly due to lack of research in different apocrine glands as compared to the amount of literature we have in exocytosis. In rat Harderian gland, observation of unstimulated secretory endpieces did not show apocrine features, they became apparent upon stimulation with secretagogue (Satoh et al., 1992; Gesase et al., 1995). In these experiments $Ca^{2+}$ was excluded from perfusion [HEPES-buffered Ringers (HR)] solution that contained 10$\mu$M CCh. One group of animals was perfused with HR without $Ca^{2+}$ and in the other group EDTA was added to the perfusion solution. In animals perfused with $Ca^{2+}$-free HR apocrine features were very few when compared with those in glands stimulated in the presence of Ca2+. Addition of 0.5 mM EDTA into $Ca^{2+}$-free HR completely inhibited apocrine secretory process an indication that apocrine secretion is a regulated process and that the presence of free cytosolic $Ca^{2+}$ is necessary during secretory process. Apocrine features have been observed in unstimulated secretory endpieces of some glands (Cohn, 1955; Kelenyi and Orban, 1965; Hashomoto et al., 1966; Testa-Riva and Pexeddu, 1980; Johnston et al., 1985; Gesase et al., 1996; Aumuller et al., 1997; Atoji et al., 1998; Baccari et al., 2000). However, such findings cannot be interpreted as constitutive secretion, because factors such as the role of free cytosolic $Ca^{2+}$ cannot be excluded (Burgoyne and Morgan, 1993). More physiological experiments are necessary in these glands.

