Studies of Frog Oviducal Jelly Secretion

II. CYTOLOGY OF SECRETORY CYCLE [1,2]

PETER A. LEE

Department of Zoology, University of Michigan, Ann Arbor, Michigan

ABSTRACT Electron microscopy reveals that the secretory glandular cells of the oviduct of *Rana pipiens* contain granules which may develop from small vesicles with smooth membranes. The latter may represent dispersed Golgi elements, although cytochemical reactions fail to reveal a localized Golgi complex within these cells. Individual granules enlarge throughout the growing season without fusing with other granules. The mature cell is packed with granules, each of which possesses uniquely an interior electron-dense (dense) part and an electron-lucid shell enclosed by a smooth membrane. The dense part first appears as a condensed body. As growth proceeds, the dense component branches to form a net-like pattern within the granule. When the granule has attained its mature size, the dense component appears to revert to a condensed pattern again. Cytoplasmic granules which are observable by light microscopy after staining with toluidine blue at pH 4.0 apparently correspond to the dense component seen in the electron microscope.

Previous studies of the aggregation of secretory products have shown that the Golgi complex participates in their formation. Proteinaceous cellular products, which accumulate as intracisternal granules in regions of rough endoplasmic reticulum of zymogenic cells of the pancreas (Palade, '56) are transported to a region of smooth-surfaced cisternae and vesicles, the Golgi zone (Caro and Palade, '64). In the pancreas, according to current concept, secretory granules consist of Golgi membranes plus an enclosed aggregate of protein. When mature, the granules move from the Golgi zone to the apical portion of the cell and discharge their contents into the glandular lumen. Autoradiographic evidence on the uptake of ^{14}C-labeled-glucose (Neutra and LeBlond, '66) and $^{35}SO_4$ (Lane et al., '64) into intestinal goblet cells indicates that these precursors first appear to be incorporated into cellular products in the Golgi zone.

This paper reports cytological details of the accumulation of a secretory product within vesicles bounded by smooth-surfaced membranes during growth of the jelly-secreting gland cells of the oviduct of a frog.

MATERIALS AND METHODS

Female leopard frogs (*Rana pipiens*) obtained from a commercial supplier in Wisconsin were induced to ovulate (Wright and Flathers, '61), fed liver three times a week to stimulate growth of reproductive organs, and were sacrificed by pithing and fixed for electron microscopy. Sacrifices were made biweekly up to two months, and monthly up to five months after ovulation. Tissues were fixed for electron microscopy for 30 minutes in 1% osmium tetroxide buffered with 0.028 M Veronal acetate at pH 7.4–7.5 and containing 0.09 M sucrose (Palade, '52; Caulfield, '57), or in 6.2% glutaraldehyde buffered with 0.1 M phosphate at pH 7.4 (Sabatini et al., '63) for 24 hours, rinsed in 7.5% sucrose and post-fixed in the osmium tetroxide solution described. Tissues were dehydrated in a graded series of ethanol, cleared in propylene oxide and embedded in Epon 812 according to Luft's method. Ultrathin sections were cut, stained with uranyl acetate for 30 minutes (Watson, '58), and observed with an RCA EMU 3E electron microscope.

Fully developed oviducts from frogs without induction of ovulation were used for cytological studies. Cytochemical reactions for various cellular constituents of jelly-

[1] This work was supported in part by a grant from the University of Michigan Cancer Research Institute and a training grant from the National Institutes of Health (USPHS Grant no. GM989).

[2] Part of a dissertation submitted at the University of Michigan in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

secreting cells were carried out according to the techniques described in the references cited below. The direct silver method of Elftmann (Lillie, '54) and localization of thiamine pyrophosphatase (Allen, '63) were used to demonstrate the Golgi apparatus. Basophilic cytoplasm was identified in sections stained with toluidine blue (Pearse, '60) before and after digestion with ribonuclease suspended in distilled water.

