AN ULTRASTRUCTURAL STUDY OF THE SEMINAL VESICLE AND ITS RESPONSE TO HORMONE TREATMENT IN THE MALE FROG RANA CLAMITANS

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ABSTRACT

AN ULTRASTRUCTURAL STUDY OF THE SEMINAL VESICLE AND ITS RESPONSE TO HORMONE TREATMENT IN THE MALE FROG *RANA CLAMITANS*

By

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The seminal vesicles in *Rana clamitans* are evaginations of the Wolffian duct consisting of a central tube that is highly branched, lined with a pseudostratified epithelium, and surrounded by connective tissue.

The cells making up the pseudostratified epithelium consist of two types, ciliated and nonciliated cells. The nonciliated cells are of four types depending on their intercellular attachments and the appearance of the cytoplasmic vesicles.

Information on the effect of HCG (human chorionic gonadotropin) on the ultrastructure of the seminal vesicle epithelium was obtained by comparing electron micrographs of HCG treated and non-HCG treated animals.

A response to HCG injection was the release of cytoplasmic vesicles followed in later time periods by the increase in the appearance of Golgi bodies and membrane-bound ribosomes. This response suggests the nearly complete release of seminal vesicle secretions followed by a return to a high level of synthesis of secretory material.

This interpretation is in agreement with other evidence that seminal vesicles secrete materials which influence fertilization and correlates with known breeding behavior of *Rana clamitans*.

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INTRODUCTION

The study of fertilization in amphibians in our laboratory has centered primarily around the role of the oviducal secretions and egg jellies in the activation of the spermatozoon. There are very few reports of observations on the structure and function of male accessory organs, the seminal vesicles, in relation to the physiological state of spermatozoa in amphibians. The seminal vesicles have been described as storage organs in which spermatozoa remain for varying periods of time, and there have been a few studies of the secretions produced by the organs in some amphibian species (Rugh, 1934, 1939; Aron, 1926; Mann, Lutwak-Mann and Hay, 1963).

The seminal vesicles of anuran amphibians are swellings or evaginations of the distal portions of the Wolffian ducts *(vasa deferentia)*. Except for teleost fishes, seminal vesicles of species in other classes of vertebrates also appear to be derived from distal evaginations of the Wolffian duct. For example, Dean and Wurzelmann (1964) have described the seminal vesicle in the mouse as consisting of a single layer of secretory epithelium, surrounded by a thin layer of connective tissue, which is confluent with the caudal portion of the *vas deferens*. The seminal vesicles of passerine birds are represented by swellings of the distal portions of the Wolffian ducts (Mann, 1964). However, in species of teleost fishes, the seminal vesicles have been described

as specialized lobes of the testes and are not homologous with the organs of the same name in other vertebrates (for references see Sundararaj, 1958). Hypertrophy and decrease in secretory activity of terminal and preterminal portions of the *vasa deferentia* in a lizard *(Anolis carolinensis)* have been reported as a result of castration, suggesting that these portions of the duct may function similarly to seminal vesicles (Parks, 1960).

Basically, the histology of the seminal vesicles as described in *Rana pipiens* (McAllister, 1973), *Rana esculenta* and *Rana temporaria* (Aron, 1926), and *Discoglossus pictus* (Mann, Lutwik-Mann, and Hay, 1963) is similar. The seminal vesicles in these species consist of a lining of epithelial cells supported by a thin layer of connective tissue. The epithelial lining was described as consisting of columnar cells, basal cells, and smaller cells.

The columnar cells of *D. pictus* (Mann, Lutwik-Mann, and Hay, 1963) contain cytoplasmic granules during the breeding season. These granules are positive for the periodic acid-Schiff (PAS) reaction, indicating that they contain mucoid or other carbohydrate-containing substances. The pseudostratified columnar epithelial cells of *R. pipiens* have been shown to contain neutral mucopolysaccharides (McAllister, 1973).

The seminal vesicles of some species of vertebrates respond to exogenous hormones or to changes in the breeding cycle. The seminal vesicles in passerine birds have been described as becoming enlarged during breeding season (Parkes, 1960). Puckett (1939) recorded an increase in the size of the Wolffian ducts and seminal vesicles of *R. pipiens* and *Bufo americanus* in response to testosterone. Rugh (1939,

1941) observed an increase in the size of the Wolffian ducts and seminal vesicles of *R. pipiens* and *Hyla crucifer* in response to injected anterior pituitary gonadotropins. The seminal vesicles of the catfish *Heteropneustes fossilis* respond to hormonal treatment, enlarging and becoming very active in response to human chorionic gonadotropin (HCG), prolactin, growth hormone, testosterone propionate and estradiol benzoate. Cyproterone, an antiandrogen, causes a regression of the seminal vesicle epithelium (Sundararaj and Goswami, 1965; Sundararaj and Nayyar, 1969).

Functions proposed for the seminal vesicles include storage, production of seminal fluids, and a source of components which interact with spermatozoa. Rugh (1939) reported masses of sperm in the Wolffian duct, nephric tubules and in the seminal vesicles after injection of anterior pituitary glands. Storage of sperm by the seminal vesicles of *H. crucifer* and *R. pipiens* has also been reported by Rugh (1941).

The fluids secreted by the seminal vesicles in *D. pictus* were analyzed by Mann, Lutwik-Mann and Hay (1963). They reported that the fluid in which spermatozoa were suspended was mucoid in nature and contained sialic acid, carbohydrate moieties, protein, lactic and citric acids. The low content of Na, K, and Cl ions rendered the fluid hypotonic. Sundararaj (1958) identified the seminal vesicles in the catfish *H. fossilis* as the source of seminal fluid. Nayyar and Sundararaj (1970) analyzed the content of seminal vesicle secretions in this species. They identified mucoproteins, mucopolysaccharides, proteases, phospholipids and proteins. They suggested that the functions of the fluid secreted by the seminal vesicles in this species are mainly nutritional and as a medium for transport.

McAllister (1973) compared the fertilizing capacity of *R. pipiens* sperm exposed to seminal vesicle extracts with that of testicular sperm. McAllister found that the sperm exposed to seminal vesicle brei retained their fertilizing capacity much longer than testicular sperm at 5°C., suggesting that the seminal vesicle contained components which prolonged the viability of the cells.

It is the object of the present investigation to describe studies of the ultrastructure of the seminal vesicles of *R. clamitans* and their response to HCG. This will provide a better understanding of the cellular basis of the function of this organ.

MATERIALS AND METHODS

Animals Used

Adult male *Rana clamitans* were obtained from Bay Biological Ltd., Port Credit, Canada, in late summer. The frogs were stored until use at 4-6°C. The frogs used weighed between 38-47 grams, with a body length of 65-73 mm.

Treatments

The frogs were divided into four groups of three frogs per group. The first group consisted of "normal" frogs that had not been injected with human chorionic gonadotropin (HCG). The remaining groups received 125 I.U. HCG (Nutritional Biochemical Corp.) injected into the dorsal lymph sacs. The seminal vesicles were then removed at two, twelve, and twenty-four hour intervals. Of each pair of seminal vesicles, one was preserved for light microscopy and one for transmission electron microscopy.

Removal of Seminal Vesicles

The frogs were rendered immobile by pithing the spinal cord. The abdominal cavity was opened by making a longitudinal cut along the ventral midline from the pelvic girdle to the shoulder girdle. The pelvic girdle was cut so that the cloaca was fully exposed. Thus, the full length of the Wolffian duct with the seminal vesicles was exposed. The removal of the seminal vesicles was done by cutting the Wolffian

duct distally as close to the cloaca as possible, to include all of the seminal vesicle. The seminal vesicles were immediately dropped into the fixative solution at 0-4°C.

Fixation Procedure

The fixative used was a mixture of 1.0% gluteraldehyde, 1.0% osmium tetroxide and 1.0% potassium dichromate in .05M cacodylate buffer at pH 7.2. The fixative was prepared by mixing 10 ml. of 2% gluteraldehyde with 2 grams potassium dichromate. The osmium tetroxide was mixed separately by adding 5 ml. of 4% osmium tetroxide to 5 ml. of .2M cacodylate buffer. The resulting 2% osmium tetroxide in .1M cacodylate (pH 7.2) solution was then added to the 10 ml. of the 2% gluteraldehyde plus potassium dichromate. The mixing of the two solutions was done at 0-4°C immediately before fixation. This fixative was selected after comparing it with 2% or 5% gluteraldehyde, Karnovsky's and picric acid paraformaldehyde.

The excised seminal vesicles were fixed for one hour. One of the pair of seminal vesicles was left intact while the other one was divided into thirds. The portion proximal to the kidney was designated upper third, and the portion most distal to the kidney was called the lower third.

Dehydration

The tissue was washed in .05M cacodylate buffer (pH 7.2) at 0-4°C for five minutes. After the buffer was removed, a dehydration series consisting of 50, 70, 90 and 100% acetone was employed. The 50 and 70% acetone solutions were at 0-4°C while the 90 and 100% acetone were at

room temperature. The solutions, with the exceptions of the 100% acetone, were changed twice during ten minutes. The 100% acetone was changed three times during thirty minutes.

Infiltration and Embedding

Two different procedures were used for infiltration and embedding for preparation of sections for light microscopy. The whole seminal vesicles were transferred out of the 100% acetone into three changes of 100% alcohol, ten minutes each change. They were then passed through three changes of xylene of five minutes each. The tissue was transferred to a 50% mixture of xylene and paraffin for one hour under a slowly applied vacuum. Finally, the tissue was transferred to a mold full of pure paraffin and then cooled. In embedding for electron microscopy, thirds of the seminal vesicle were taken from 100% acetone into a 50% mixture of Spurr's and Acetone embedding medium. They were then exposed to vacuum for thirty minutes. The vacuum was released and the tissue transferred to pure Spurr's embedding medium for another thirty minutes of exposure to vacuum. After this the pieces were removed and further divided by razor into approximately 1 mm lengths and placed into appropriately labeled flat rubber molds containing pure Spurr's embedding medium for one hour in a desiccator. The molds were then placed into an oven at 70°C for at least eight hours to harden.

Sectioning and Staining

Sectioning for light microscopy was done at 10 microns. The sections were mounted serially on glass slides and stained with Ehrlich's hematoxylin and eosin. A treatment with 1% hydrogen peroxide

was included in the staining procedure to remove the osmium tetroxide prior to staining.

Tissue for electron microscopy was sectioned on a Porter-Blum ultramicrotome, MT-2. Sections taken were between 60 and 90 nm as determined by interference colors. The sections were picked up on .2% formvar-coated Anthene copper grids, mesh size 100. Immediately before and after thin sectioning, 4 to 5 1-micron thick sections were transferred to glass slides and stained with 1% methylene blue in 1% borax for observation by light microscopy.

The grids were stained for three hours with 0.5% uranyl acetate in 25 ml. absolute methyl alcohol and 75 ml. of 70% ethyl alcohol, succeeded by a rinse consisting of the alcohol mixture alone (G. R. Hooper). The grids were then stained with Reynolds lead citrate for 15 minutes, rinsed with .02N sodium hydroxide and filtered distilled water.

The grids were then examined in a Philips electron microscope, Model 300, and pictures were taken on 3¼ x 4 inch cut film (Kodak fine grain positive film).

