ULTRASTRUCTURE OF THE SECRETORY CELLS OF THE PROCTODEAL GLAND IN MALE AND FEMALE COTURNIX COTURNIX JAPONICA (AVES)

by

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INTRODUCTION

The proctodeal gland (Glandula proctodealis) (Klemm et al., '73) is an exocrine gland present in the dorsal wall of the proctodeum of the Common-Coturnix quail (Coturnix coturnix japonica Temminck and Schlegel). Tissue similar to the proctodeal gland appears to be present, although in a less well-developed state in the Single Comb White Leghorn Chicken (Gallus domesticus) (Calhoun, '54). In the Common-Coturnix the gland extends caudad from the cloacal bursa almost to the end of the dorsal lip of the cloaca; it extends slightly down the lateral walls of the cloaca (Klemm et al., '73). Although the gland exhibits considerable hypertrophy in the adult, sexually-active male (Coil and Wetherbee, '59; Nagra et al., '59; Tamura and Fujii, '67), a corresponding hypertrophy is not present in the adult, sexually active-female (Tamura and Fujii, '67; Klemm et al., '73).

Light microscopy studies by Klemm et al, ('73) have suggested that this gland is an "aggregate gland composed of numerous discrete glandular units each of which is most closely comparable in structure to simple branched alveolar glands". The simple branched alveolar classification was confirmed in studies of the embryogenesis of the gland by Schafersman and Klemm, ('77).

The secretory cells of the glandular units are columnar shaped (Nagra et al., '59; Sachs, '67). Ikeda and Taji ('54); Fujii and Tamura ('67) and Klemm et al., ('73) have evaluated some histochemical properties of the exudate with conflicting

results as to the nature of the secretion. Renzoni ('72) using paper chromatography, suggested that the secretion was, in part, a glycoprotein.

The specific function of the secretion and of the proctodeal gland has not been determined, but most reports strongly suggest a relationship with the reproductive process (Ikeda and Taji, '54; Coil and Wetherbee, '59; McFarland et al., '68; Klemm et al., '73) or with fertility (Ogawa et al., '73).

Despite the possible importance of this gland and its secretion to reproduction in the Common Coturnix, there is no information on the cellular events of the secretory process beyond that inferred from the gross, light microscopy, and histochemical studies. Still the Common-Coturnix, as was suggested by Howes and Ivey ('61), continues to be used as an experimental pilot animal in a wide variety of studies on avian biology.

To provide additional data, I initiated a study of the ultrastructure of the secretory cells of the proctodeal gland. The objectives of this study were:

1) To characterize the ultrastructural features of the secretory cells of the proctodeal gland of adult, sexually-active male and female Common-Coturnix, at various stages of the secretory process; and,
2) To compare the ultrastructure of the secretory cells of the Common-Coturnix with similarly appearing secretory cells in various other glands.

Terminology:

Cytosegresome (Ericsson and Trump, '64): This term will be used to describe all myelin-like structures enclosed in mature secretory droplets.

Condensing vacuoles (Caro and Palade, '64): This term will be used to describe all membrane bound structures produced by the Golgi apparatus of the cell; as used in this study, the term is synonymous with the term presecretory droplet as used by Zeigel and Dalton ('62).

LITERATURE REVIEW

In 1955 the Common-Coturnix Quail was imported from Japan by the Missouri Conservation Commission in an attempt to increase the game bird population in the United States (Padgett, '58; Padgett and Ivey, '61). Although the Common-Coturnix was unable to survive in the wild (Keeler, '67), its potential for avian research, especially as an experimental pilot animal for poultry research, was recognized (Padgett and Ivey, '59; Howes and Ivey, '61), and the species became a widely used research animal.

Adult, sexually-active males, i.e., older than seven weeks post hatch, are most readily characterized by a large reddish, ovoid protuberance which extends to the cloacal vent (Coil and Wetherbee, '59). This protuberance is produced by the proctodeal gland (Klemm et al., '73), located in the dorsal wall of the proctodeal cavity. On a sagittal plane, the gland measures approximately 10.0 mm at greatest length; on a transverse plane, it measures 11.0 mm at greatest width. Thickness, measured dorsoventrally, is 1.5 to 3.0 mm in the sexually-active male (Coil and Wetherbee, '56; Klemm et al., '73).

The external protuberance is not seen in the adult, sexually-active female. However, the proctodeal gland is present. It is shorter (8.0 mm in length) and thinner (0.9 to 1.6 mm thick) (Klemm et al., '73).

Details of the proctodeal gland morphology and some studies on the histochemistry of the glandular secretion have been described for the adult bird by several investigators; (Coil

and Wetherbee, '59; Tamura and Fujii, '67; Fujii and Tamura, '67; McFarland et al., '68; Renzoni, '72; Klemm et al., '73; Schafersman and Klemm, '77).

The proctodeal gland is composed of individual glandular units each of which opens into the proctodeal cavity through a small excretory pore (Coil and Wetherbee, '59), located at the end of a short terminal papilla (Klemm et al., '73). Each glandular unit contains tall, columnar, PAS-positive secretory cells. A rounded to oval shaped nucleus is located in the basal one third of each cell. The nuclei stain darkly with Hematoxylin and Eosin (Coil and Wetherbee, '59; Tamura and Fujii, '67; Klemm et al., '73). As seen in the light microscope, the structure and staining reaction of the secretory cells in the male and female are basically similar; however, some differences are present. Klemm et al. ('73) measured the heights of the secretory cells in males and females; overall the cells were significantly shorter ($P < 0.05$) in the females (16.24 ± 0.2 mm), than in the males (22.14 ± 0.48 mm). The glandular lumina were smaller and excretory canals narrower in the female than in the male. The intraglandular septae were wider in the female than in the male due to an increased amount of connective tissue separating the individual glandular units (Klemm et al., '73).

The chemical composition and staining reactions of the secretory cells have been studied by several investigators: Coil and Wetherbee, ('59); Fujii and Tamura ('67); McFarland et al. ('68). Both the secretion and the secretory droplets

were resistant to digestion by ribonuclease (Coil and Wetherbee, '59), and malt diastase (Klemm et al., '73). Coil and Wetherbee ('59) also reported that the secretion removed from the bird, smeared on a slide and air dried, gave the same PAS-positive reaction as did the secretion found in the glandular lumina of the excised tissue. Fujii and Tamura ('67) suggested that the secretion was a non-sulfated acid mucopolysaccharide in the immature male and mature female; in the mature male it was reported to be a sulfated acid mucopolysaccharide. As sulfated acid mucopolysaccharides are PAS-negative, Klemm et al., ('73) questioned the results of Fujii and Tamura ('67) suggesting instead that the secretion was probably a neutral mucopolysaccharide, a mucoprotein or a glycoprotein. Studies by Renzoni ('72) demonstrated by paper chromatography that the secretion was, in part, a glycoprotein.

The pH of the secretion ranges from 6.3 to 6.6 (Ikeda and Taji, '53) and, according to Perez and Sandoval ('66) the secretion is "rich in nitrogen bodies", although no definition of that term was given.

Two explanations have been advanced for the frothy nature of the secretion: 1) that it results from a squeezing action of the sphincter muscles of the cloaca which aerates the secretion (Tamura and Fujii, '67); and 2) as suggested by McFarland et al., ('68) that carbon dioxide and water, produced by Escherichia coli and Proteus mirabilus present in the proctodeal cavity, were held in the secretion by a cohesive force, thus causing the foam. Neither of these postulates have been confirmed.

Other studies on the gland were predominantly concerned with males. Nagra et al. ('59) reported that the proctodeal gland in castrated males was similar to that in laying females. Nagra et al. ('59), Sachs ('67) and McFarland et al., ('68) reported that castration inhibited the growth and secretory activity of the proctodeal gland. Adkins and Adler ('72) found that the administration of testosterone-propionate to photocally castrated males and females caused a hypertrophy of the proctodeal gland in the male equivalent to that seen in adult non-castrated males; females showed a small degree of hypertrophy, but, in addition, did produce a foamy secretion in small amounts, identical to that of the adult male.

Similar responses were reported by Franks and Barton ('60) for the mouse prostate in which castration decreased the activity of the prostate gland and administration of testosterone propionate to castrated males and immature males and females increased the growth and activity of the prostate gland.

Thus, the proctodeal gland has been: 1) implicated as having some type of reproductive-association function; 2) shown to produce a secretion which is, at least in part, a glycoprotein; and 3) found to be testosterone sensitive in both male and female in a manner similar to that seen in the prostate gland which it resembles structurally (Klemm, personal communication).

All of the data to date on the proctodeal gland have been based solely on a few experimental studies on the relationship of the gland to reproductive activity or light and histochemical

studies of the glandular tissue and its secretory product. No studies have been done which describe the ultrastructural characteristics of the proctodeal gland secretory cells in the male and female or which relate these ultrastructural characteristics to the secretory process of the cell or to other similarly secreting cells.