RESULTS

The cells which synthesize jelly in the middle part of the oviduct form the lining of tubular glands which open into the lumen between ridges capped by mucous-secreting and ciliated epithelial cells (fig. 1). Electron micrographs show that the jelly-secreting cells of the mature oviduct are filled with large secretory droplets containing electron-dense bodies embedded in a light matrix (fig. 2).

Cytochemical reactions

Basophilic granules throughout the cytoplasm of glandular cells of the oviduct in preovulatory animals are evident after staining with toluidine blue at pH 4.0 to show localizations of RNA (fig. 3). Pretreatment of sections with ribonuclease resulted in reduction of intensity of the staining with toluidine blue, both in granules and in background cytoplasm (fig. 4). Control sections incubated in distilled water showed no reduction of intensity.

Neither the Elftmann nor the thiamine pyrophosphatase technique demonstrated a localized Golgi region in glandular cells, which indicates that the Golgi apparatus is probably dispersed in these cells.

Electron microscopy

Electron microscopic observations were made on oviducts from frogs sacrificed periodically from the time of spawning, until growth of the oviducts was again complete. Some animals were fed in the laboratory for various periods before sacrifice. Others were sacrificed at the time of purchase. Accumulation of secretory granules in developing glands is concomitant with striking ultrastructural changes. After secretion of jelly, the gland cells of the oviduct retain only a few scattered, unsecreted granules. Cells are much smaller, and their lateral borders interdigitate extensively with those of their neighbors, especially toward the apical and basal regions of cells. The basal border of the cells is irregularly undulated, and a typical basal lamina follows its undulations. There are large empty vacuoles scattered among the typical organelles of the cytoplasm (fig. 6).

In the cells just after secretion (fig. 5), many vesicles bounded by smooth membranes are observed. The cytoplasm contains free ribosomes but only limited rough endoplasmic reticulum (fig. 7). Nuclei are centrally located (fig. 6). Mitochondria are distributed throughout the cells but at this stage are most dense at apical ends (figs. 5, 6). Microvilli project from the inner surfaces into the glandular lumen (fig. 5). Tight junctions and desmosomes bind adjacent glandular cells together (fig. 6).

Changes in the appearance of glandular cells as time progresses are a result of the aggregation of secretory product in separate vesicles. At the beginning of the period of secretory accumulation during the first two weeks after spawning, small granules are present throughout the cytoplasm but are often massed in the apical end of glandular cells (figs. 6, 7). Secretory granules possess an electron-dense (dense) core and a much less dense shell within a membrane. Golgi elements are evident at this stage, although light microscopic cytochemistry shows no discrete Golgi apparatus at any stage of development.

Secretory granules are present, but chiefly at the apical end of the cell, at two weeks after spawning. By one month, they have accumulated and grown so that they fill the cells to their basal ends. Some of the granules at one month are relatively small with a central dense core and little or none of the electron-lucid (light) component (fig. 8). Larger granules in the cells at one month after spawning contain a dense component which branches and forms a net-like pattern ramifying through the surrounding light component. Granules subsequently grow for two or three months until the cell more than triples its size and fills with secretory droplets (fig. 9). In animals sacrificed from 3–5 months

after induced ovulation, glandular cells contain varying proportions of granules with spherical or filamentous electron-dense components (fig. 2). Toward maturity, elements of the branching dense components appear to coalesce again so that the fully mature granule possesses a compact, dense core surrounded by a lucid shell (fig. 10). This dense core may account for the basophilic granules observed by light microscopy (fig. 3). An animal fed for five months in the laboratory still had granules with both branching and condensed dense material, although a female feeding naturally, outdoors, for this length of time apparently possessed only granules of the latter type (fig. 10).

The dense component of the secretory granule is often at one edge of a secretory granule, apparently separated from it only by the membrane of the secretory granule. The outer electron-lucid component accounts principally for increase in bulk as granules grow. This light component contains subunits about 70Å in diameter. Although it cannot be said whether the number of granules increases as growth proceeds, it is evident that the major increase in size of a glandular cell is due to enlargement of those granules present or produced early in growth.