OBSERVATIONS

The seminal vesicle of *R. clamitans* is an enlargement of the Wolffian duct (Figure 1). This enlargement extends posteriad from the kidney to the junction with the cloaca and the bladder. The diameter of the seminal vesicle ranges from approximately one millimeter at its anterior end to 3 to 4 mm. at its widest, narrowing again at the posterior end to approximately 2 mm. The lateral surface of the seminal vesicle is convex while the medial surface is straight to slightly concave. The external appearance is that of a spongy tissue. The length of the seminal vesicle varies widely, from 8 to 14 mm. Unlike the seminal vesicles of *R. pipiens*, the seminal vesicles of *R. clamitans* are not bordered laterally by rudimentary oviducts.

The internal structure of the *R. clamitans* seminal vesicle is shown in a longitudinal section (Figure 2). A central lumen is seen with numerous side channels wich arborize. The arborization is most prevalent on the lateral side, which accounts for the convex curvature of this side. The arborization begins gradually at the anterior end, increasing posteriorly. The lumen in all parts of the seminal vesicle is lined with a single layer of pseudostratified epithelium (Figures 3, 4, 5 and 6).

The epithelial layer is composed of columnar cells (Figure 4). These cells fall into two main groups, ciliated and nonciliated. They

apparently lie at oblique angles to the lumen, since cells are seldom seen to run from the basement membrane to the lumen.

Morphology of Ciliated Cells

The ciliated cells are distributed sporadically along the entire length of the seminal vesicle (Figures 3 and 4). They are numerous and appear along the main lumen as well as along the side branches.

The cilia appear to be typical with the fibers arranged in the 9+2 arrangement. They have a tubular asymmetrical basal body with striated rootlet and a basal plate (Figures 7 and 8).

The cytoplasm appears granular and contains membrane-bound vesicles (Figures 9 and 10), microfilaments (Figure 8), mitochondria, Golgi bodies, lysosomes, and glycogen granules (Figures 7, 8, 9 and 10). The mitochondria are numerous and distributed mainly at the apical end of the cell, only a few being seen toward the basal end of the cell (Figure 7). The term apical refers to the side of the cell bordering the lumen; basal refers to that portion of the cell against the basement membrane. The mitochondria are approximately 40 nm in diameter and are quite long (200 nm). They have well developed cristae.

The Golgi bodies are found close to the nucleus and are distributed from the equator of the nucleus to the apical side, in a semicircular fashion about the nucleus (Figures 9 and 10). The glycogen granules are found throughout the cytoplasm, although their concentration is denser around mitochondria and Golgi bodies (Figure 7).

Vesicles found (Figures 9 and 10) in the ciliated cell vary considerably in size (17 nm-21 nm). Their electron density, as shown by

osmium, also varies. Lightly stained vesicles are fewer than the clear vesicles.

The Golgi bodies appear to produce vesicles whose contents exhibit different staining properties. It also appears that some clear vesicles have their origins as pinched-off protrusions of the nuclear membrane. The concentrations of vesicles seem to rise toward the end of the cell adjacent to the lumen.

The cell has large numbers of microfilaments (Figures 7 and 9). Although some can be found throughout the cell, the majority lie close to the cell membrane.

The free surface of the ciliated cell shows few microvilli or protrusions other than those associated with the cilia. The lateral membrane (Figure 7) shows many folds and has tight junctions at the apical ends. There are also desmosomes connecting adjacent cells (Figure 10).

The nonciliated cells of the lining epithelium seem to fall into four types. The types are differentiated by either their attachment to adjacent cells or by the appearance of their cytoplasmic vesicles.

Morphology of Type I Nonciliated Cell

The Type I cell (Figure 11) is found distributed throughout the entire epithelium of the seminal vesicle. It is characteristically in loose contact with neighboring cells. The intercellular spaces are quite large. The cells are attached to each other at the apical border by tight junctions. Along the sides there is a loose interdigitation with other cells by means of their cytoplasmic projections. Internally, these cells contain numerous vacuoles that are not stained or contain

a lightly stained material. The cells contain well developed Golgi bodies as well as numerous mitochondria. There is very little rough endoplasmic reticulum and very few free ribosomes. The nucleus of this type of cell appears darker staining in comparison with the other types, and the nuclear membrane appears swollen. There are large, dense bodies, 40 nm to 45 nm in diameter, which may be lysosomes. The cytoplasm also contains large numbers of glycogen granules. The very wide intercellular spaces do not stain with the exception of a small amount of scattered flocculent material.

Morphology of Type II Nonciliated Cell

The second type of nonciliated cell (Figure 12) has an altogether different appearance from that of Type I. The Type II cell is closely attached to the adjacent cells by tight junctions at the apical end, and by desmosomes at intervals along the sides (Figure 13). Also, there is much interdigitation of cell membranes with those of adjacent cells. The mitochondria are numerous (Figures 13 and 14) and are concentrated at the apical end of the nucleus as in the ciliated and Type I cell. The Golgi bodies are well developed and appear to be giving off small vesicles filled with a lightly stained material (Figures 13 and 14). The special characteristics of these cells are their vesicles (Figures 13 and 15) which are approximately 14 to 20 nm in diameter and have very lightly stained contents. Their walls stain darkly. The vesicles are found primarily at the apical end of the cell (Figure 15).

Morphology of Type III Nonciliated Cell

The third type of nonciliated cell differs from Type I and II in the size and staining properties of vesicles present in the cytoplasm (Figures 16 and 17). In Type III the darkly staining vesicles are more numerous and larger than the lightly stained vesicles of Type II. In size they vary from 14 nm to 22 nm (Figure 18). The staining reaction with osmium is such that they are dark gray to black. The clear vesicles present in the cytoplasm appear to be the same as found in the Type II cell. However, they are much less numerous. Both types of vesicles are concentrated at the apical end of the cell (Figures 17 and 18).

Morphology of Type IV Nonciliated Cell

The Type IV nonciliated cell appears to be a goblet cell (Figure 19). Its apical cytoplasm is filled with membrane bound vesicles that stain a light gray. Mitochondria almost completely fill the remaining space between the lateral margins of the cell and the space between the nucleus and vesicles. There are considerably more membrane-bound ribosomes present than in any of the other types of cells observed from normal seminal vesicles (Figure 20). The cytoplasmic vesicles are quite large (36 to 125 nm) and are grouped in clusters at the apical end of the cell (Figure 19). Around the cluster of vesicles appears a complex of membranes associated with ribosomes (Figure 20).

Numerous vesicles of various sizes are always seen near the apical borders of Type I, II, III, and IV nonciliated epithelial cells. They are also rich in Golgi bodies. The cell membrane bordering the lumen is very rough, having many projections. These observations taken

together suggest that these epithelial cells may be secretory in nature. However, there is a noticeable scarcity of membrane-bound ribosomes in Types I, II, and III nonciliated epithelial cells. The significance of this observation will be discussed.

Figures 5 and 6 show sections taken near the end of a side channel off the main lumen. These two figures give an overall view of the relationships of the epithelial cells to the lumen. Also, they show the presence and location of the vesicles in relation to the lumen in the non-HCG injected animal.

Type IV epithelial cells or goblet cells were not evident in the post-HCG-injected animals. Type I, II, and III nonciliated cells showed definite changes. In the 2, 12, and 24 hour post-injected animals the epithelial cells show a decrease in the number of vesicles present in the cells (Figures 21 through 26). In the cells in which some vesicles are left, they are found very near the apical border of the cell (Figures 21, 23, and 26). In the cells which appear to have lost their granules and vesicles, the luminal membrane is smoother than the membrane in the preinjected animals (Figures 22 and 23). In those cells which still have granules present, the membrane bordering the lumen is still rough, and darkly stained material is found in the lumen next to the cell membrane (Figure 26). There also appears to be an increase in the number of Golgi bodies (Figures 24, 27, 29, 30, 31 and 32). Small segments of membrane with bound particles can now be found, especially in the post 12 and 24 hour animals (Figures 28, 29, 30, 31, 32 and 33). These bound particles are found on the outer nuclear membrane and also on the endoplasmic reticulum.

LIST OF ABBREVIATIONS USED IN FIGURES

ant - anterior end

B - urinary bladder

bb - basal body

bp - basal plate

bv - blood vessel

C - cloaca

c - collagen fibers

cc - ciliated cell

ci - cilia

CM - cell membrane

cp - cytoplasmic projection

ct - connective tissue

D - desmosome

dv - dark vesicles

ep - epithelial layer

FMA - folded membrane giving rise to vesicles

G - Golgi body

gg - glycogen granules

Is - intercellular space

K - kidney

l - lower third

lat - lateral side

lu - lumen

lv - light vesicles

M - mitochondria

m - middle third

MBR - membrane-bound ribosomes

mbv - membrane-bound vesicle

med - medial side

mf - microfilaments

MN - membrane network

mn - main channel

MV - microvilli

N - nucleus

Ncc - nonciliated cell

P - vesicle in process of being expelled

post - posterior end

PV - possible vesicle formation

RV - possible vesicle release

se - secretory epithelium

sp - side pocket

SV - seminal vesicle

T - testes

tj - tight junction

u - upper third

VE - *vas efferens*

WD - Wolffian duct

2 - Type II cell

3 - Type III cell

Figure 1. Diagrammatic representation of the urogenital system of an adult male *Rana clamitans*.

VE - *vas efferens*
T - testes
K - kidney
WD - Wolffian duct
SV - seminal vesicle
C - cloaca
B - urinary bladder

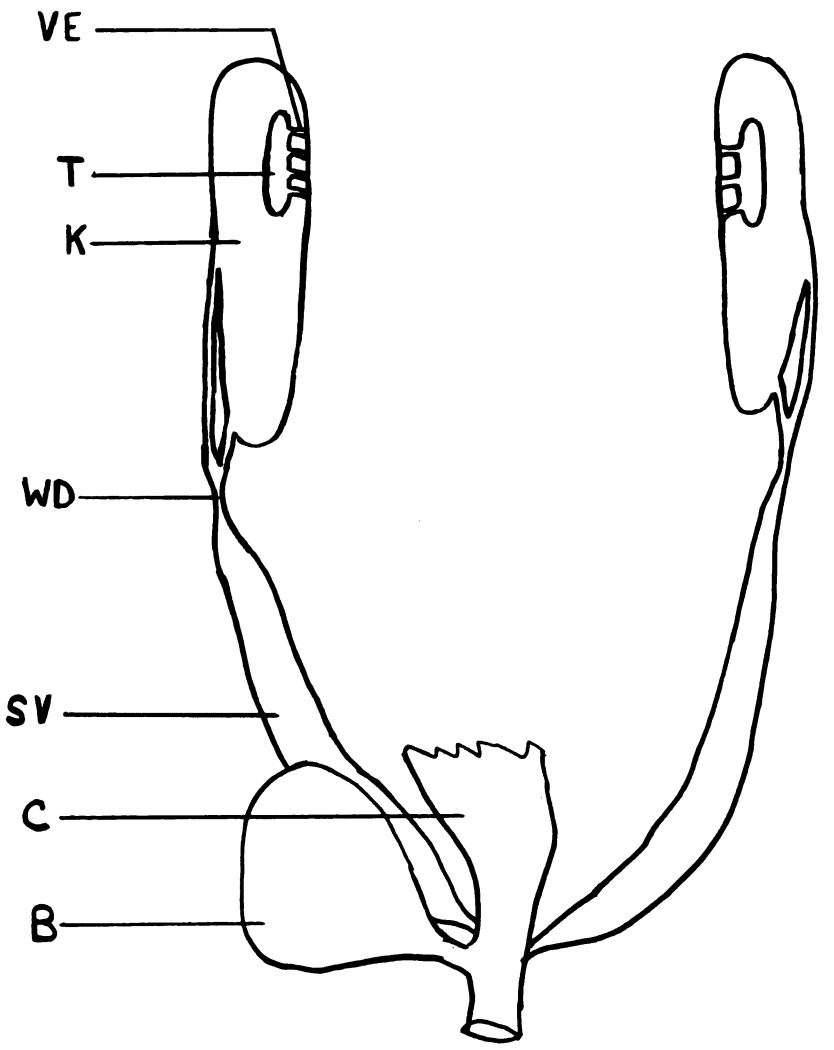


Figure 1

Figure 2. Longitudinal and transverse sections of paraffin embedded seminal vesicle taken from a noninjected frog.