MATERIALS AND METHODS

Secretory cells of the proctodeal gland were studied in 12 healthy, sexually-active, adult Common-Coturnix quail (6 males and 6 females). Specimens were selected from the breeding stock maintained by the Laboratory of Vertebrate Morphology in the College of Veterinary Medicine, Kansas State University. All specimens were held under a photoperiod of 16 hours light and 8 hours dark with free access to food and water.

Ages of the birds varied from 14 to 16 weeks post hatch; weight ranged from 100.0 - 117.0 grams in the males and from 128.0 to 138.0 grams in the females.

The birds were anesthetized with sodium pentabarbitol (15.0 mg/ml) injected into the medial plantar tarsal vein (0.045g/0.45kg body weight) using a 1.0 cc tuberculin syringe with a 26 gauge disposable needle. The appropriate plane of anesthesia was determined as that at which the bird no longer responded with a toe flexion reflex when pinched. After the removal of all cloacal feathers, a median section of gland was removed by making two cuts on either side of the median line and one cut, transversely, just caudal to the cloacal bursa.

The excised tissue was immediately immersed in 2.5% glutaraldehyde fixative at $4^{\circ}C$, mixed 1:1 with 0.15 M cacodylate buffer (pH 7.2; 330 mOsm, Hayat, '70 modified -- see Appendix I). All tissue was kept at $4^{\circ}C$ until dehydration. The cloacal musculature which overlies the glandular tissue was removed and the glandular tissue cut into pieces no greater than 1.0 mm

x 1.0 mm, with acetone cleaned, double edge razor blades, used once per cut. The final shape of each tissue was recorded for future reference. Each tissue sample was transferred individually from the initial fixative via pipette to a 15.0 x 45.0 mm, 1 dram screw cap vial filled with fresh fixative, and placed in a refrigerator for 2 hours.

Following fixation, the tissue was rinsed for 15 minutes in three changes of 0.15 M cacodylate buffer (pH 7.2; 330 mOsm, Hayat, '70) and post-fixed for 1 hour in 2.0% osmium tetroxide mixed 1:2 with 0.5 M cacodylate buffer (Caufield, '57 Modified, see Appendix I). Following postfixation the tissues were rinsed for one to two minutes in three changes of double distilled water, stained en bloc in aqueous uranyl acetate (Hayat '69) for 16-20 hours and again rinsed for one to two minutes in three changes of double distilled water.

Tissues were dehydrated in increasing concentrations of ethanol, placed in propylene oxide for 20 minutes and embedded in Epon/Araldite (Newcomb, undated--See Appendix I). Thin sections (< 0.1 μm) were cut with glass knives on a Sorvall MT-2 ultramicrotome, placed on uncoated grids, stained with lead citrate (Venable and Coggeshall, '65) and examined on an RCA EMU 3G electron microscope at an accelerating voltage of 50 kV.

Thick epon sections (± 1.0 μm) were cut with a glass knife and stained with Toluidine blue 2.0% sodium borate solution (Richardson '62) for examination under the light microscope.

OBSERVATIONS

Light microscopy:

Thick epon sections (1.0 μm) were taken of the glandular epithelial tissue from the proctodeal gland of male and female Common-Coturnix. These sections, which had been stained en bloc with uranyl acetate, were further stained with toluidine blue and viewed in the light microscope. In both the male and the female, the cells of this epithelium (figs. 1,2) are columnar in shape and are oriented with their apex toward the lumen of the glandular unit; a heterochromatic nucleus is located in the basal one-third of the cell. In general, the cells appear taller in the male than in the female; however, it is doubtful that the cells were perfectly bisected when sectioned and, consequently, measurements of cell heights were not made. Randomly scattered basal cells are seen along the base of the glandular epithelium between adjacent secretory cells; these cells were not studied and only their presence is reported here. Both the secretory and the basal cells rest on a basal lamina beneath which is a layer of connective tissue in the form of septae; these septae separate adjacent layers of glandular epithelium.

As viewed in the light microscope, the glandular epithelium is composed of cells exhibiting a wide variety of staining characteristics; in some the cytoplasm appears homogeneous and dark-staining; in others there is a variety of staining intensities of the supranuclear region caused primarily by the relative numbers of secretory droplets present (figs. 1,2).

However, on the basis of the staining characteristics of the cells, it is possible to identify and describe three basically different cell types: dense-staining cells, intermediate-staining cells, and light-staining cells.

Dense-staining cells. These are, in general, the least numerous cells seen in the glandular tissue. In thick section they appear to show a few refractile secretory droplets near the apex of the cell. The cytoplasm is chromophilic throughout most of the length of the cell, except near the basal portion where the deep staining, heterochromatic nucleus is located.

Intermediate-staining cells. These are the most numerous cells in thick sections. They possess extensive and numerous clear secretory droplets in the apical one-third of the cell. The droplets are large, refractile and appear relatively homogeneous in shape. The chromophilic material of the cell cytoplasm, staining less intensely than in other cells, is located around the nucleus and extends into the middle third of the cell. The nucleus is similar to that of the dark-staining cells.

Light-staining cells. These are the second most numerous of the cells seen in thick section. The vacuolated appearance suggests that large secretory droplets of a variety of shapes and degrees of homogeneity are present. These droplets are dispersed throughout the entire cell length except in the area immediately adjacent to the base of the cell. Near the apical region, the droplets appear to coalesce and to release their contents into the lumen of the glandular unit. Very little chromophilic material is visible in the cells. The nucleus

appears slightly less intensely stained than in the dark- and intermediate-staining cells and is slightly more vesicular in appearance.

Electron microscopy:

General. As with the appearance of the glandular epithelium seen in the light microscope, the secretory cells when viewed in the transmission electron microscope exhibit a variety of differences in organellar organization and appearance. These variations appear to reflect sequential steps in the secretory process of the cell. Within these variations it is possible to identify three characteristically different patterns which can be used to describe changes occurring in the rough endoplasmic reticulum (RER), the Golgi complexes and in the apical region of the cell during the secretory process. The three stages are designated as stage 1, stage 2, and stage 3. Stage 1 cells can be used to describe the basic ultrastructural characteristics of a proctodeal gland secretory cell which is presumed to be at the beginning of the secretory process; stages 2 and 3 appear to represent sequential steps in the secretory process in which changes in the structure of the organelles associated with the secretory activity may be described.

As the secretory cells of the male and female gland exhibited only quantitative differences in certain measurements, the secretory cell of the male will be used as a basis for description. Only one micrograph, either of the male or the female, will be used to show the general features of the cell

at each of the stages; however, micrographs of identical areas in both the male and female will be referenced to document the basic similarity in secretory cell ultrastructure in the male and female.

The proctodeal gland secretory cell - Stage 1. For descriptive purposes the cell can be divided into three regions (figs. 3,4); these are: 1) a basal or perinuclear region containing the nucleus and the major concentration of RER; 2) a supranuclear region in which both the centrally located Golgi complexes and, when present a few condensing vacuoles are located; and 3) the apical region distinguished by the presence of a terminal web, a microvillous luminal surface and, when present, accumulating and releasing secretory droplets.

Basal region. This region is occupied primarily by the nucleus and the RER (figs. 3,4). The heterochromatic nucleus (figs. 5,6) is located in the central portion of the basal region; it is round to oval in shape and contains a prominent, granular nucleolus. Chromatin material is coalesced into a narrow osmiophilic band near the inner nuclear membrane; other aggregates of chromatin are dispersed throughout the nucleoplasm. Nuclear pores are present between the inner and outer nuclear membrane and a narrow (27.0 - 40.0 nm wide, male; 27.0 - 36.0 nm wide, female) perinuclear cistern surrounds the nucleus. The external border of the perinuclear cistern consists of a ribosome-studded membrane which appears to be continuous with that of the adjacent RER (fig. 6).

A few elongated mitochondria are present in the basal region. Where present, here and throughout other regions of the cell, these mitochondria are characteristically surrounded by a layer of RER (figs. 4,5,6). The majority of the RER, however, is located in the basal region of the cell and extends distad on either side of the nucleus up to the supranuclear region. The RER is present as an extensive tubular system consisting of compact, parallelly arranged membranes lined with ribosomes and enclosing narrow flattened cisternae (fig. 7) which measure 47.0 - 71.0 nm wide in the male, 37.0 - 64.0 nm wide in the female.

Supranuclear region. This region (fig. 3) is characterized by the presence of Golgi complexes, an abundance of RER-encircled, elongate mitochondria, transition elements of RER, transfer vesicles and, frequently, a few membrane-bound droplets. The Golgi complexes (figs. 4,8,9) consist of several arrays of compacted, elliptical-shaped lamellae; they are arranged in a U-shaped to circular pattern in the central portion of the region. Each complex consists of a forming face (convex-shaped and consisting of the outer Golgi saccules) and a mature face (concave-shaped and consisting of the inner Golgi saccules). The forming face is generally oriented toward the nucleus; the mature face toward the apex of the cell (figs. 8,9). The lamellae of the Golgi complexes consists of a continuous array of four to five membranes; saccules enclosed by the lamellae are somewhat flat and measure between 36.0 and 90.0 nm in width.