Organelles characteristic of young cells become localized because of the crowding by growing granules. As granular growth proceeds, the nucleus is pressed against the basal border of the cell and assumes a distorted shape. Mitochondria are squeezed between enlarging secretory granules. The Golgi complex has not been identified in cells loaded with granules, even though typical arrays of Golgi membranes can be seen in the cytoplasm of cells not laden with secretory granules. Microvilli appear to be constant in length throughout the growth cycle but are not so densely distributed upon an enlarged cell as on a cell without secretory droplet accumulation.

During secretion, granules are apparently released individually (fig. 5). Glandular cells shrink immediately. No residual remnants of either light or dense components persist in the cell after granular discharge, at least in the form they possessed before secretion. Presumably membranes surrounding the granules fuse with the plasma membrane during the secretion. Vacuoles in the cytoplasm of cells which have released their jelly may persist for several weeks (fig. 6).

DISCUSSION

Fabrication of secretory granules by jelly glands in the oviduct of the frog appears to differ in some respects from the pattern described for other secretory granules, e.g., protein in the pancreatic acini (Caro and Palade, '64) and mucoid material in goblet cells (Neutra and LeBlond, '66). The present study has demonstrated that secretory granules in oviducal glandular cells consist of three components: (1) a compact (figs. 2, 11) or spongelike (fig. 2), osmiophilic interior mass staining positively with toluidine blue; (2) osmiophobic, finely granular substance surrounding the dense component, staining positively with the periodic acid-Schiff reaction; and (3) an external smooth-surfaced membrane. Zymogen granules of the pancreas (Palade, '59) appear to be membrane-bound inclusions containing protein distributed homogenously.

Accumulation of secretory product within membranes of rough-surfaced endoplasmic reticulum, as is characteristic for zymogen granules (Caro and Palade, '64), has not been observed in jelly-secreting cells of the oviduct. Indeed, membranes of the rough-surfaced variety are scarce in glandular cells even at the beginning of the phase of growth of secretory granules. Ribosomes are abundant in cells at this period but are often free or clustered as polyribosomes. Cells at the beginning of the growth phase possess occasional Golgi elements in the form of clusters of flattened cisternae with associated vesicles; but these clusters are not abundant. The large droplets of jelly precursors may originate from small vesicles of the Golgi complex; but a direct association between young secretory granules and Golgi vesicles has not been verified in the present study. Secretory granules first appear membrane-bound and may originate from small, smooth-membraned vesicles dispersed throughout the cytoplasm (fig. 5) and apparently then grow to maturity individually. It is possible that these scat-

tered vesicles are derived from elements of the Golgi system.

Histochemical analysis (Lee, '67) and cytochemical observations suggest that the electron-dense component of secretory bodies, as seen in the electron microscope, may be the same as the basophilic component which stains positively for protein with bromphenol blue, or for RNA with toluidine blue. The electron-lucid component of the granule apparently accounts for the positive PAS reaction throughout the cytoplasm of mature glandular cells previously reported (Lee, '67). The basophilic component, largely surrounded with PAS-positive material, often appears to be to one side of the secretory granules, possibly connecting with adjacent cytoplasm. Evidence that the basophilic body contains both protein and RNA, leads one to speculate that it may contain the enzymes for synthesis of its own protein and also for the carbohydrate of the PAS-positive material which becomes the predominant component of the enlarging granule. Polymerization of the carbohydrate moiety may take place after secretion into the lumen of the oviduct.