Approximate magnification of longitudinal section
Approximate magnification of transverse sections

mn - main channel, sp - side pockets off main channel, se - secretory epithelium, ant - anterior end, post - posterior end, med - medial side, lat - lateral side, u - upper third of seminal vesicle, m - middle third of seminal vesicle, l - lower third of seminal vesicle.

Figure 2

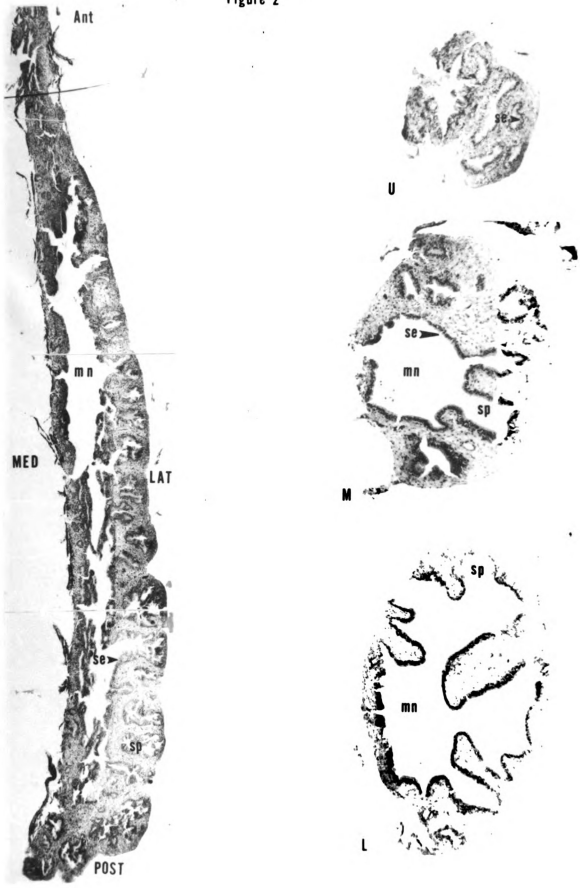


Figure 2

Figure 3. Section from control frog.

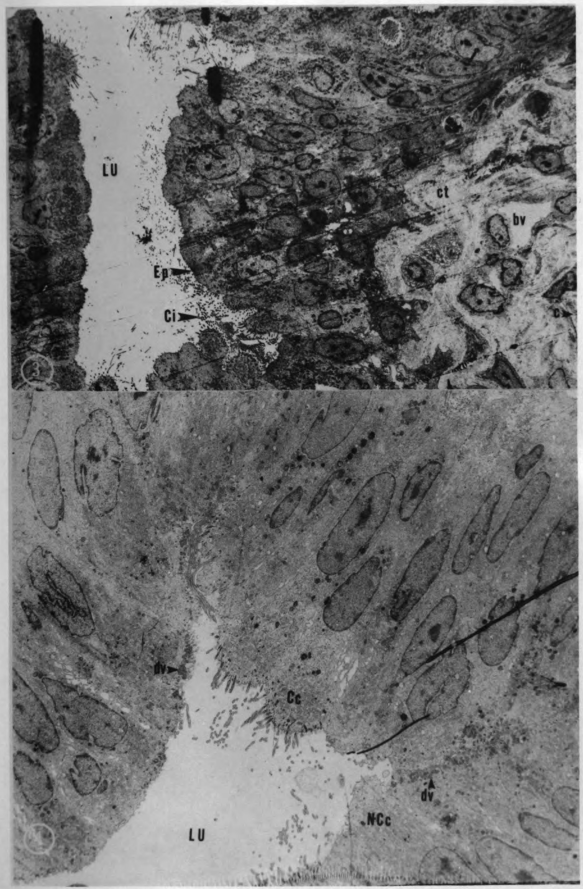
Approximate magnification 1,152x

lu - lumen, ep - epithelial layer, ct - connective tissue, c - collagen fibers, bv - blood vessel, ci - cilia

Figure 4. Section from control frog.

Approximate magnification 2,500x

Cc - ciliated cell, Ncc - nonciliated cell, dv - dark vesicles



Figures 3 and 4

Figure 5. Cross section taken at the tip of a side channel, noninjected.

Approximate magnification 4,500x

dv - dark vesicles, Lu - lumen, 3 - Type III cell, 2 - Type II cell, c - collagen fibers in connective tissue

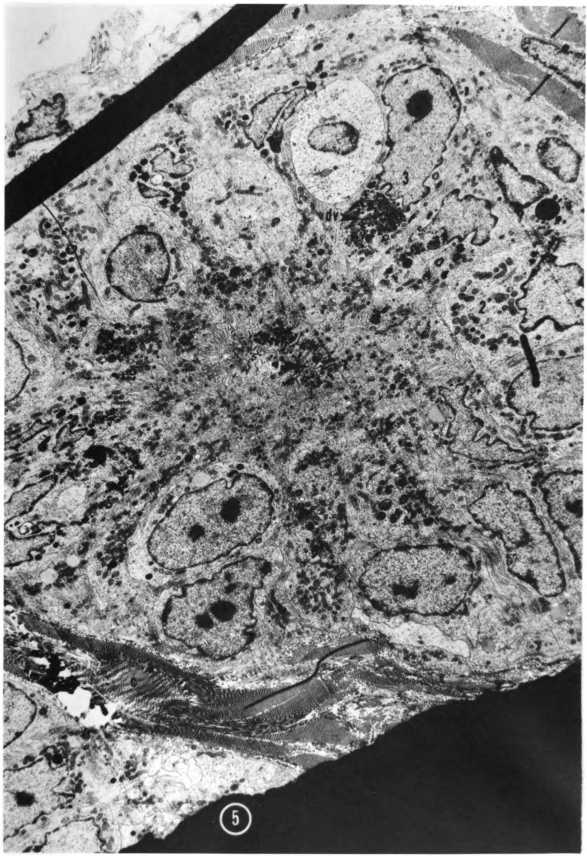


Figure 5

Figure 6. Cross section of secretory epithelium showing lumen along a side pocket, noninjected.

Approximate magnification 4,500x

Lu - lumen, 2 - Type II cell, 3 - Type III cell, lv - light vesicles, dv - dark vesicles, tj - tight junction

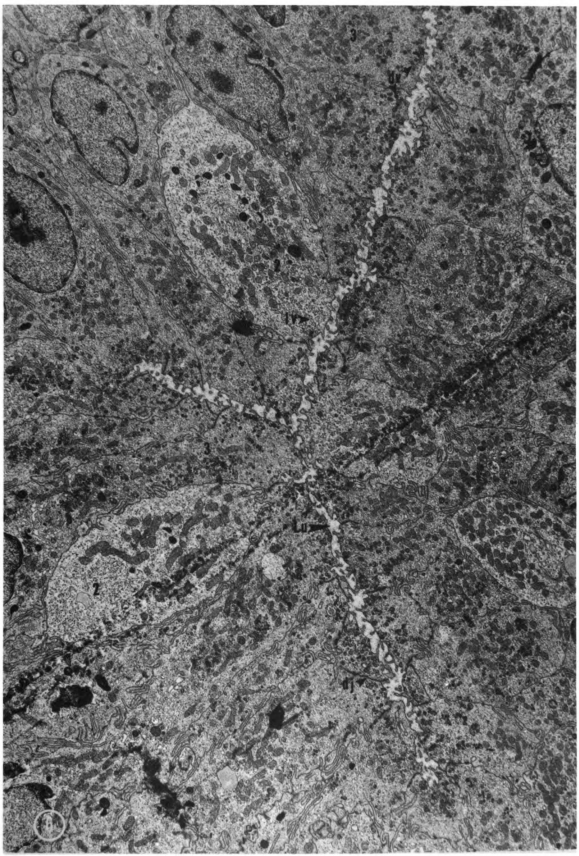


Figure 6

Figure 7. Ciliated cell, noninjected.

Approximate magnification 13,800x

Bb - cilia basal body, tj - tight junction, D - desmosome, gg - glycogen granules, M - mitochondria, G - Golgi bodies, N - nucleus, Is - intercellular space

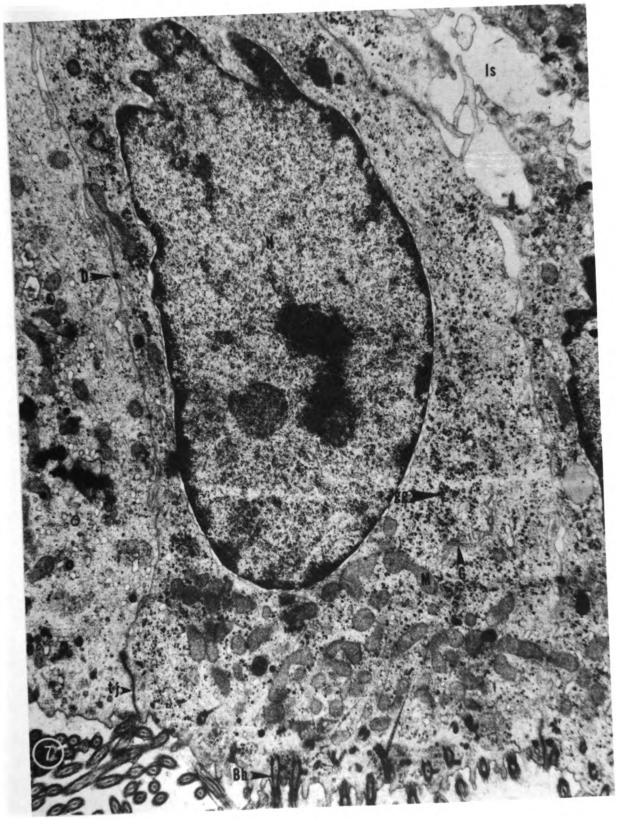


Figure 7

Figure 8. Enlargement of area in Figure 7.