RER is not extensive in this area; that which is present appears to be extensions of that located basally. Transition elements of the RER (fig. 8) are found close to the Golgi complex; these elements which are part granular and part agranular appear to bud off the numerous transfer vesicles seen in the region of the Golgi complexes.

A few secretory droplets and condensing vacuoles (400.0 nm to 900.0 nm in greatest diameter) are located in association with the Golgi complexes (figs. 3,4,8,9). These vacuoles are membrane-bound and non-uniform in shape; in some, small vesicles and whorls of electron dense material resembling cytosegresomes, are present (figs. 8,9). Mature secretory droplets, identifiable by a fine, evenly distributed precipitate are seldom seen in stage 1 cells.

Apical region and plasma membrane. The apex of the cell (figs. 3,4,10) is a relatively thin zone consisting of a series of tonofilaments which form a terminal web. The filaments appear to extend into microvilli which line the apical border and project into the glandular lumen. The microvilli are short (0.3 - 0.5 μm) narrow (0.09 - 0.10 μm) and are regularly spaced along the luminal surface of the cell. A few small membrane-bound microvesicles and free ribosomes are also present in this region as are a few mature secretory droplets. A typical junctional complex (fig. 11) is located on the lateral borders of the apex of the cell. This complex consists of an outermost zonula occludens, and intermediate zonula adherens and an innermost macula adherens to which the tonofilaments

of the terminal web appear to attach. Distal to this junctional complex the lateral plasma membrane of adjacent cells is thrown into a series of complex interdigitating folds (fig. 12) which extend the length of the cell. The plasma membrane at the base of the cell rests on the basal lamina (figs. 3,4); it lacks any complex folds although it is sometimes irregularly contoured.

Stage 2 cells. Cells in this stage (fig. 13) exhibit a high state of secretory activity as indicated by the presence of: 1) branched RER enclosing dilated cisternae; 2) numerous condensing vacuoles and sometimes a few mature secretory droplets in the supranuclear region; and 3) dilated Golgi saccules with condensing vacuoles.

Other than a possible increase in the number of nuclear pores (figs. 14,15), the nucleus (fig. 13) shows little change from that of a stage 1 cell. The perinuclear cistern has enlarged (width: 49.0 to 147.0 nm male; 50.0 to 120.0 nm in the female). The continuity seen between the perinuclear cisterns and those of the RER in stage 1 cells is clearly evident in cells of this stage (fig. 15).

Flattened, parallel arrays of RER characteristic of stage 1 cells have changed to a highly branched system with enlarged cisternae containing a flocculant precipitate; the cisternal widths ranged from 217.0 to 285.0 nm in the male and from 149.0 to 244.0 nm in the female (figs. 14,15). Numerous transfer vesicles are present in association with the Golgi complex (figs. 16,17) and transitional elements of the RER. The Golgi saccules are enlarged (67.0 - 168.0 nm wide in the male; 70.0 - 137.0 nm wide in the female) and the inner Golgi saccules,

along the mature face, contain a fine grained precipitate similar to that visible in the secretory droplets forming from the saccules, in nearby condensing vacuoles, and in the mature secretory droplets.

Many condensing vacuoles (400.0 -900.0 nm dia.) and mature secretory droplets (720.0 - 1500.0 nm dia.) are present throughout the supranuclear region and extend up to the area immediately beneath the terminal web (figs. 13,16,17). As in stage 1 cells, cytosegresomes are present in a few of the mature secretory droplets. Other than the shortening of the region produced by the accumulation of secretory droplets, the apex of the cell (figs. 18,19) shows little change from that of stage 1. RER in this region, as with that throughout the cell, shows visibly enlarged cisternae.

Stage 3 cells. In this, the releasing stage of the secretory process, the major features of the cell (fig. 20) are: 1)extremely dilated cisternae of the RER; 2) large, distended and somewhat disorganized Golgi saccules; and 3) presence of large numbers of mature secretory droplets throughout the length of the cell. At the apex, the terminal web and the regularly-spaced microvillous surface has been replaced by coalescing secretory droplets which bulge and release their contents into the lumen of the glandular unit.

The nucleus of the stage 3 cell (figs. 21,22) remains oval to round and is heterochromatic. The number of nuclear pores present seems to have decreased. The dilated perinuclear cistern (72.0 - 184.0 nm wide, male; female, 45.0 - 137.0 nm)

is interrupted and contiguous with cisternae of the RER. The RER (figs. 21,22) consists of a loose meshlike network of irregularly arranged membranes as the results of extreme dilation and distention (475.0 - 859.0 nm wide in the male; 441.0 - 570.0 nm in the female) of the cisternae and the intertubules of the RER. A granular precipitate is visible in the cisternae.

The Golgi complex (figs. 23,24) is highly distorted and consists of discontinuous and disordered arrays of membranes enclosing greatly distended saccules (width: 462.0 - 627.0 nm, male; 251.0 - 320.0 nm, female). Relatively few transition elements of the RER or transfer vesicles are present compared with stages 1 and 2.

Large condensing vacuoles are dispersed throughout the cell. At the apex (figs. 25,26) several secretory droplets have fused with each other and with the apical plasma membrane; the integrity of the membranes of the droplets is lost in many instances and mitochondria, ribosomes and other cellular material is intermixed with the secretory product. These organelles are released into the lumen of the glandular unit along with the secretory product.

DISCUSSION

The secretory cycle and proctodeal gland secretory cells

This study describes for the first time the ultrastructural appearance of secretory cells of the proctodeal gland from six (each) male and female Common-Coturnix. The study has related these features to three discernible stages of the secretory cycle. When compared with data published on other secretory cells, this study indicates that the secretory process of the proctodeal gland is similar, but not identical, to that which occurs in other glands. While sharing basic similarities with all secretory cells, the proctodeal gland exhibits characteristics which are specific to the gland itself and which appear to be determined: 1) by the cyclic nature of secretory activity which it undergoes; 2) by the type of secretory product produced; and 3) by its response to exogenous hormone stimulation.

Palade ('75) has described the series of events which occur in protein synthesis in the pancreatic acinar cell. This secretory process can be applied to many protein-secreting cells. Essentially, the process consists of: 1) synthesis of protein by ribosomes attached to the rough endoplasmic reticulum (RER), and transfer of the protein from the ribosomes to the cisternae of the ER; 2) elaboration of smooth surfaced vesicles from the cisternae which then transport the protein to the Golgi complex; 3) accumulation of the secretion which then buds off as membrane-bound condensing vacuoles; and 4) enlargement and movements of the condensing vacuoles to the apex of the cell where they are released into the glandular lumen. In some

instances, as is the case in the proctodeal gland, a carbohydrate moiety is added to the protein in the Golgi complex to form a glycoprotein secretion (Renzoni, '72).

The secretory process described by Palade ('75) can be correlated with the ultrastructural features of the three stages of cells identified in this study to describe the secretory cycle of proctodeal gland cells. Stage 1 cells are those in which the organelles have reorganized following completion of one secretory cycle and are initiating a new cycle. In stage 1, the compacted cisternae of the RER contain little or no evidence of accumulation of synthesized material, the Golgi complex is composed of flattened lamellae in a single array, and few transfer vesicles are found between the Golgi complex and the RER cisternae. The few secretory droplets located in the supranuclear and apical regions are possible remnants of those produced in the previous secretory cycle. With increased synthetic activity, the cell moves into stage 2 configuration in which more material is being passed into the RER cisternae and distending them. Numerous transfer vesicles form and carry this protein to the Golgi complex which also begins to enlarge, form and bud off condensing vacuoles. The secretory droplets mature and begin to move to the apex of the cell. By the time the cells have arrived at stage 3, synthesis has decreased or ceased; the RER is disorganized and the Golgi complex, which is also highly distended, is in the process of forming the last secretory droplets of the cycle. At this stage the apex of the cell shows numerous coalescing mature secretory droplets distending the apical

plasma membrane. The droplets are released from the apex which appears devoid of microvilli except at the perimeter of the cell; disappearance of the microvilli probably is the result of distention of the coalescing secretory droplets. The presence of small portions of cellular debris, including some mitochondria, in the lumen of the glandular unit, suggests that a type of apocrine release is carried out by these cells. The cellular material may be released as the result of fusion and breakdown of secretory droplet membranes within the cell allowing some cytoplasmic elements to be intermixed with the secretion product and released into the glandular lumen.