Secretion of jelly precursors from a cell packed with separate granules poses a somewhat different problem than secretion from the pancreatic acinus, as visualized by Caro and Palade ('64). Hundreds of bodies are extruded simultaneously from the jelly-secreting cell, whereas in the exocrine cells of the pancreas, granules are continually breaking away from the Golgi zones, migrating to the cell surface, fusing with plasma membranes and emptying their contents into the acinar lumen. The jelly-secreting cell shrinks markedly as a result of elimination of its secretory droplets but the pancreatic acinar cell retains its shape throughout its secretory activity. The exact manner in which bodies reach the surface for extrusion has not been elucidated. Presumably those close to the surface fuse and empty their contents into the glandular lumen, much as do granules in the pancreatic cell. Deeper lying droplets may fuse those above them progressively so that layer by layer they reach the receding cell surface and are extruded. Shrinkage of the cell could provide a means for bringing successive layers of secretory bodies to the surface without their active migration. There is no evidence that cytoplasm of the glandular cell is lost during the process of granular extrusion.

After secretion of the droplets, the shrunken cells possess an abundance of ribosomes and smooth-surfaced vesicles. Secretory product begins to accumulate in some of these vesicles as the phase of growth ensues. How the raw materials are delivered to sites of developing granules, how synthesis of secretory product occurs, and how secretory vesicles grow are questions for further research.

SUMMARY

The distrubution of secretory product during the period of accumulation and discharge has been studied by ultrastructural and cytochemical means in the jelly-secreting cells in the oviduct of a frog (*Rana pipiens*). Secretory product is presumed to originate and accumulate within smooth-surfaced membranes that may be related to Golgi membranes. Secretory bodies develop separately, crowding the cytoplasm to the periphery of the cell as they enlarge. They are distinctive in possessing an electron-lucid shell and an electron-dense core. The dense core appears to contain protein and RNA and may be involved in the synthesis of the electron-lucid material. Glandular cells shrink markedly after discharge of their secretory granules.

ACKNOWLEDGMENT

The author is grateful to Dr. Norman E. Kemp for suggestions and counsel.

LITERATURE CITED

Allen, J. M. 1963 The properties of Golgi-associated nucleoside diphosphatase and thiamine pyrophosphatase: I. Cytochemical analysis. J. Histochem. and Cytochem., *11:* 529-541.

Caro, L. G., and G. E. Palade 1964 Protein synthesis, storage and discharge in the pancreatic exocrine cell. An autoradiographic study. J. Cell Biol., *20:* 473–496.

Caulfield, J. B. 1957 Effects of varying the vehicle for OsO_4 in tissue fixation. J. Biophys. Biochem. Cytol., *3:* 827–830.

Lane, N., L. Caro, L. R. Otero-Vilardebo and G. C. Godman 1964 On the site of sulfonation in colonic goblet cells. J. Cell Biol., *21:* 353–366.

Lee, Peter A. 1967 Studies of frog oviducal jelly secretion. I. Chemical analysis of secretory product. J. Exp. Zool., *166:* 99–106.

Lillie, R. D. 1954 Histopathologic Technic and Practical Histochemistry. Blakiston, New York.

Neutra, M., and C. P. LeBlond 1966 Synthesis of the carbohydrate of mucus in the Golgi complex as shown by electron microscope autoradiography of goblet cells from rats injected with glucose-H^3. J. Cell Biol., *30:* 119–136.

Palade, G. E. 1952 A study of fixation for electron microscopy. J. Exp. Med., *95:* 285–298.

——— 1956 Intracisternal granules in the exocrine cell of the pancreas. J. Biophys. Biochem. Cytol., *2:* 417–422.

——— 1959 Functional changes in the structure of cell components. In: Subcellular Particles, T. Hayashi, ed. Ronald Press, New York, pp. 64–83.

Pearse, A. G. E. 1960 *Histochemistry, Theoretical and Applied.* Second Edition. Little, Brown, Boston.

Sabatini, D. D., K. Bensch and R. J. Barrnett 1963 Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity and aldehyde fixation. J. Cell Biol., *17:* 19–58.

Watson, M. L. 1958 Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol., *14:* 475–478.