Approximate magnification 42,000x

Lu - lumen, bb - basal body, bp - basal plate, mf - microfilaments, dv - dark vesicles, gg - glycogen granules, lv - light vesicles

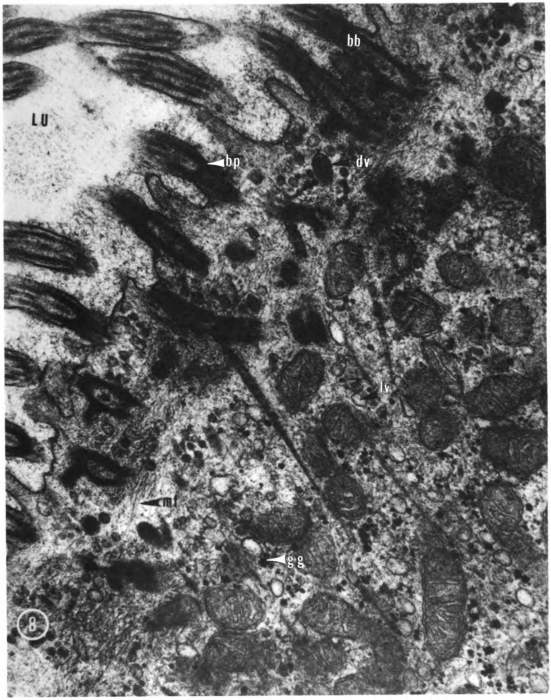


Figure 8

Figure 9. Ciliated cell, noninjected frog.

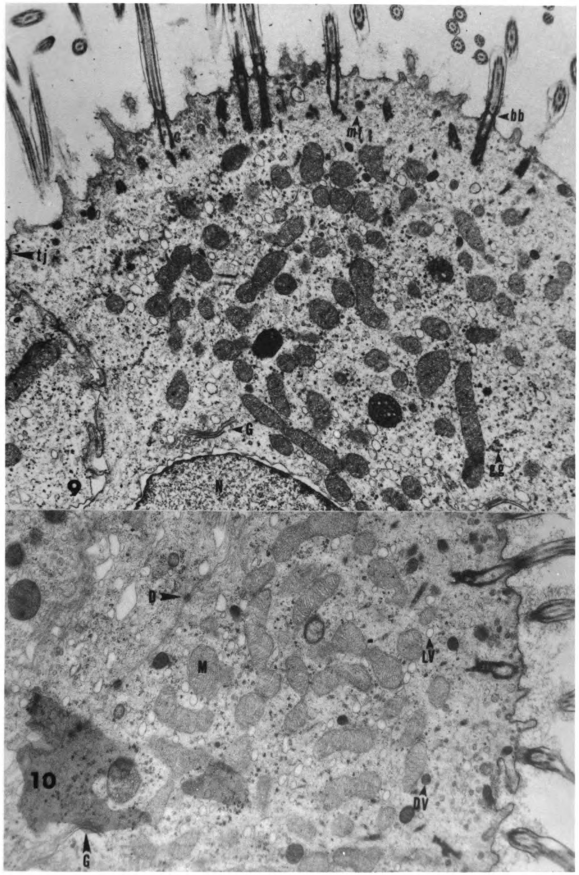
Approximate magnification 16,200x

G - Golgi apparatus, N - nucleus, mf - microfilaments, bb - basal body of cilium, tj - tight junction, gg - glycogen granules

Figure 10. Ciliated cell, noninjected frog.

Approximate magnification 20,000x

D - desmosome, G - Golgi apparatus, lv - light vesicles, dv - dark vesicles, M - mitochondria



Figures 9 and 10

Figure 11. Type I nonciliated cell taken from noninjected frog.

Approximate magnification 8,000x

N - nucleus, Is - intercellular space, tj - tight junction,
cp - cytoplasmic projections

Inset

Approximate magnification 25,000x

G - Golgi, M - mitochondria

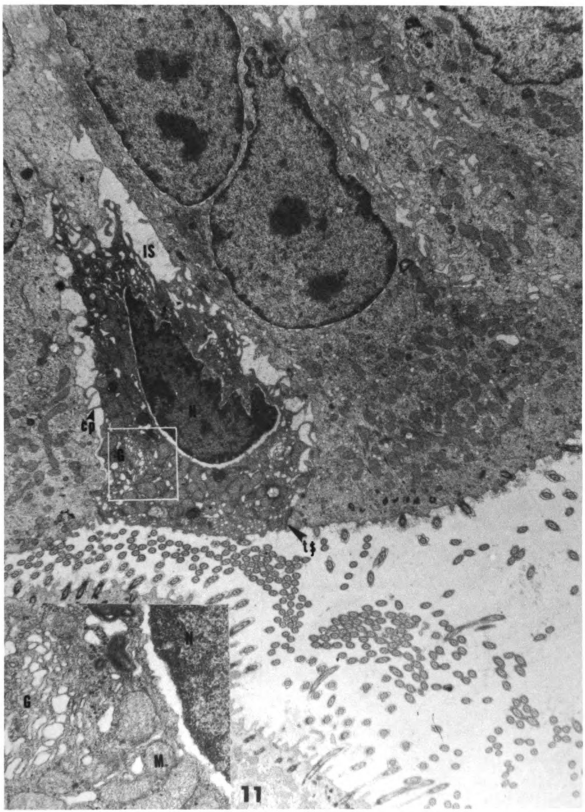


Figure 11

Figure 12. Diagrammatic representation of a Type II non-ciliated cell from a noninjected adult male *Rana clamitans*.

N - nucleus
CM - cell membrane
G - Golgi body
D - desmosome
LV - light vesicle
TJ - tight junction
DV - dark vesicle

Figure 13. Type II nonciliated cell from noninjected frog.

Approximate magnification 11,500x

tj - tight junction, D - desmosome, lv - light vesicle, M - mitochondria, CM - cell membrane showing folding, MV - vesicles arising from loop of folded cell membrane, gg - glycogen granules, dv - dark vesicle

Inset A

Golgi apparatus with associated vesicles

Approximate magnification 34,375x

Inset B

Portion of cell next to lumen

Approximate magnification 34,375

lv - light vesicles, dv - dark vesicles, tj - tight junction, D - desmosome

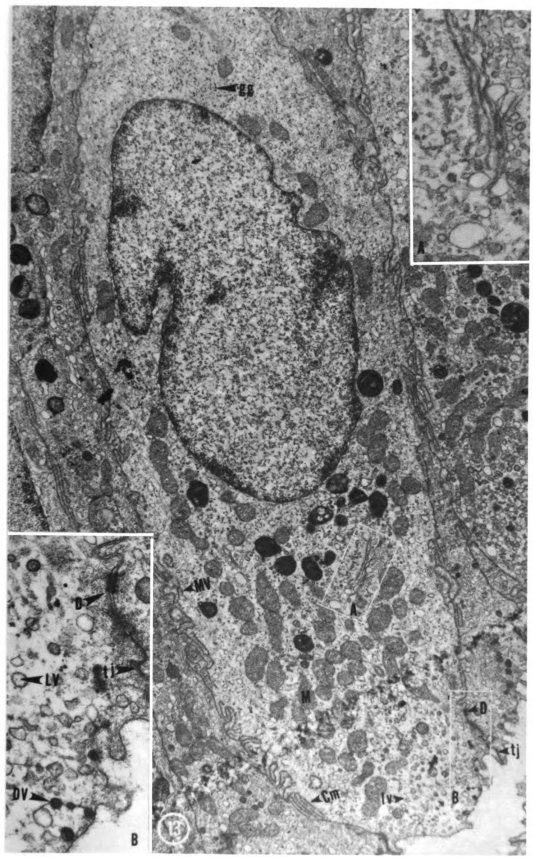


Figure 13

Figure 14. Portion of a Type II nonciliated cell.

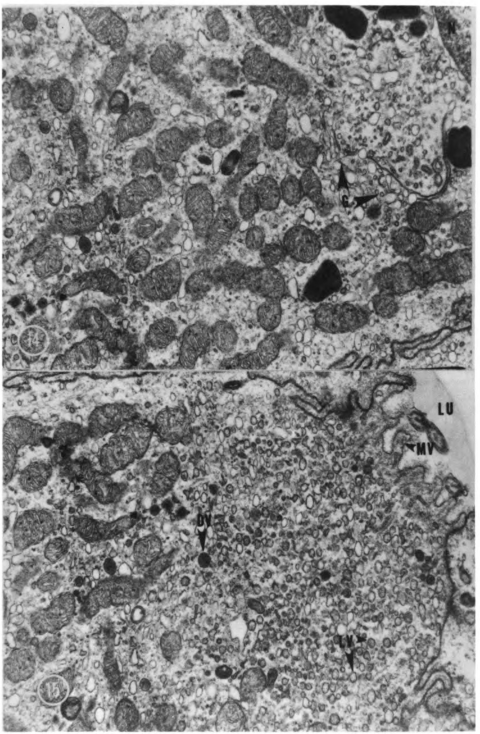
Approximate magnification 28,770x

G - Golgi apparatus and associated light vesicles, N - nucleus

Figure 15. Apical portion of Type II nonciliated cell.

Approximate magnification 28,770x

LV - light vesicles, concentrated at apical end of cell, DV - dark vesicles, MV - microvilli, LU - lumen



Figures 14 and 15

Figure 16. Diagrammatic representation of a Type III non-ciliated cell from an injected adult male *Rana clamitans*.

N - nucleus
CM - cell membrane
D - desmosome
G - Golgi body
DV - dark vesicle
M - mitochondria
LV - light vesicle
TJ - tight junction

Figure 17. Type III nonciliated cell.

Approximate magnification 18,975x

DV - dark vesicles, LV - light vesicles, MV - microvilli, LU - lumen

Inset A

Approximate magnification 43,375x

G - Golgi apparatus, DV - dark vesicles

Inset B

Approximate magnification 34,375x

DV - dark vesicles, N - nucleus

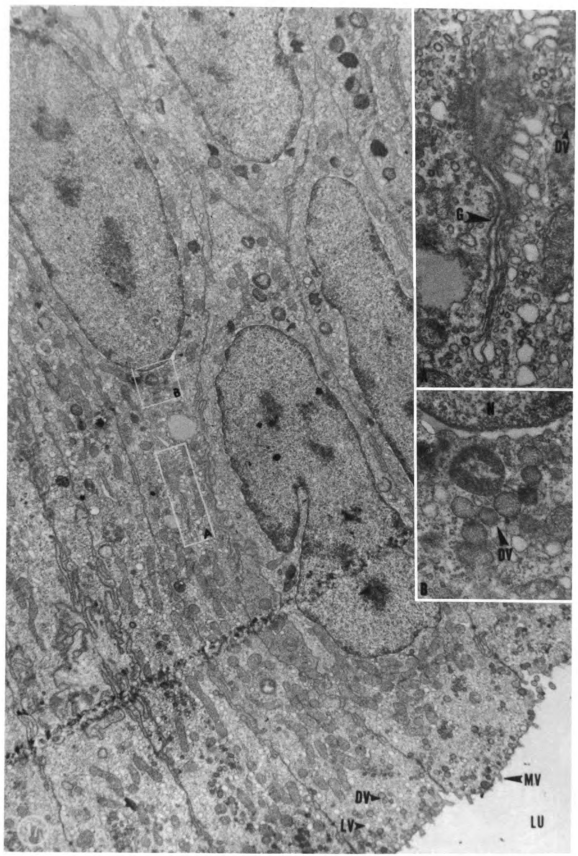


Figure 17

Figure 18. Apical end of a Type III nonciliated cell from a noninjected frog.

Approximate magnification 43,375x

MV - microvilli, DV - dark vesicles, LV - light vesicles, D - desmosome, LU - lumen

Large arrow indicates a dark vesicle which has possibly fused with cell membrane.