The cellular changes which occur in the secretory cycle can be observed only on the ultrastructural level; however, cells in the three stages described can also be identified in the light microscope. Thick sections of tissue stained en bloc with uranyl acetate and stained additionally with toluidine blue exhibited cells of three staining intensities: dark, intermediate, and light. Toluidine blue stains the cytoplasmic elements of the cell, including the RNA on ribosomes of the compact RER; uranyl acetate stains membranes. Those cells in which the Golgi complex and the cisternae of the RER are compact, and in which few secretory droplets are present, stain most intensely. As the RER cisternae and the Golgi saccules enlarge and more secretory droplets appear, the stain becomes less intense, in part because the ribosomes become more dispersed. On this basis, one can identify the dark staining cells as being comparable to stage 1, (i.e. active protein

synthesis beginning); the intermediate staining cells as comparable to stage 2, (i.e. packaging in the Golgi complex and secretory droplet formation); and the light staining cells as comparable to stage 3, (i.e. secretory droplet release and disorganized Golgi complex and RER).

There is no published evidence available to document that a given secretory cell of the proctodeal gland undergoes a series of sequential secretory cycles. However, indirect evidence suggests they do. Klemm (unpublished data) has observed that mitotic figures are only rarely seen in glandular tissue of adult sexually-active male and female Common-Coturnix. Franks and Barton ('60) observed this phenomenon in the prostate gland in the mouse, as did Woodhouse and Rhodin ('63) in Harder's gland in the mouse. The proctodeal gland of the adult male Common-Coturnix produces copious amounts of a foamy secretion continuously throughout its adult life. The absence of high mitotic activity in the cells suggests that the secretion must come from cells which are either in a constant state of secretion or which cycle through many secretory periods. The existence of stages in the secretory process described in this study indicates that the cells cycle from a period of active synthesis and secretion to one of cellular reorganization followed by initiation of a new cycle. The actual secretory process which they undergo, however, appears identical to that which takes place in many cells.

A major ultrastructural characteristic of the three stages described in this study is the progressive dilation of the

cisternae of the RER and the Golgi saccules. It is unlikely that this dilation is due to fixation artifact since I could identify all three stages of the secretory cells randomly dispersed throughout a given section; in some instances a stage 1 cell was adjacent to a stage 3 cell. It would be difficult to ascribe dilated cisternae of a stage 3 cell to poor fixation when an adjacent stage 1 cell lacks any apparent fixation artifact. I believe these stages show actual cellular alterations which occur in the normal cyclic secretory process of cells of the proctodeal gland.

Comparison with ultrastructural features of other secretory cells

Studies on other secretory cells in which specific stages of a secretory cycle have been defined, are few. Woodhouse and Rhodin ('63) described stages of a secretory cycle in Harder's gland of the male and female mouse; Brandes ('66) has reported ultrastructural features of the RER cisternae and of Golgi saccules in various lobes of male mammalian prostate gland which are like those seen in the stages described in this study. Brandes ('66) has also described secretory and "resting" stages of the male rat prostate gland in which the ultrastructural features of the cells are closely similar to stages 1 and 3, respectively, of this study.

A characteristic of the stages of the secretory cycle described by Woodhouse and Rhodin ('63) and implied by Brandes ('66) is the progression of cisternae of the RER or the Golgi saccules, or both, from a compacted to a distended, disorganized

state during the progress of the secretory cycle. At the end of the cycle these organelles reorganize, return to a compacted state, and begin a new cycle. In Harder's gland the Golgi complex shows this cyclic nature (Woodhouse and Rhodin, '63); RER is present and is described as "extensive" (Cowan, '71), but was not referred to as progressing through or not progressing through secretory stages. In the male prostate, however, the alterations of the cisternae of the RER and of the Golgi are sequentially identical to those described in this study for cells of the proctodeal gland (Brandes, '66).

In glands which produce a glycoprotein secretion, as the proctodeal gland (Renzoni, '72), the Golgi complex appears as is described in this study for the proctodeal gland. Neutra and LeBlond ('66), in their description of the structure of the Golgi complex in the glycoprotein-secreting goblet cells of the intestine, refer to a large U-shaped Golgi complex in early stages of secretion giving way to formation of several arrays of Golgi complexes with dilated saccules. The goblet cells, unlike the proctodeal gland cells, show no coalescence of secretory droplets at the apex of the cell; instead, each droplet fuses with the plasma membrane and releases its contents individually into the glandular lumen. Neither the structure of, or changes in, the RER were discussed by Neutra and LeBlond ('66).

References to sexual differences in glandular secretory cells are also scarce. Woodhouse and Rhodin ('63) studied cells from both male and female Harder's glands of the mouse and found no sexual difference detectable between the cells;

presumably this would be expected as these glands show no evidence of sex hormone sensitivity or sex-related functions. The prostate gland, on the other hand, is well-documented to be reproductively associated and hormone sensitive. Smith et al ('68) reporting on the prostate gland of male and female rodents (Praomys (Mastomys) natalensis) found major differences between the male prostate and its female homologue. The ultrastructural features of the male prostate in Praomys, however, as with that described by other investigators of prostate gland ultrastructure (Brandes et al. '64, Mao and Angrist, '64, Brandes '66) were similar to those described in this study for cells of the male proctodeal gland. Smith et al. ('68) ascribed the sexual differences in the prostate of Praemys to a greater sensitivity of the male prostate to androgens than of the female homologue to ovarian hormones. The effect of testosterone on prostatic secretory cells, recorded in the mouse by Franks and Barton ('60) indicated that one effect of the hormone was that testosterone-treated cells were "taller" than non-treated cells. Klemm et al. ('73) reported that secretory cells of the proctodeal gland of the male were significantly higher than those of the female. Unlike the extreme ultrastructural differences noted in testosterone-treated cells versus non-treated cells of the mouse prostate (Franks and Barton, '60) this study has shown no difference in appearance of male and female secretory cells.

The lack of any significant differences in appearance of secretory cells of the male and female proctodeal gland can be

explained by a possible androgen sensitivity on the part of cells of both male and female Common-Coturnix. The androgen sensitivity of the male proctodeal gland has been well-documented (Nagra et al., '59, Sachs, '67, McFarland et al., '68); these studies suggest that the visible enlargement of the proctodeal gland and production of copious amounts of a foamy secretion in the male Common-Coturnix is due to androgen sensitivity of the gland. No direct data has been published on hormonal sensitivity of the female proctodeal gland; however, a study by Adkins and Adler ('72) suggests that the gland is sensitive, at least to a degree, to androgens. Adkins and Adler ('72) induced enlargement of the female proctodeal gland to approximately one-half the size of the gland found in normal males. Concurrent with this glandular enlargement was a production of a small amount of foamy secretion by the female. These effects were accomplished by administering 8-10 injections each of 5.0 mg testosterone propionate to photocastrated females. Photocastrated males, given the same injections, showed glandular development and secretory activity identical to that found in normal, sexually active adult males. These data suggest that the proctodeal gland of normal female Common-Coturnix is sensitive to androgen. In the normal female sufficient testosterone may be produced by the ovary, or the adrenal glands, or both, to stimulate the proctodeal gland to secrete; circulating titers of testosterone, however, may be insufficient to stimulate the gland to the high secretory state seen in the male. In addition, the inability of the female gland to respond as fully as the

male to identical concentrations of testosterone (Adkins and Adler, '72) suggests a lower degree of sensitivity of the gland to the hormone in the female.

These circumstantial pieces of evidence on hormonal sensitivity of proctodeal gland secretory cells suggests: 1) that the similarities in ultrastructural appearance of the cells results from a sensitivity on the part of cells of both sexes to testosterone; but 2) a greater sensitivity to, or a higher circulating titer of, androgen -- or both -- in the male which accounts for the more copious secretory activity and larger size of the gland in the male.

The proctodeal gland is similar to the prostate gland and to Harder's gland in that the cells of these glands undergo a cyclic pattern of secretory activity which is evident in changes in the RER cisternae and Golgi saccules; it is also similar to the male prostate gland in that both are androgen sensitive. Cells of the proctodeal gland are similar to glycoprotein-secreting goblet cells as far as cyclic patterns of the secretory droplet release. Based on the limited data available on the secretory process of glandular cells, it would thus appear that the basic secretory process is the same in all of these cells but that each cell shows individual characteristics. These characteristics are dependent upon the type of secretion produced, on the method of secretory droplet release, on whether or not a single cell undergoes many repeated secretory cycles --with concurrent cellular reorganization following each cycle --and on whether or not exogenous factors such as hormones

act on and modify the secretory cell function or structure. All of these factors appear to influence the secretory process in the proctodeal gland; their effect can be demonstrated on the ultrastructural characteristics of these secretory cells.