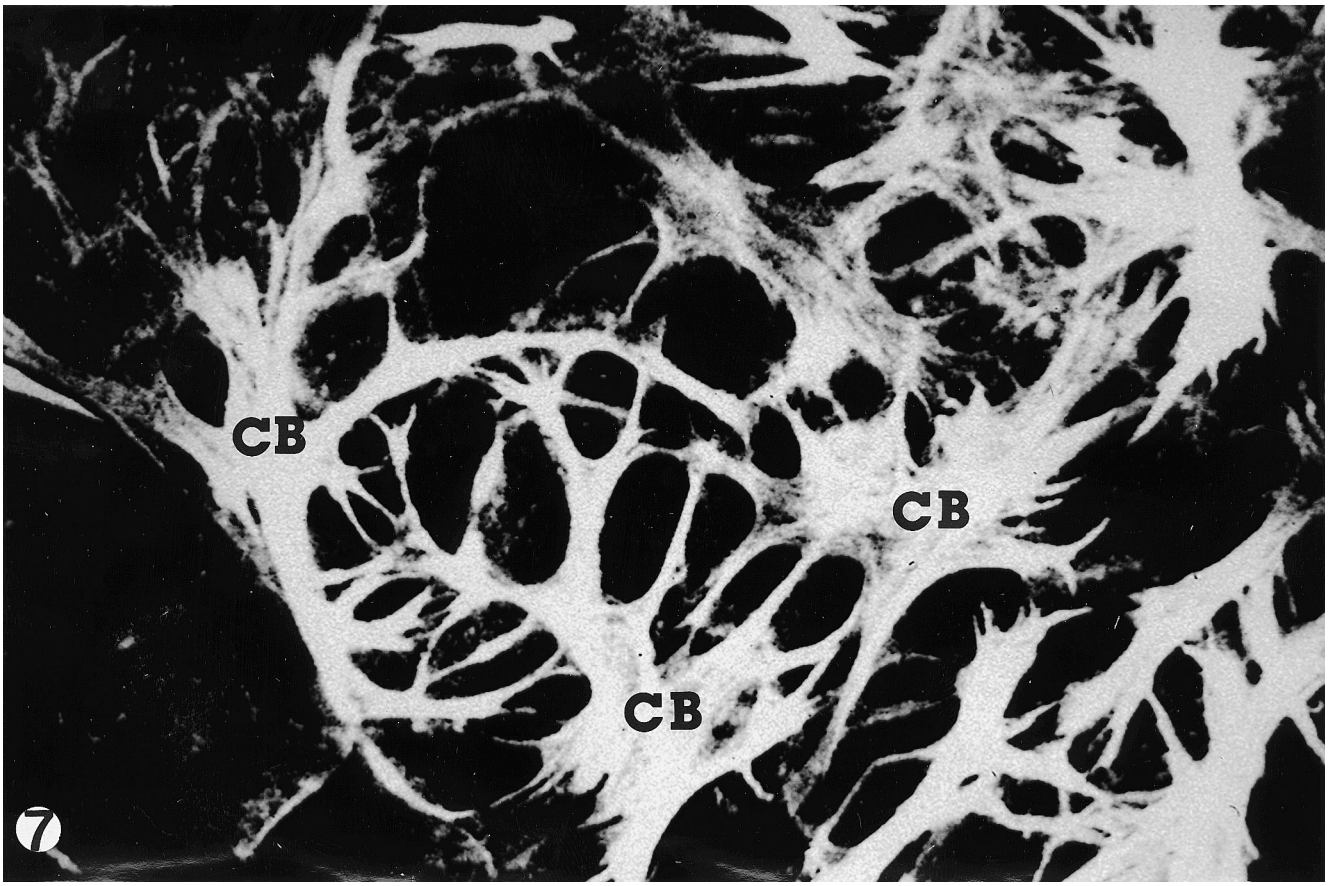



**Fig. 7.** Light micrograph showing the network of the myoepithelial cells (CB) on the basal surface of the glandular endpieces of the golden hamster Harderian gland. The actin filaments were stained with Bodipy-phalacidin after fixation by 4% parafomaldehyde. x 800

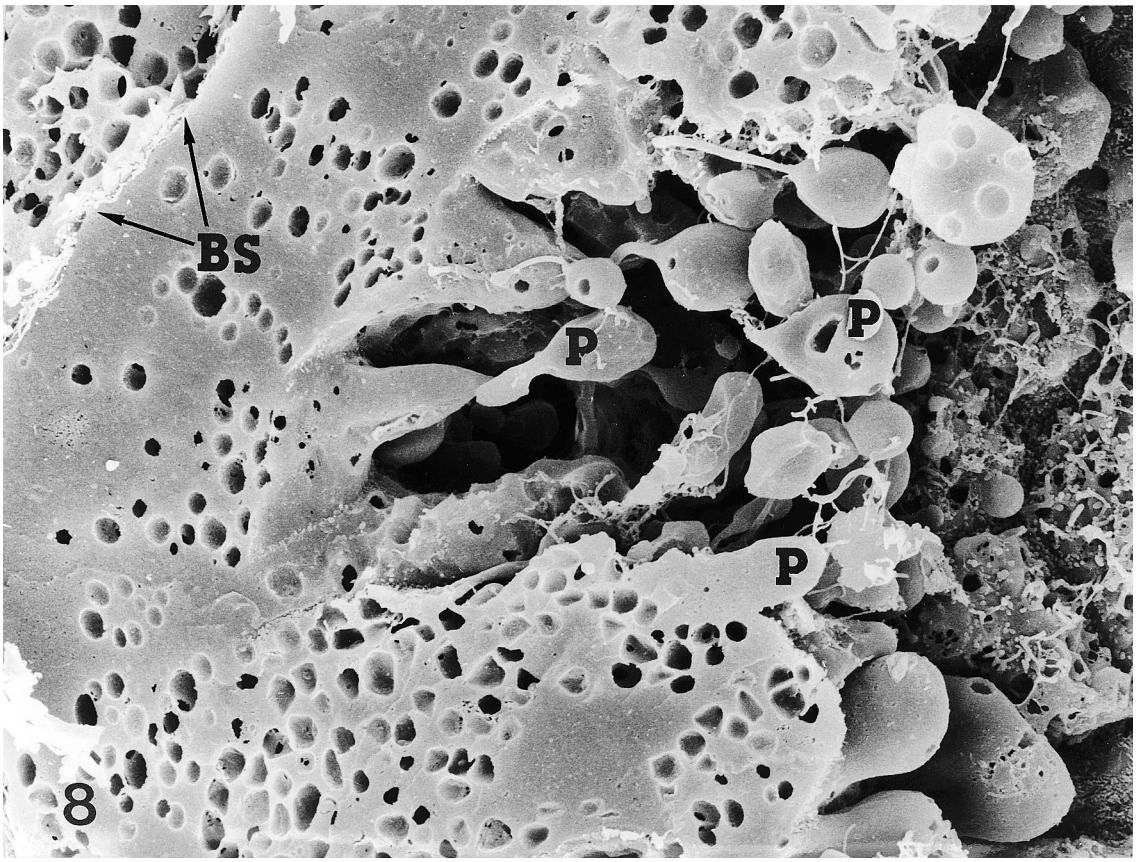



**Fig. 8.** Scanning electron micrograph of the Harderian gland from a rat perfused with papaverine in HEPES-buffered Ringers solution containing 10 $\mu$l of carbamylcholine. Apical protrusions (P) seen on the apical membrane, the basal surface (BS) remains smooth with no deformations. x 5,000

Apocrine secretory mechanism is complex and cannot be explained in one sentence. There is a clear specie/cell difference on how it occurs. Morphology of apocrine glands is different, the nature of secreted material is different and the physiological functions of released materials are also different. Research into apocrine secretion must be assessed in individual gland in order to increase our understanding on the pathways that regulate apocrine secretion.

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