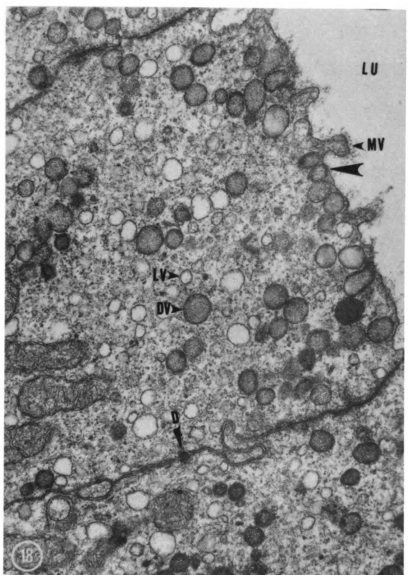


Figure 18

Figure 19. Type IV nonciliated cell, goblet cell from a noninjected frog.

Approximate magnification 14,500x

LU - lumen, P - vesicle in process of being expelled into lumen, MBV - membrane-bound vesicle

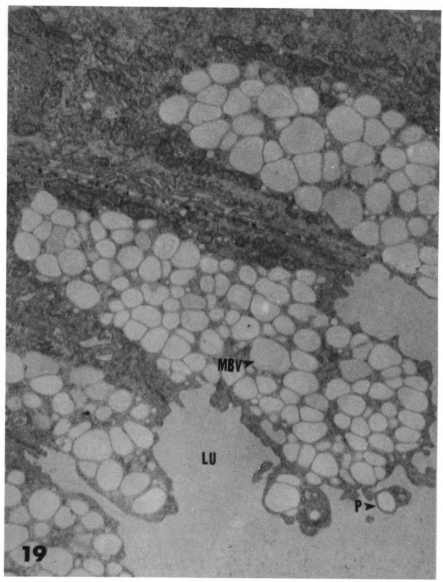


Figure 19

Figure 20. Enlarged view of secretory vesicles of a Type IV nonciliated cell from noninjected frog.

Approximate magnification 20,700x

MN - membrane network that surrounds the vesicle, MBR - membrane-bound ribosomes, N - nucleus, M - mitochondria, MBV - membrane-bound vesicles

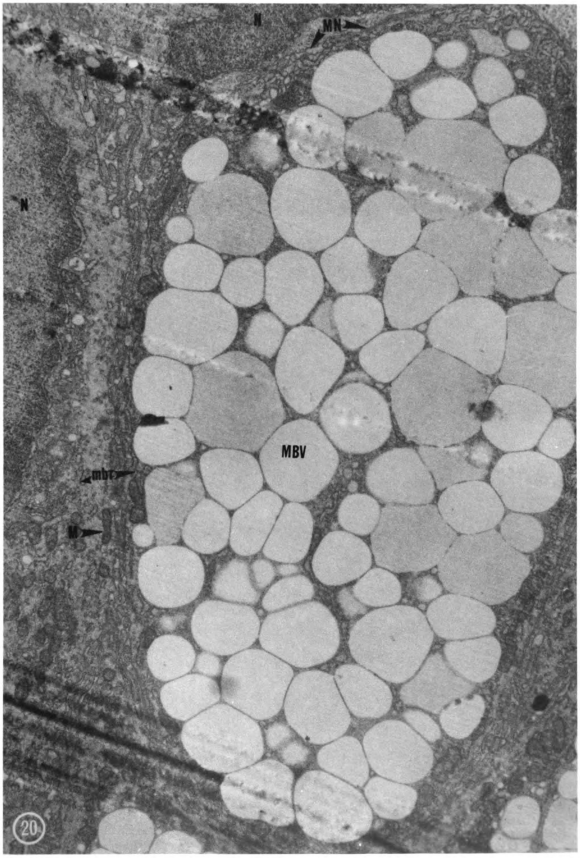


Figure 20

Figure 21. Picture taken 12 hours after injection of HCG. Area shown is comparable to Figures 5 and 6, which are from noninjected frogs.

Approximate magnification 3,700x

LU - lumen, Ct - connective tissue, DV - dark vesicle, 2 - Type II cell, 3 - Type III cell

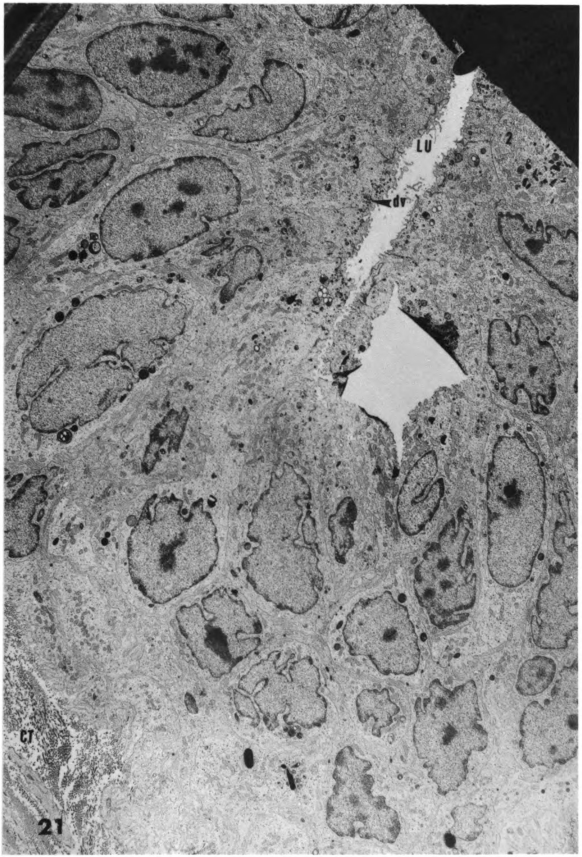


Figure 21

Figure 22. Area of section comparable to Figure 6. Section taken from animal 2 hours after injection of HCG. Shows the decrease in both light and dark vesicles. Also, the lumen has considerable amount of granular material which may have been released from the vesicles.

Approximate magnification 9,750x

DV - dark vesicles, LV - light vesicles, LU - lumen

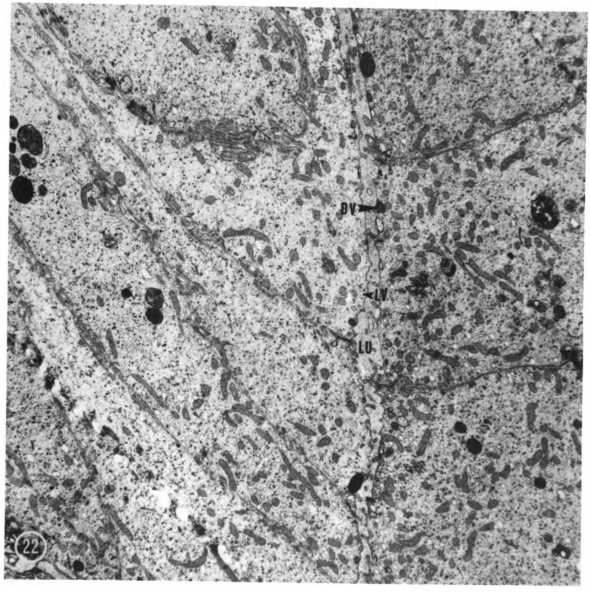


Figure 22

Figure 23. Enlarged view of the cell membrane bordering the lumen showing a decrease in vesicles. Section taken 2 hours post-injection.

Approximate magnification 17,000x

MBR - membrane-bound ribosomes, DV - dark vesicles, tj - tight junction

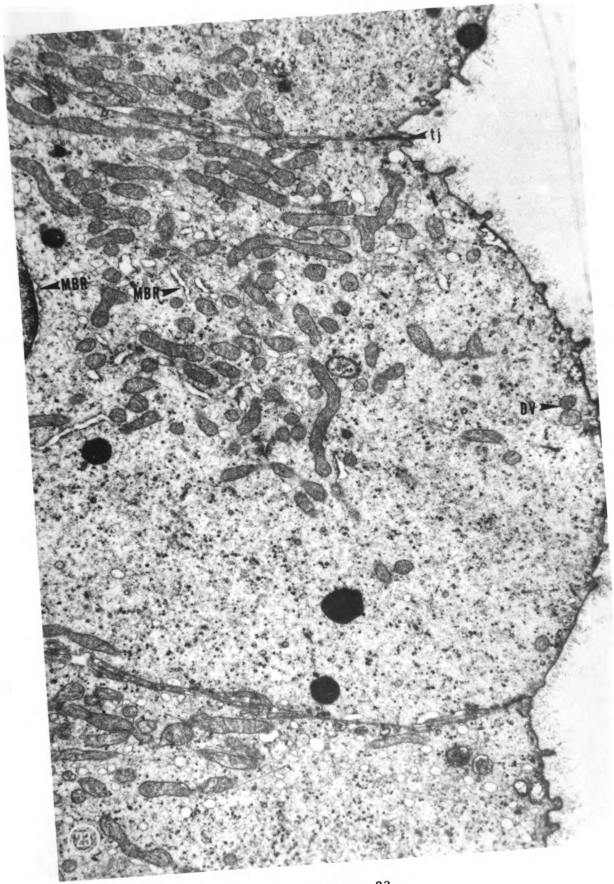


Figure 23

Figure 24. Type I nonciliated cell 2 hours post-injection showing a decrease in number of small vesicles.

Approximate magnification 17,000

Arrows indicate membrane-bound ribosomes. G - Golgi apparatus, GG - glycogen granules, IS - intercellular space

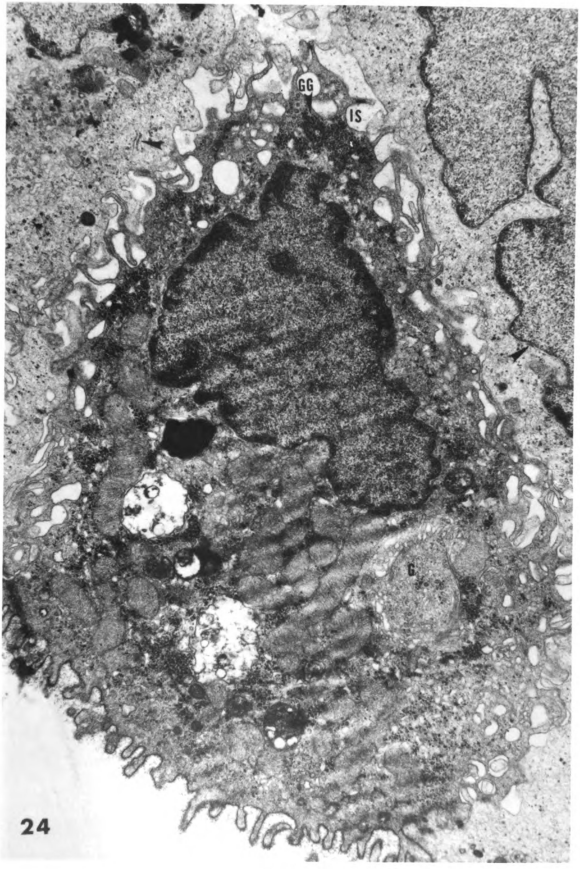


Figure 24

Figure 25. Type II or III nonciliated cell showing an increase in ribosomes and membrane-bound ribosomes. Section taken 12 hours post-injection.