APPENDIX I

MATERIALS AND METHODS

1. Cacodylate Buffer (Hayat, '70)
   0.15 M Cacodylate Acid MW 160.1 6 gm/250 ml H_2O
   Refrigerate to 4^oC
   Adjust pH to 7.2 and mOsm to 330

2. Glutaraldehyde Fixative (Hayat, '70 modified)
   2.5% glutaraldehyde @ 4^oC
   Remove Acids
   Barium Carbonate (1 spatula/50 ml glutaraldehyde)
   Stir
   Filter
   Adjust pH to 7.0
   Mix 1:1 with 0.15 M Cacodylate buffer
   Adjust pH to 7.2 and mOsm to 330

3. Osmium tetroxide Post-fix solution (Caufield, 1957, modified)
   2% OsO_4 0.5 gm/25 ml double distilled H_2O
   Mix 1:2 with 0.15 M Cacodylate buffer
   Adjust pH to 7.2

4. Aqueous Uranyl Acetate (Hayat, 1969)
   25 ml 15% Acetone
   0.5 gm Uranyl Acetate
   0.5 ml DMSO
   Mix on stirrer for approximately one hour using small beaters in a corked 50.0 ml flask.

5. Epon Araldite (Newcomb, undated)

Epon 812	31.0 gm
Araldite 502	21.5 gm
DDSA	60.0 gm
DMP-30	63 drops via Pasteur pipette

Mix each ingredient in first before adding the next; motor stir the solution in disposable beaker.
Makes 100 ml which can be stored at $4^{o}C$ in sealed 10cc syringes.

6. Lead Citrate (Venable and Coggeshall, 1965)
10.0 ml double distilled, carbon dioxide free, water
0.02 gm Lead Citrate (PbCi)
0.1 ml 10 N NaOH
Mix all ingredients in a screw top vial. Shake vigorously until all lead citrate is dissolved.

7. Toluidine blue - Sodium borate (Richardson, 1962, Modified)

Toluidine blue	2 gm
Distilled water	100.0 cc
Sodium borate	2 gm
Distilled water	100.0 cc

Mix equal amounts of the toluidine blue - sodium borate solutions (usually one drop of each will do) on a dry glass slide holding the sections. Heat until edges of the stain start to turn green. Do not let stain dry at this stage. Rinse in distilled H_2O. Heat again to dry.

ABBREVIATIONS

BC	Basal cell
BL	Basal lamina
C	Chromatin
CIS	RER cisternae
CS	Cytosegresome
CT	Connective tissue septum
CV	Condensing vacuole
FF	Forming face - Golgi
GC	Golgi complex
GS	Golgi saccule
IF	Interdigitating fold
IGS	Inner Golgi saccule
M	Mitochondrion
MA	Macula adherens
MF	Mature face - Golgi
MSD	Mature secretory droplet
MV	Microvilli
N	Nucleus
NC	Nucleolus
NP	Nuclear pore
OGS	Outer Golgi saccule
PC	Perinuclear cistern
PM	Plasma membrane
PPT	Precipitate
R	Ribosome
RER	Rough endoplasmic reticulum
TE	Transition element
TF	Tonofilament
TV	Transfer vesicle
TW	Terminal web
V	Vesicle
ZA	Zona adherens
ZO	Zona occludens

1	Dark-staining cell
2	Intermediate-staining cell
3	Light-staining cell

PLATE I

Photomicrographs of thick (1.0 um) Epon-Araldite sections of the secretory tissue of the proctodeal gland of adult, sexually-active male (fig. 1, X750) and female (fig. 2, X784) Common-Coturnix. Three types of cells are visible: 1) dark-staining, 2)intermediate-staining, and 3) light-staining. Basal cells are sometimes present between the basal borders of the secretory cells. Both the secretory and basal cells rest on a basal lamina; connective tissue septae separate adjacent layers of the secretory epithelium. Toluidine blue.

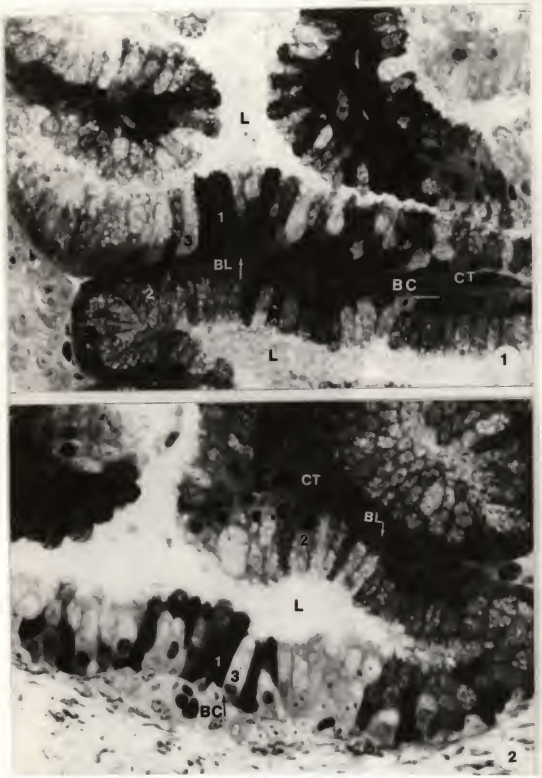
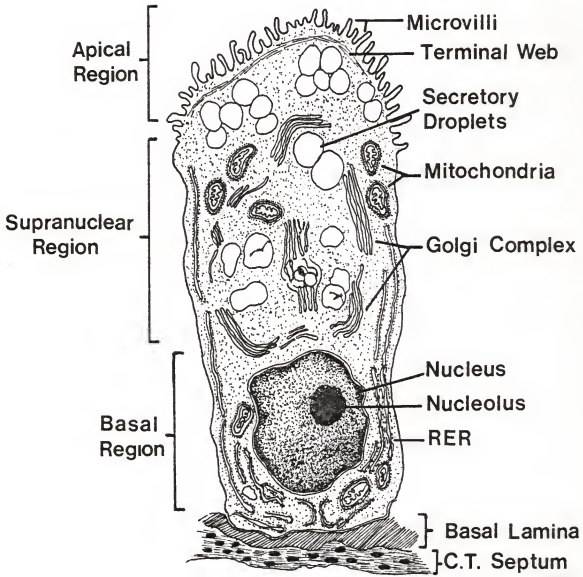

L
1
3
BL
2
BC
CT
L
1
CT
BL
2
L
1
3
BC
2

PLATE II

Fig. 3 A diagrammatic representation of the ultrastructure of a typical secretory cell of the proctodeal gland. For descriptive purposes the cell may be organized into three major regions as shown. Major structural features of each region are indicated.

GLANDULAR LUMEN



③

PLATE III

Fig. 4 Electron micrograph of a stage 1 secretory cell in an adult, sexually-active male Common-Coturnix showing: 1) in the basal region, a heterochromatic nucleus with two nucleoli, surrounded by RER; 2) the supranuclear region containing the extensive Golgi complex with a few condensing vacuoles and numerous RER-encircled mitochondria both above and below the Golgi complex; and 3) the apex of the cell in which a few condensing vacuoles are also present immediately beneath the microvillous-lined luminal surface. Uranyl Acetate, Lead Citrate. X 13,720.

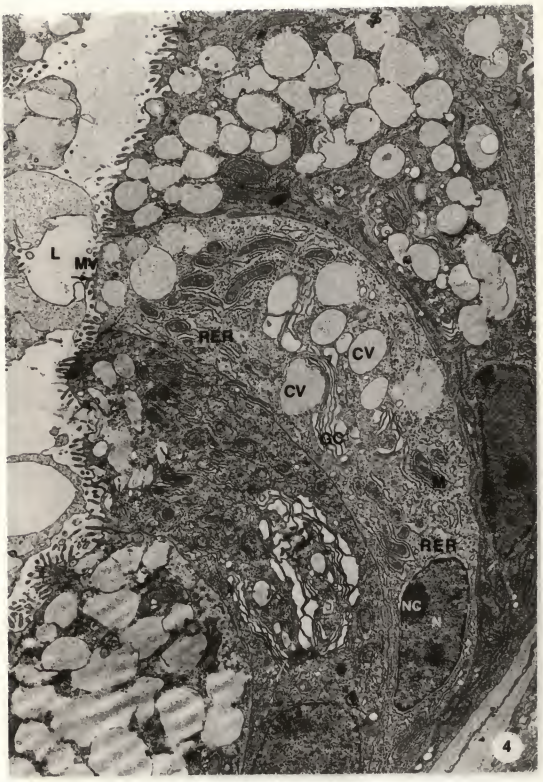

L
MV
RER
CV
CV
GC
RER
NC
N
4

PLATE IV

Electron micrographs comparing the nuclei in stage 1 secretory cells in the male (fig. 5, X 16,800) and in the female (fig. 6, X 23,680). Chromatin material is dispersed throughout the nucleoplasm and is condensed into a narrow border near the inner nuclear membrane. Nuclear pores and a narrow perinuclear cistern are visible as are flattened cisternae of the RER and a few RER-encircled mitochondria. Note (fig. 6, arrow) the continuity between the outer nuclear membrane and the RER. The insert (fig. 6) is an enlargement of a region similar to that outlined in fig. 6 showing a nuclear pore, the perinuclear cistern and the ribosomes on the outer nuclear membrane. Uranyl Acetate, Lead Citrate, X 63,640.