Approximate magnification 25,000x

Arrows indicate ribosomes or membrane-bound ribosomes, GG - glycogen granules, G - Golgi apparatus, LV - light vesicles

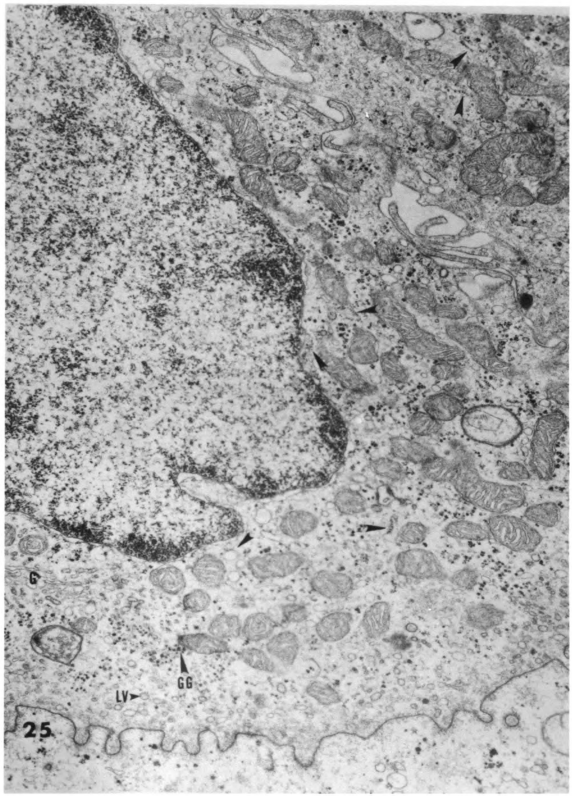


Figure 25

Figure 26. Type III nonciliated cell 24 hours post-injection, showing the dark vesicles reduced in number and lying against the membrane bordering the lumen.

Approximate magnification 20,500x

Large arrows indicate vesicles which appear to have fused with cell membrane, small arrows indicate membrane-bound ribosomes, G - Golgi apparatus, LU - lumen

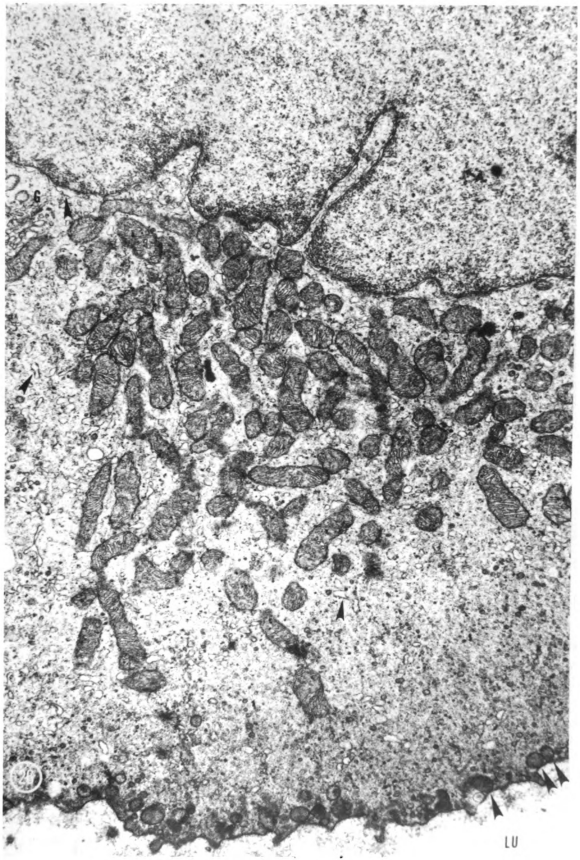


Figure 26

Figure 27. Enlarged area of Figure 24 showing the Golgi body, 2 hours post-injection.

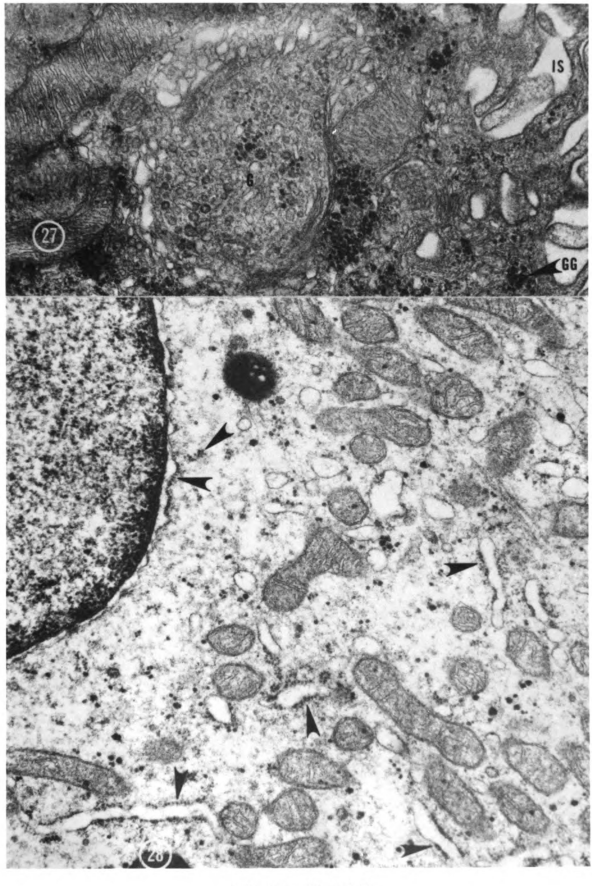
Approximate magnification 40,000x

G - Golgi body, GG - glycogen granules, IS - intercellular space

Figure 28. Section from a 24 hour post-injected frog of an area close to the nucleus of a Type II or III cell, showing membrane-bound ribosomes.

Approximate magnification 40,000x

Arrows indicate membrane-bound ribosomes



Figures 27 and 28

Figure 29. Section from a 12 hour post-injected frog of a Type II or III cell.

Approximate magnification 25,000x

G - Golgi bodies, arrows indicate ribosomes, MF - microfilaments, FM - folded membrane that seems to give rise to vesicles, DV - dark vesicles perhaps in the process of forming

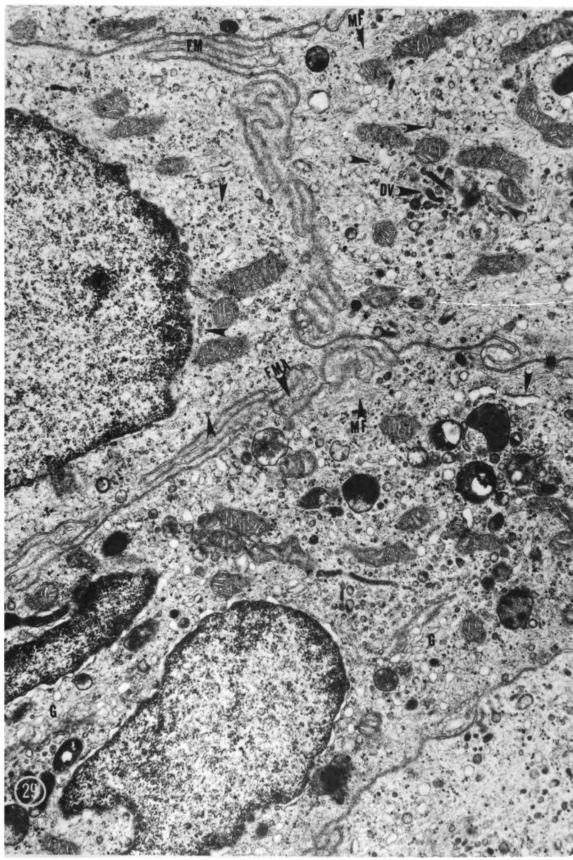


Figure 29

Figure 30. Type II or III cell from a 2 hour post-injected animal.

Approximate magnification 17,000x

G - Golgi body, arrows indicate membrane-bound ribosomes, PV - possible formation of secretory vesicles

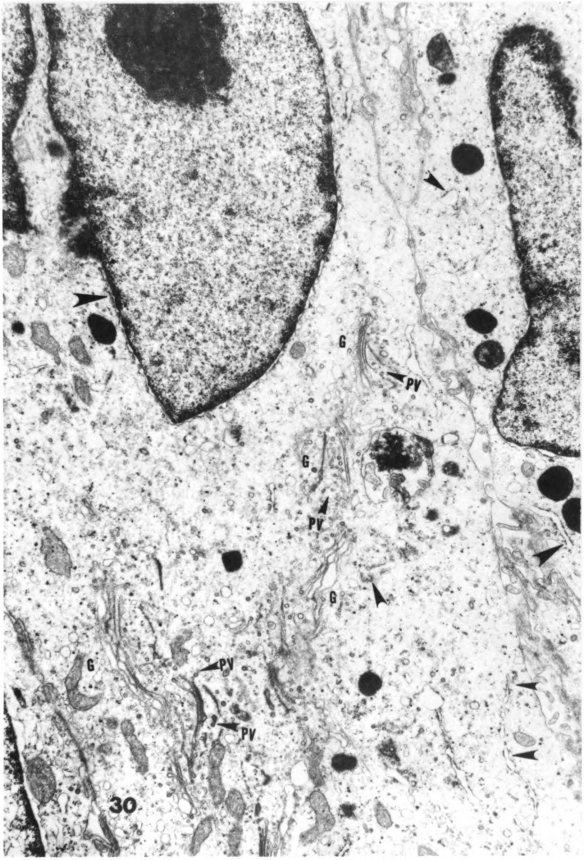


Figure 30

Figure 31. Section of a Type II or III cell 24 hours post-injection of hormone.

Approximate magnification 20,500x

G - Golgi body, LV - light vesicles, arrows indicate ribosomes or membrane-bound ribosomes

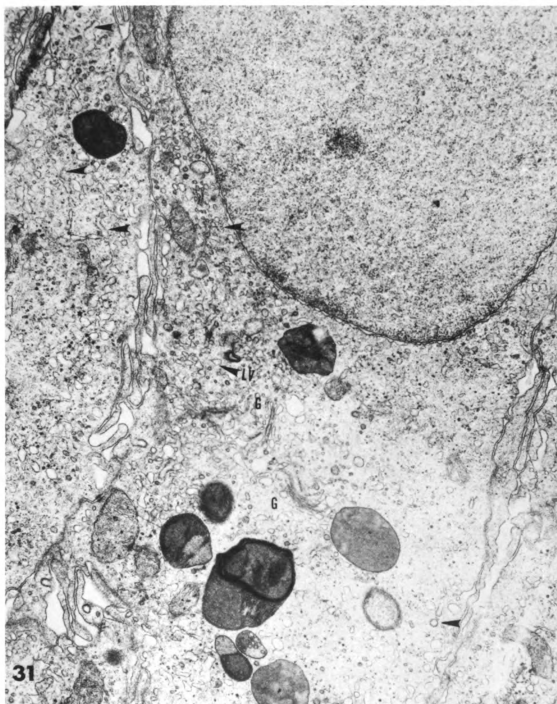


Figure 31

Figure 32. Section of a Type II or III cell 24 hours after post-injection showing Golgi bodies, membrane-bound ribosomes and light vesicles.

Approximate magnification 20,500x

G - Golgi body, arrows indicate membrane-bound ribosomes, LV - light vesicles often associated with Golgi

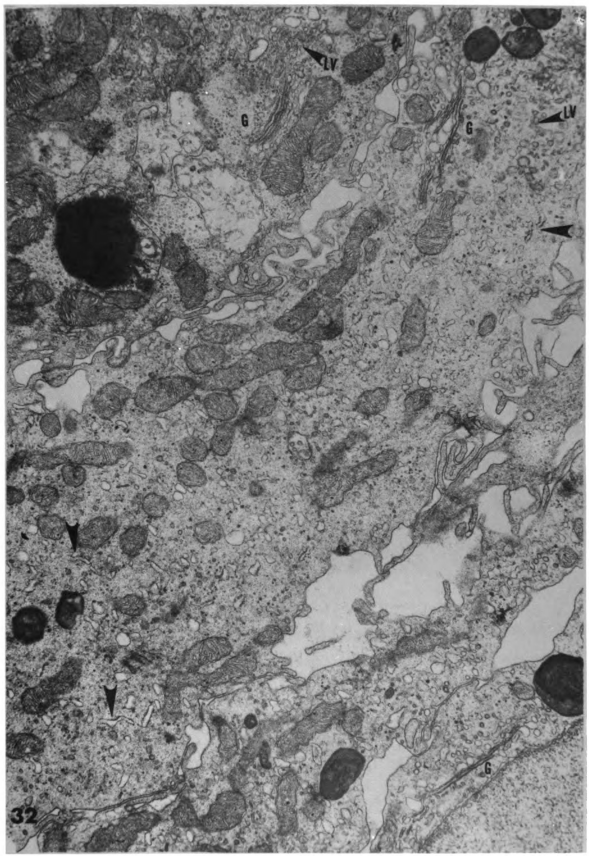


Figure 32

Figure 33. Section of a Type II or III cell 24 hours post-injection showing membrane-bound ribosomes.

Approximate magnification 40,000x

Arrows indicate membrane-bound ribosomes

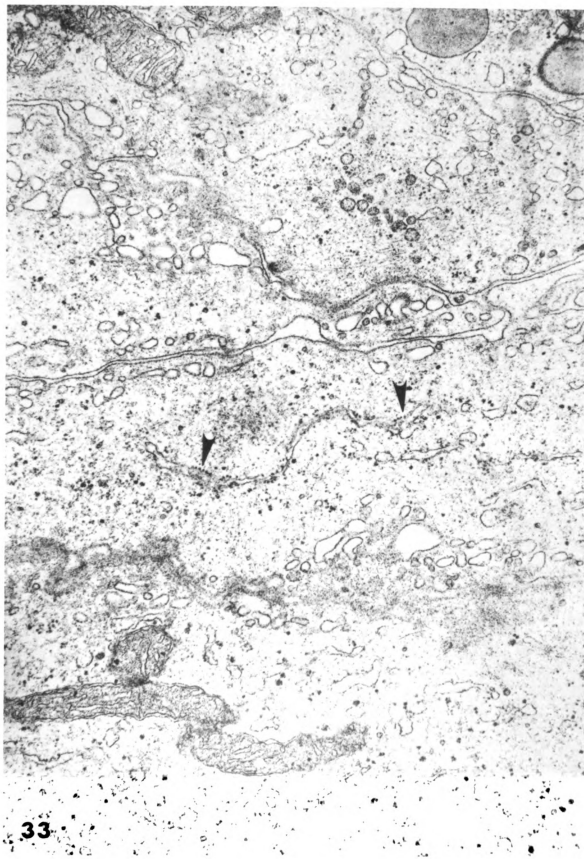


Figure 33

Figure 34. Section showing membrane border area of Type III cell from a noninjected frog.

Approximate magnification 25,000x

Arrows indicate what appears to be fusion between dark vesicles and cell membrane

Figure 35. Section of area similar to Figure 34 but with a Type II cell from a noninjected frog.

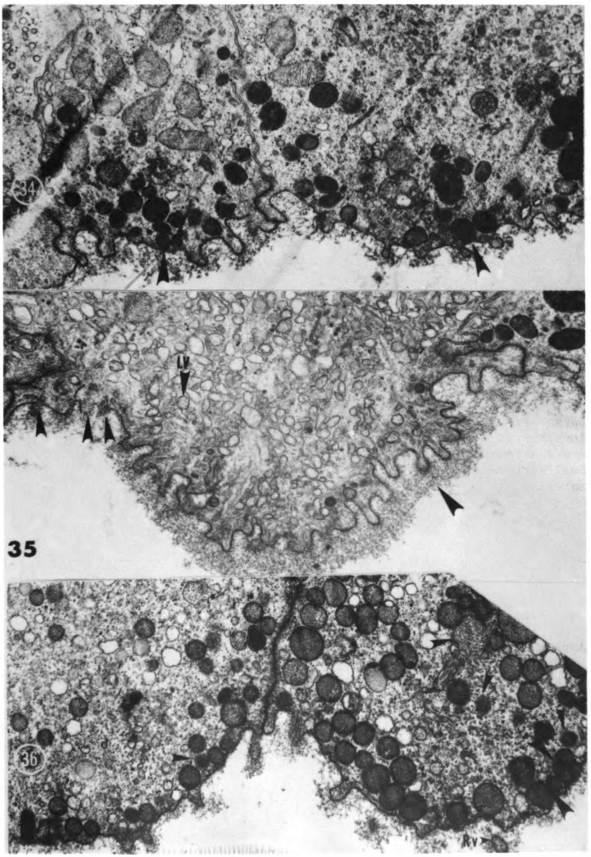
Approximate magnification 25,000x

Large arrows indicate granular material in lumen that is similar in appearance to contents of LV - light vesicles. Light vesicles are scarce along the most extreme border of the lumen of the cell. Small arrows indicate areas which seem to be releasing granular material.

Figure 36. Area of Type III cell from a noninjected frog.

Approximate magnification 31,250x

Large arrows point to fusion between vesicles, small arrows indicate possible vesicle breakdown. RV indicates possible vesicle released into lumen.



Figures 34, 35 and 36

Figure 37. Section of a Type III cell from a noninjected animal showing the lumen border of the cell.

Approximate magnification 31,250x

PV - possible vesicles released from cell into lumen, large arrows indicate fusion of vesicles. Small arrows indicate vesicle breakdown.

Figure 38. Section of a Type III cell showing the location of dark vesicles along the border of the lumen. Section is from a noninjected frog.

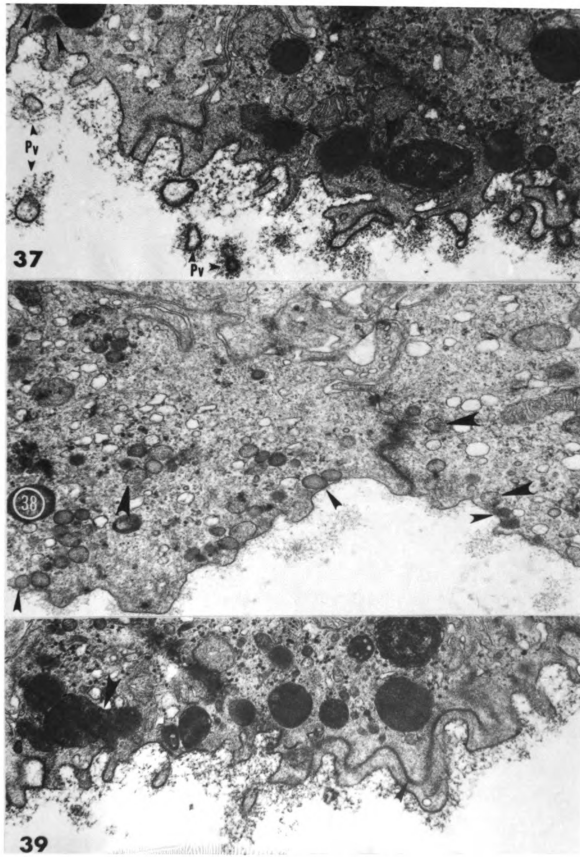
Approximate magnification 31,250x

Large arrows indicate possible breakdown of vesicles. Small arrows indicate vesicle and cell membrane fusion.

Figure 39. Area similar to Figures 37 and 38, section from noninjected frog.

Approximate magnification 31,250

Large arrows indicate fusion of two vesicles. Small arrows indicate possible membrane formation by fused vesicles.



Figures 37, 38 and 39

Figure 40. Enlarged view of Golgi bodies from a noninjected animal.

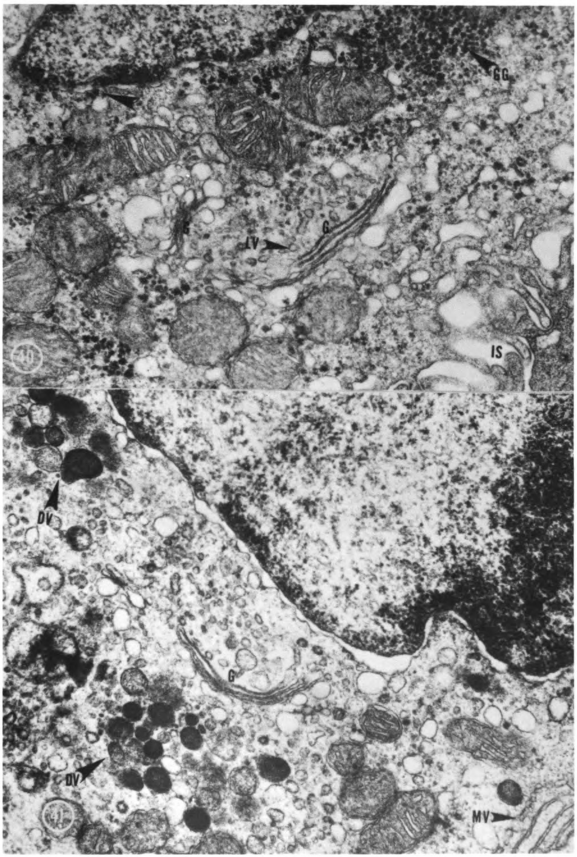
Approximate magnification 40,000x

GG - glycogen granules, G - Golgi body, LV - light vesicles, IS - intercellular space, arrow indicates vesicle formed from nuclear membrane

Figure 41. Section showing Golgi body and groups of dark vesicles. This grouping of dark vesicles perhaps indicates the area of their formation.

Approximate magnification 52,500x

G - Golgi, DV - groups of dark vesicles, MV - vesicles which appear derived from cell membrane



Figures 40 and 41

Figure 42. Section taken from a noninjected animal showing one possible source of vesicles which may form from the Golgi body.

Approximate magnification 14,000x

Arrow indicates Golgi body and clear vesicles. IS - intercellular space.

Figure 43. Enlarged Golgi area of Figure 42.

Section shows possible source of clear vesicles to be the pinching off of the lateral cell membrane.

Approximate magnification 50,000

IS - intercellular space, CV - clear vesicles, LV - light vesicles

Figure 44. Section taken from noninjected animal showing possible source of vesicles.