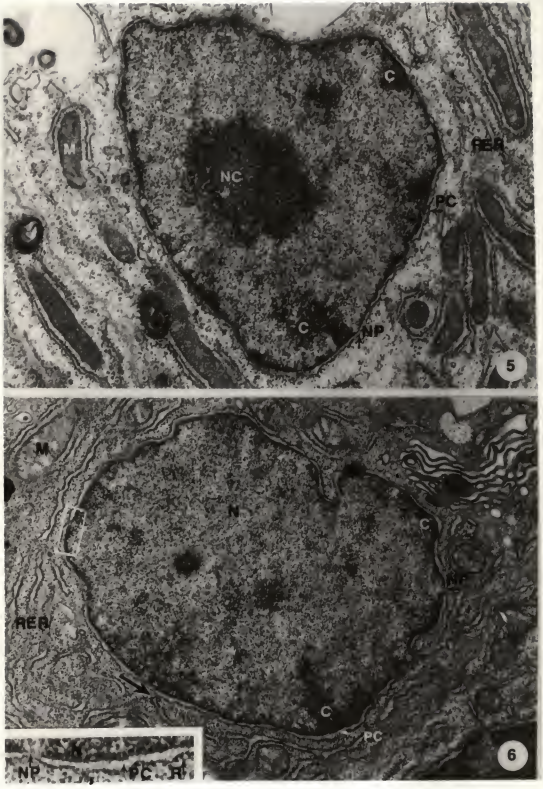
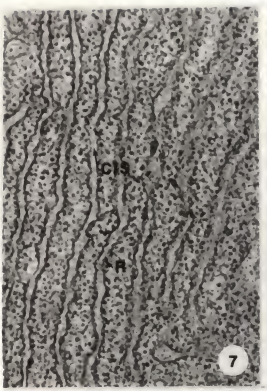

C
M
NC
RER
PC
C
NP
5
M
N
C
NP
RER
C
PC
NP
PC
R
6

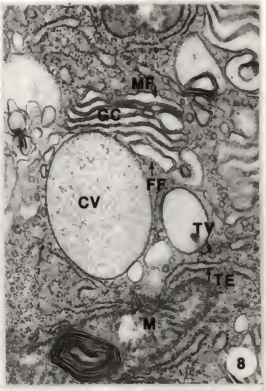
PLATE V

Fig. 7 Electron micrograph of a stage 1 cell of the female showing the flattened, parallel array of compacted RER cisternae. Ribosomes are visible on the outer membrane; the cisternae contain a fine fibrogranular substance.

Fig. 8 Electron micrograph from a stage 1 secretory cell of the female showing the forming face and the mature face of a Golgi complex. Note the part granular and part agranular transition elements of the RER and transfer vesicles which appear to bud from them. A large condensing vacuole is present near the forming face. Below the condensing vacuole is a vacuolate-appearing mitochondrion containing a cytosegresome. Uranyl Acetate, Lead Citrate, X 34,040.

Fig. 9 Electron micrograph of a stage 1 secretory cell of the male showing a U-shaped Golgi complex in which large condensing vacuoles are located between the saccules. Outer Golgi saccules of the forming face and inner Golgi saccules of the mature face are identified. The condensing vacuoles contain a fine fibrogranular substance and an occasional osmiophilic cytosegresome. Uranyl Acetate, Lead Citrate, X 18,400.


CIS
R
7


MF
GC
FR
CV
JY
TE
M
8

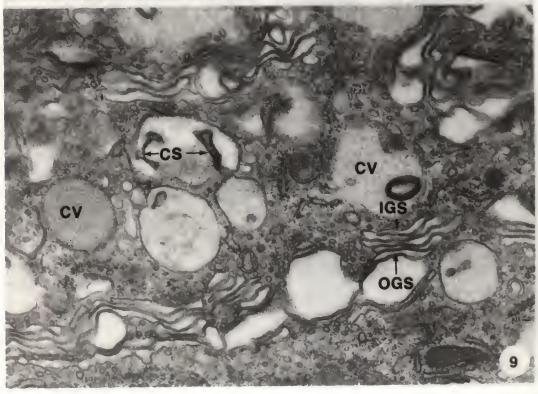

CS
CV
CV
IGS
OGS
9

PLATE VI

Fig. 10 Electron micrograph of the stage 1 secretory cell of the male. The apical region shown in the micrograph contains a terminal web, free ribosomes, tonofilaments and small vesicles. Tonofilaments appear to extend into the regularly-spaced microvilli which project into the glandular lumen. Uranyl Acetate, Lead Citrate, X 26,320.

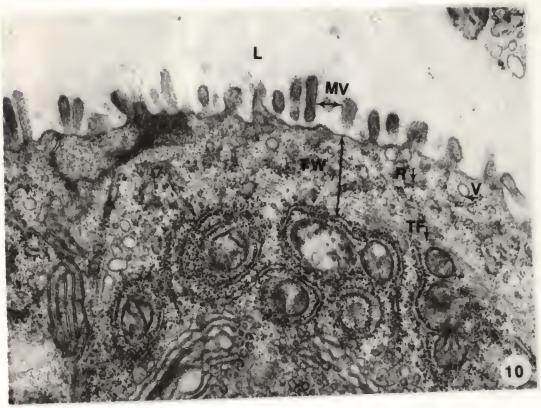
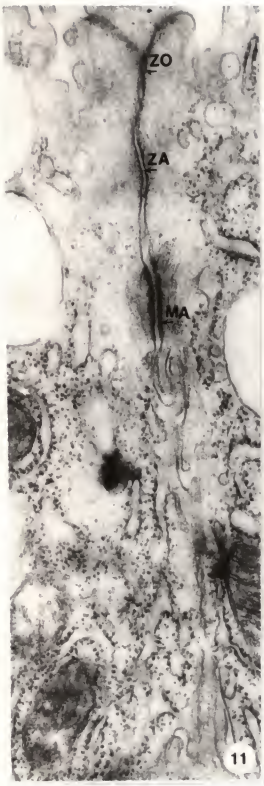

L
MV
PW
R
V
TF
10

PLATE VII

Fig. 11 Electron micrograph of adjacent secretory cells in the male showing the junctional complex of the plasma membrane in the apical region. Note the zona occludens, the zona adherens, and the macula adherens to which tonofilaments of the terminal web appear to attach. Uranyl Acetate, Lead Citrate, X 21,000.

Fig. 12 Electron micrograph of the male illustrating the interdigitating folds of the lateral plasma membrane of adjacent secretory cells. Uranyl Acetate, Lead Citrate, X 30,150.


ZO
ZA
MA
11

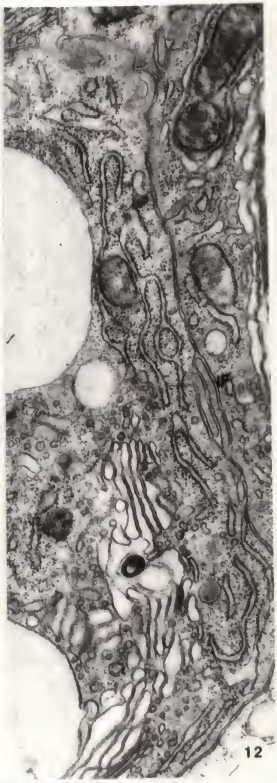

12

PLATE VIII

Fig. 13 Electron micrograph of a stage 2 secretory cell in the female. The cell is in a state of active secretion as suggested by cellular changes evident by comparison with stage 1 cells (see fig. 4). In the basal region the nucleus is surrounded by the RER which consists of dilated intertubules and cisternae. The supranuclear region extends from immediately above the nucleus almost to the apex of the cell. The Golgi complex contains dilated saccules and appears separated into several arrays by the presence of many condensing vacuoles and a few mature secretory droplets present in the region. The apical region appears somewhat thinner than in stage 1 (see fig. 4) but a terminal web and regularly spaced microvilli are still present. RER-encircled mitochondria are dispersed throughout the cell from the basal region up to the region of the terminal web. Uranyl Acetate, Lead Citrate, X 12,060.

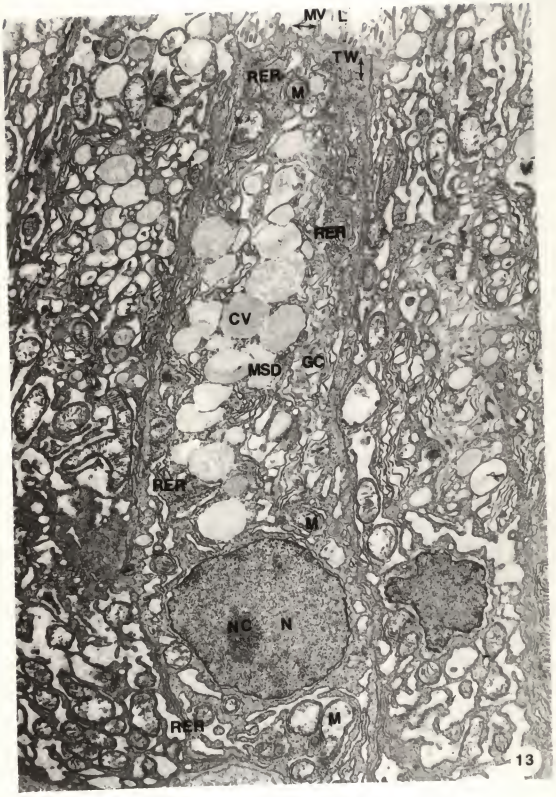
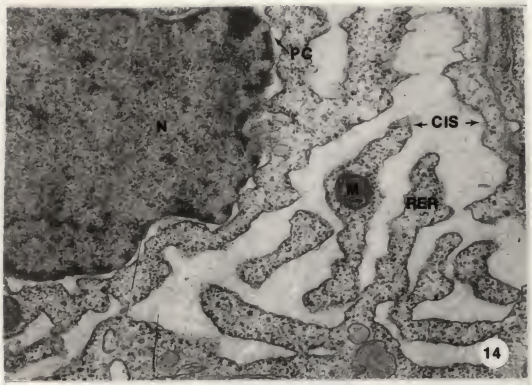

MV
L
TW
RER
M
RER
CV
MSD
GC
RER
M
NC
N
RER
M
13

PLATE IX

Electron micrographs comparing the perimeter of the nuclei and the RER in stage 2 secretory cells of the male (fig. 14, X 37,740) and female (fig. 15, X 37,740). Note the numerous nuclear pores and the enlarged perinuclear cistern. Accumulation of a fibrogranular substance within the cisternae and intertubules of the RER give it a branching appearance; the cisternae also appear more dilated in the male than in the female.


PC
N
CIS
M
RER
14

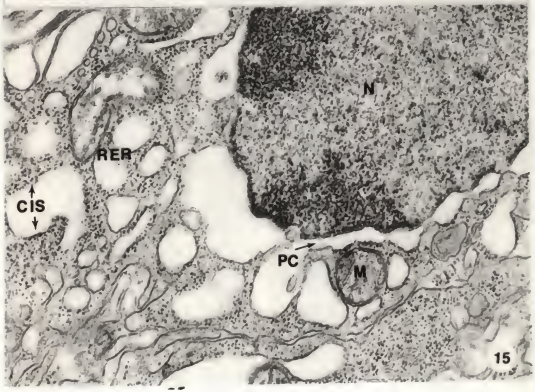
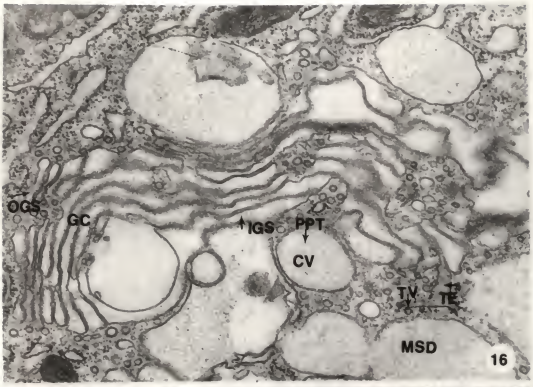

N
RER
CIS
PC
M
15

PLATE X

Electron micrographs comparing the Golgi complex in stage 2 secretory cells in the male (fig. 16, X 34,780) and the female (fig. 17, X 34,780). Transition elements of the RER, part granular and part agranular, are visible as are transfer vesicles formed from the transition elements. The transfer vesicles are numerous and are in close proximity to the outer Golgi saccules. The saccules of the Golgi complex are more dilated than in stage 1 (see figs. 8,9) and numerous condensing vacuoles containing a flocculent precipitate are present in the area of the outer Golgi saccules at the mature face. Coalescing mature secretory droplets are also present in the region.


OGS
GC
IGS
PPT
CV
TV
TE
MSD
16

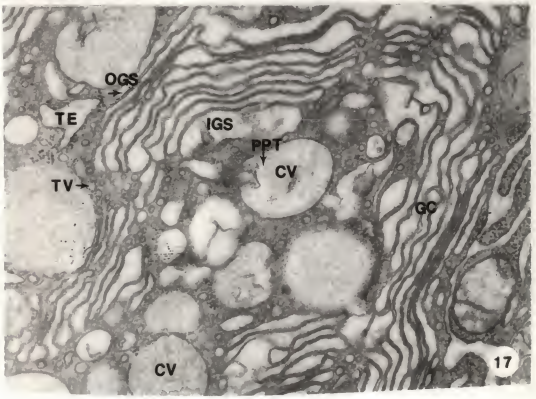
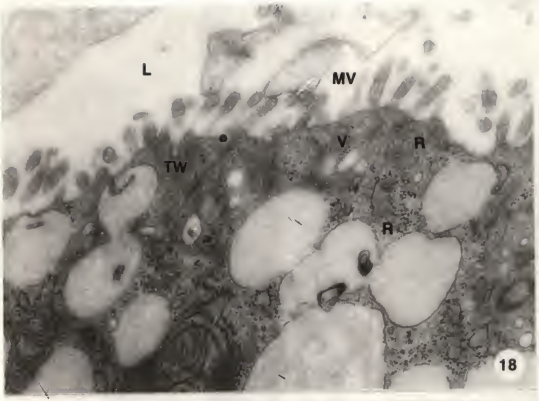

OGS
TE
IGS
PPT
CV
TV
GC
CV
17

PLATE XI

Electron micrographs of the apical region of stage 2 secretory cells in the male (fig. 18, X 35,520) and in the female (fig. 19, X 35,520). The region is only slightly changed from that seen in stage 1 (see fig. 10) with the basic difference being a shortening of the region. A terminal web is still present as are numerous free ribosomes and small vesicles. Regularly spaced microvilli line the luminal surface of the cell. The presence of mature secretory droplets in the male than in the female suggests that the male was in a more advanced phase of stage 2 than was the female.


L
MV
V
R
TW
R
18

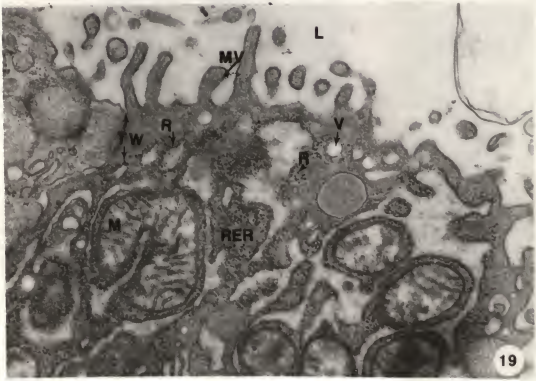

L
MV
R
TW
V
R
M
RER
19

PLATE XII

Fig. 20 An electron micrograph of a stage 3 secretory cell in the male in which the secretory product is being released into the glandular lumen. The basal region contains only the nucleus and RER which show considerably distended cisternae. The supranuclear region occupies the majority of the cell; the Golgi complex is distorted, membranes are disordered and saccules highly dilated. The major feature of the region is the presence of a few condensing vacuoles and numerous mature secretory droplets which have coalesced with each other. The apical region is devoid of any evidence of a terminal web and consists primarily of large numbers of mature secretory droplets which have fused with the apical plasma membrane and are bulging into the glandular lumen. Microvilli on the apical surface have disappeared except at the lateral margin of the cell. Mitochondria, surrounded by RER with distended cisternae are dispersed throughout the cell. Uranyl Acetate, Lead Citrate. X 10,620.