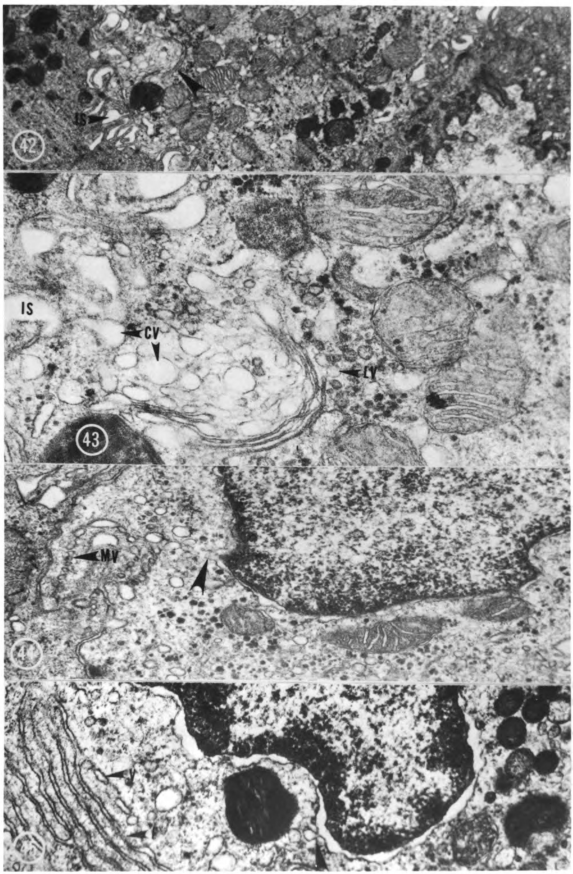
Approximate magnification 31,250x

MV - vesicles derived from a loop of folded cell membrane, large arrow indicates vesicles which appear to be derived from nuclear membrane

Figure 45. Section from noninjected frog showing folded cell membrane as a source of vesicles as well as vesicle formation from nuclear membrane.

Approximate magnification 52,500x

F - folded cell membrane, V - vesicles from membrane, large arrow indicates vesicle forming from nuclear membrane



Figures 42, 43, 44 and 45

Figure 46. Section from a 2 hour post-injected frog showing areas which appear to be involved in the production of dark vesicles.

Approximate magnification 20,500x

DV - dark vesicles appear in groups near the nucleus and also oblong section near the lumen end of the cell. These elongated vesicles may be parts of Golgi or smooth endoplasmic reticulum. Arrows indicate membrane-bound ribosomes. D - desmosome, MF - microfilaments, LV - light vesicles, LU - lumen

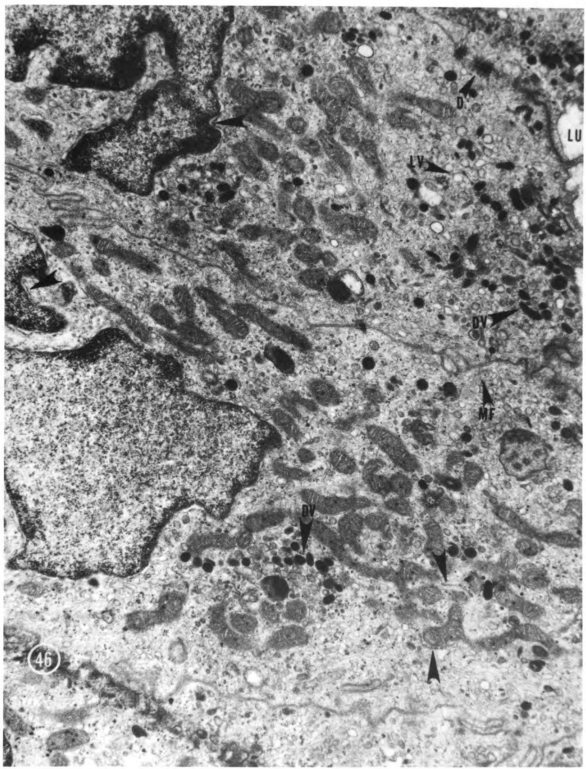


Figure 46

DISCUSSION

The visible result of HCG on the ultrastructure of the seminal vesicle is the release of materials from the epithelial cells lining the organ. This result is suggested by the significant decrease in cytoplasmic vesicles after HCG injection as shown by comparing the low power pictures on Figures 5 and 6 with Figures 21 and 22. The dark staining vesicles of Type III cells and the smaller lightly stained vesicles of Type II cells are prominent in the apical region of these cells before injection of the hormone (Figures 5 and 6). In contrast to these pictures, Figures 21 and 22 (post-injection 2 and 12 hours) show that the vesicles of both Type II and III cells are much reduced in number. This same observation can be made by comparing Figures 13 and 18, which are from control animals, to Figures 23, 25 and 26, which are from post animals 2, 12 and 24 hours after injection. In general, the release of vesicles was so complete by 24 hours after injection that it became impossible to tell Type II and Type III cells apart. The Type IV cell was not identifiable in any of the injected animals, suggesting that these cells may have released their vesicles.

The luminal surface of the epithelial cells in the injected animals has a different appearance from that of the control animals. The luminal surfaces of the control cells, which contain large numbers

of vesicles, seem to have more microvilli (Figures 6, 34, 35 and 36) than the luminal surface of the cells from the injected animals (Figures 22 and 23). It is possible that the surface activity, as indicated by the microvilli, may reflect a chronic level of secretory activity in cells of control animals. Thus, epithelium from control animals which has not released all of its secretions would have more microvilli than the epithelial cells of the injected animals which have lost most of their vesicles and are consequently not secreting. The surface projections of the seminal vesicle epithelium have been taken as an indication of seminal vesicle cellular activity in the human (Riva, 1967) and the mouse (Dean and Portar, 1960). Also, Dean and Wurzelmann (1965), in describing the postnatal differentiation of the mouse seminal vesicle, place the development of the microvilli in the same time period as the appearance of dense secretory material in Golgi cisternae and the lumen. Toner and Baillie (1966), in describing the effect of castration on the mouse seminal vesicle, listed the disappearance of microvilli as well as a decrease in Golgi bodies and disorganization of the endoplasmic reticulum as ultrastructural changes resulting from castration.

Examples of the actual release of the vesicles or their contents after HCG injection are scarce. This is possibly due to the dose of HCG being too large, resulting in the overstimulation of the epithelial cells and the release of the vesicle before samples were taken for observation. Hence, there are insufficient observations to make definite statements on the methods of secretion. The few observations that could be made indicate several methods.

One method is the release of the vesicles in entirety into the lumen. Such an interpretation could be applied to Figure 37. This plate shows what appear to be vesicles in the lumen similar to the vesicles along the apical edge of the epithelial cell. The lighter density of the released vesicles could be explained by the escape of the vesicular material into the lumen. This would also account for the granular material clustered around these vesicles. A somewhat similar method has been described in the mouse seminal vesicle by Dean (1963). In this study the vesicles contain a granular which does not fill the entire vesicle. The vesicle moves to the lumen border and releases the granule plus any other contents of the vesicle into the lumen. The main difference between this description in the mouse and the images seen in the present study is that in the former the vesicular membrane becomes incorporated into the cell membrane with only the vesicular contents being released. However, a few images seen in this present study could be interpreted as indicating that some of the vesicles fuse with the luminal cell membrane, as has been described in the mouse seminal vesicle (Figures 18, 34, 36 and 38).

Another possible variation is indicated in Figure 39. The vesicles themselves seem to fuse as they approach very close to the cell membrane bordering the lumen. The vesicle membrane nearest to the lumen breaks down while that portion which does not connects with the cell membrane. Then the vesicle contents are released into the lumen along with the cell membrane between the point of connection.

There is also evidence that the vesicles break down near the border of the lumen, and their material passes through the cell

membrane (Figures 34, 36, 37 and 38). There is frequently a zone on the luminal side of the cell membrane that contains a fairly granular material. This material resembles, in less compact form, that material found in the dense vesicles and in the cytoplasm along the border of the cells (Figures 34, 36, 37 and 38). It is possible, of course, that the secretion of materials from these cells is a process of which several phases have been captured in the images described above.

The Type IV cell seems to release its vesicles *en masse*. The group of vesicles is pushed out at the apical end of the cell into the lumen. These clusters of vesicles break away from the cell into the lumen (Figure 19).

As has been mentioned before, the epithelial cells of the seminal vesicle have the characteristics of secretory cells. That is, they have well developed Golgi, many mitochondria, and secretory vesicles. However, there is an absence of a well developed system of membrane-bound ribosomes. This system, which is well developed in the human seminal vesicle (Riva, 1967), mouse (Dean, 1963; Dean and Porter, 1960), and rat (Szirmai and Van Der Linde, 1962), has been associated with secretory cells (Porter and Melampy, 1952; Wilson, 1962; Kochakian, 1964).

The small amount of membrane-bound ribosomes in the seminal vesicle epithelium of *R. clamitans* may be due to removal of them by the fixative. However, the other cellular organelles were well preserved, and the Type IV cells had abundant amounts of membrane-bound ribosomes. Also, in the post-HCG-injected animals there were membrane-bound ribosomes (Figures 23, 25, 28, 29, 30, 32 and 33). An apparent

increase in the amount of membrane-bound ribosomes in proportion to the time after HCG injection may have been due to the effect of the fixative on cells in differing physiological states or could have represented a real difference.

Unlike the human, rabbit or mouse, the frog breeds only once a year, usually in early to late spring, depending on environmental factors and the species. After breeding, the frog builds up a supply of gametes, during the summer before going into hibernation, which will be released the following spring.

The frogs used for these observations were late summer frogs. Therefore, their breeding cycle probably had been completed approximately one or two months previously. The control frogs may have been in later stages of synthesis needed to produce the seminal vesicle secretion. Therefore, the lack of membrane-bound ribosomes could be due to an earlier phase of synthesis having been completed. The increase in the membrane-bound ribosomes noticed in the post-injected samples may reflect an increase in synthesis of secretory material, the injected HCG having caused the release of the secretory vesicles, starting the secretory cycle over.

The Golgi bodies which are present in all the epithelial cell types described seem to be more prevalent in the post-injected samples (Figures 11, 13 and 17, compared to Figures 24, 27, 30, 31 and 32). In the seminal vesicles from the control samples, one or two Golgi bodies per cell section were seen. In the post-injected animals, particularly the post-12 hour and post-24 hour samples, three or more Golgi bodies per cell section were seen. The exception to this was the

Type I cell, which had very extensive Golgi bodies both pre- and post-HCG injection. The Type I cell, post-injected, did seem to have more extensive Golgi bodies that stained a dark gray (Figure 11 compared with Figures 24 and 27). This noticeable increase in the "activity" of the Golgi bodies is consistent with the possible increase in the synthesis of secretory material in the post-injected animal.

The origins of cytoplasmic vesicles seen within the cells cannot be determined with accuracy, nor can they be differentiated as to whether they will eventually be of the type which will be secreted. However, there seem to be three sources of cytoplasmic vesicles.

One apparent source is the Golgi bodies (Figures 13, 17, 31, 40 and 41). Vesicles given off by the Golgi bodies may or may not be lightly stained. Another source of cytoplasmic vesicle is the nuclear membrane (Figures 44 and 45).

The actual source of the secretory vesicles, that is, the vesicles found in the apical regions of Type II and Type III cells, is uncertain. The Golgi produce vesicles that are similar to those most common in the Type II cells. The vesicles that are typical of the Type III cells seem to be found in clusters near the nucleus. They may be derived from the Golgi bodies by fusing and condensation of the smaller vesicles produced by the Golgi (Figures 41 and 46).

In summary, the observations presented suggest that the seminal vesicle of *R. clamitans* is secretory in nature, that they do release substances in response to HCG which also causes sperm release. The synthesis of cytoplasmic vesicles, their release and the sequence of appearance of cell organelles, such as Golgi bodies and membrane-bound

ribosomes, are consistent with a cyclic reproductive behavior. Also, the epithelial cells of the *R. clamitans* seminal vesicles are very organized in the distribution of various cell organelles.

It is possible that the observed secretory vesicles contain a substance which may maintain the fertilizability of sperm that is stored in it.

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