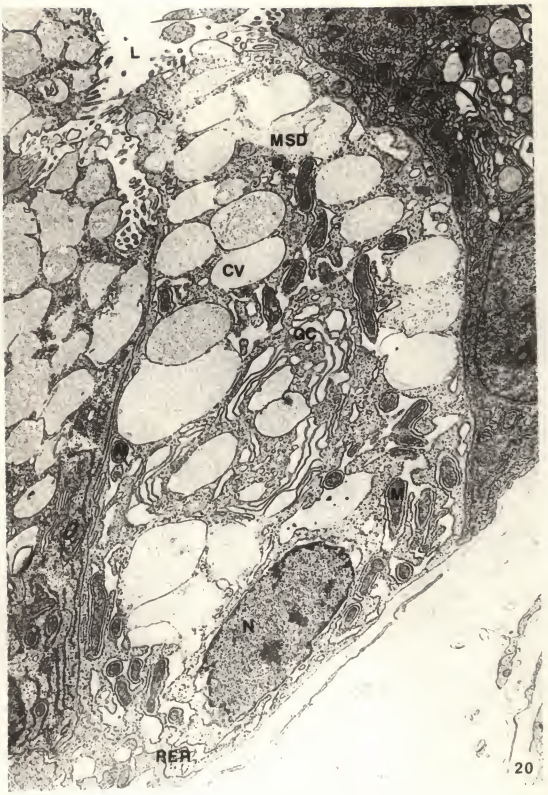
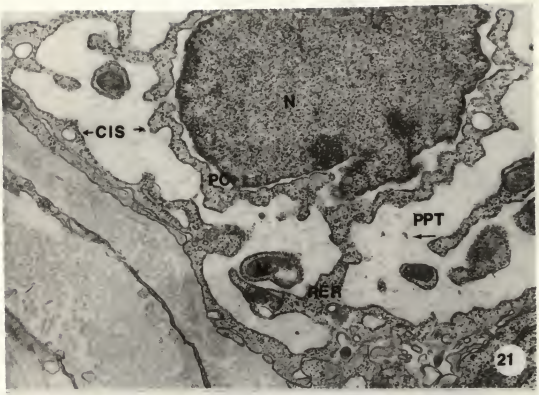

L
MSD
CV
GC
N
RER
20

PLATE XIII

Electron micrographs comparing the perimeter of the nucleus and the RER in stage 3 secretory cells of the male (fig. 21, X 34,100) and the female (fig. 22, X 35,100). Note the nuclear pores and the greatly enlarged perinuclear cistern. The cisternae of the RER are extremely dilated and contain small aggregations of a granular appearance substance. Uranyl Acetate, Lead Citrate.


N
CIS
PC
PPT
RER
21

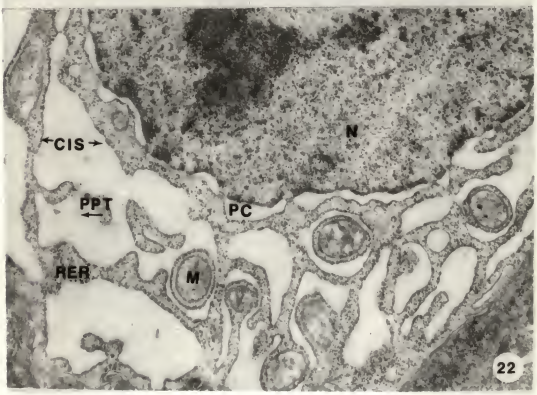
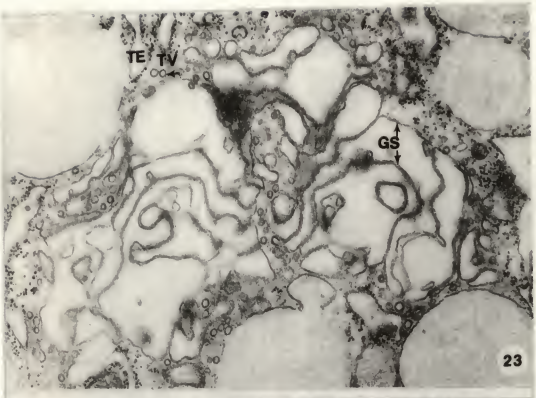
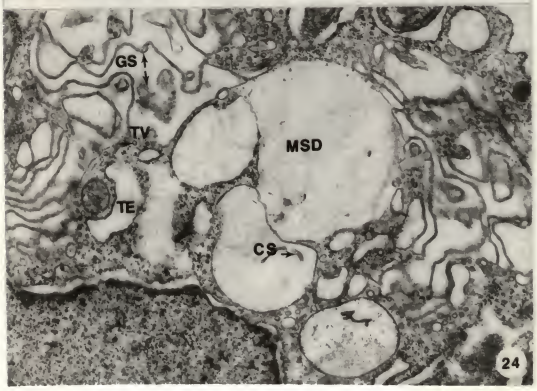

CIS
N
PPT
PC
RER
M
22

PLATE XIV

Electron micrograph comparing an array of the Golgi complex in stage 3 secretory cells of the male (fig. 23, X 31,500) and the female (fig. 24, X 31,500). Note the greatly enlarged transition elements of the RER, the presence of transfer vesicles and especially the disordered membrane pattern and disorganized, highly dilated Golgi saccules. A cytosegresome is present in one mature secretory droplet (fig. 24). Uranyl Acetate, Lead Citrate.


TE
TV
GS
23


GS
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MSD
TE
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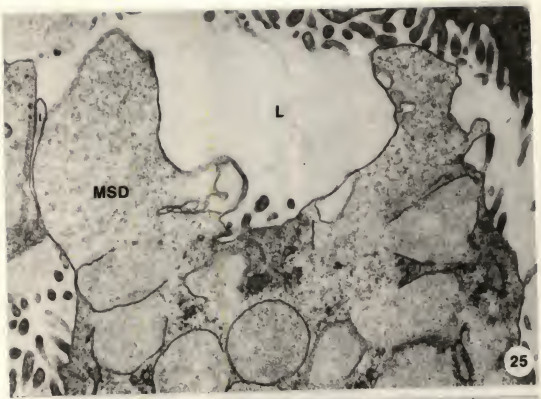
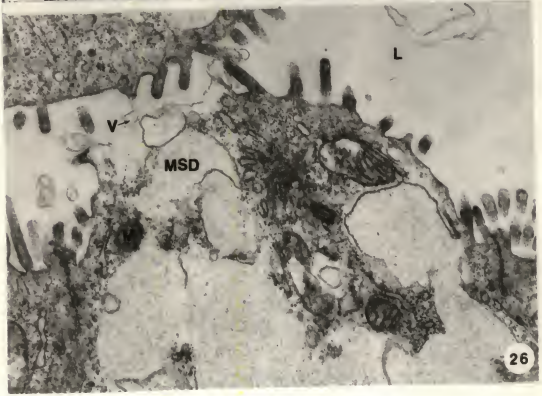
24

PLATE XV

Electron micrographs of the apex of a stage 3 secretory cell from males showing the appearance of the surface immediately prior to release of the product (fig. 25, X 21,600) and during release (fig. 26, X 25,200) into the glandular lumen. Mature secretory droplets coalesce at the surface of the cell, the membranes break down and cellular material is intermixed with the secretory product (fig. 25); microvilli disappear with the bulging of the apical plasma membrane. At release (fig. 26) the plasma membrane of the cell breaks down and the contents of the secretory droplet, which may include ribosomes and an occasional mitochondrion (not shown) are released into the glandular lumen in an Apocrine type of secretion. Uranyl Acetate, Lead Citrate.


L
MSD
25


L
V
MSD
26

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THE ULTRASTRUCTURE OF THE SECRETORY CELLS OF THE PROCTODEAL GLAND IN MALE AND FEMALE *COTURNIX COTURNIX JAPONICA* (AVES)

by

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B.A., Westmar College, 1974

A MASTER'S THESIS

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requirements for the degree

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1979

ABSTRACT

The ultrastructure of secretory cells of the proctodeal gland of the Common-Coturnix (Coturnix coturnix japonica) was studied in 12 adult, sexually-active male and females. Secretory cells described by light microscopy as dark-staining, intermediate-staining, and light-staining, could be identified ultrastructurally as representing sequential stages in a secretory process. These stages were identified as stage 1, stage 2 and stage 3 cells. In stage 1 cells, at the initial phase of the secretory process, the cells exhibited: 1) compacted, rough endoplasmic reticulum (RER); 2) a U-shaped Golgi complex and a few condensing vacuoles; and 3) absence of any major accumulation of secretory droplets. At stage 2, the cells were in a high state of secretory activity in which: 1) cisternae and intertubules of the RER were dilated; 2) dilated Golgi saccules with numerous condensing vacuoles were present; and 3) an accumulation of condensing vacuoles and mature secretory droplets was visible near the apex of the cell. By stage 3, the releasing stage, the cells contained: 1) extremely dilated RER cisternae which appeared as a loose meshwork of irregularly arranged membranes; 2) highly distended Golgi saccules with distorted membranes; and 3) accumulation of mature secretory droplets which coalesce with each other and fuse with the apical plasma membrane to release their contents into the glandular lumen.

There were no detectable sexual differences in the secretory cells other than a lesser degree of dilation of the RER cisternae and Golgi saccules in the female. The absence of sexual differences is related to variations in sensitivity of the secretory cells to androgens in the male and female.

Ultrastructural characteristics of the proctodeal gland were compared with those of the mammalian Harder's and prostate glands. Although differences in ultrastructure exist in the three glands, all three undergo a continuous secretory cycle in which a cell synthesizes (stage 2) and releases (stage 3) a secretory product, then reorganizes and begins a new secretory cycle (stage 1).