Wright, P. A., and A. R. Flathers 1961 Facilitation of pituitary-induced frog ovulation by progesterone in early fall. Proc. Soc. Exp. Biol. Med., *106:* 346–347.

PLATE 1

EXPLANATION OF FIGURES

1 Longitudinal section of glands from secretory region of oviduct. Cells show nuclei which are slightly displaced basally, although no heavy accumulation of secretory granules is evident. Mucous and ciliated epithelial cells cover the epithelial tufts protruding into the oviducal lumen. × 400.

2 Longitudinal section of oviducal secretory cell from animal maintained four months after induced ovulation showing glandular cells packed with jelly secretion. The most distal portions of the cells shown here are at the upper right. The nucleus (N) of the middle cell is pressed against the basal margin as a result of heavy accumulation of secretory granules. Note that the cells appear to be divided into compartments. Each compartment (arrow) is a secretory body bounded by a smooth surfaced membrane and containing numerous electron-dense spheroid bodies or filaments plus an electron-lucid material surrounding them. × 3,075.

3 Cross-sections of oviducal glandular cells showing fine cytoplasmic granules staining orthochromatically with toluidine blue. × 540.

4 Adjacent section to figure three incubated with ribonuclease before staining with toluidine blue. Granules are less pronounced. × 540.

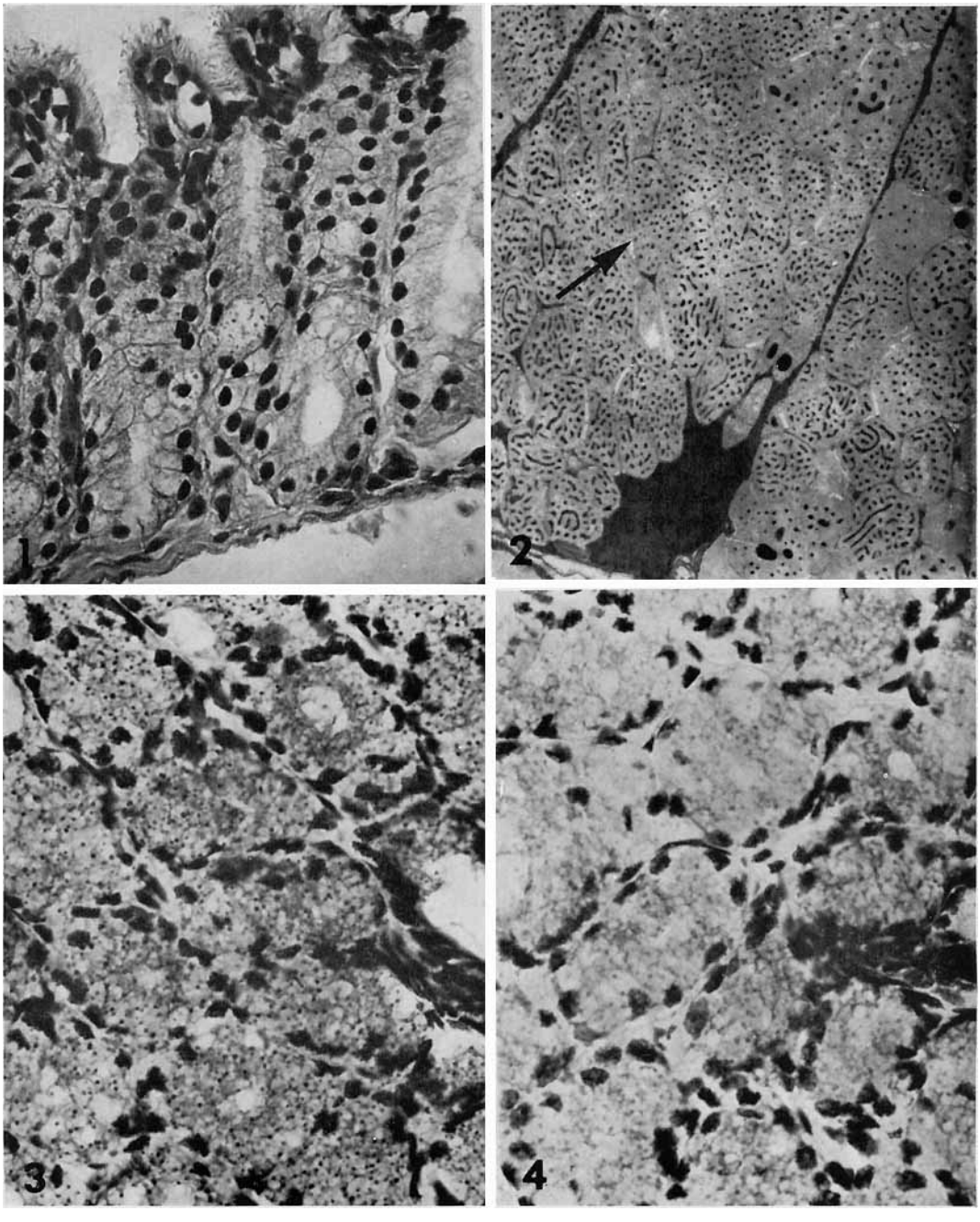

1
2
3
4

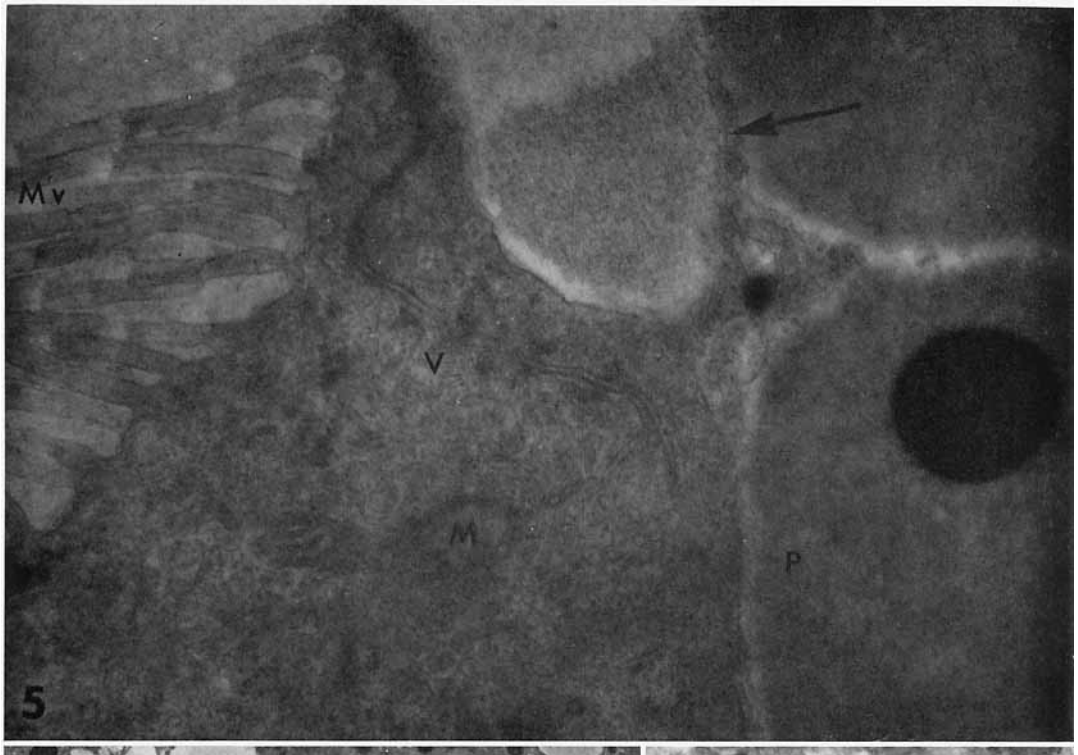
PLATE 2

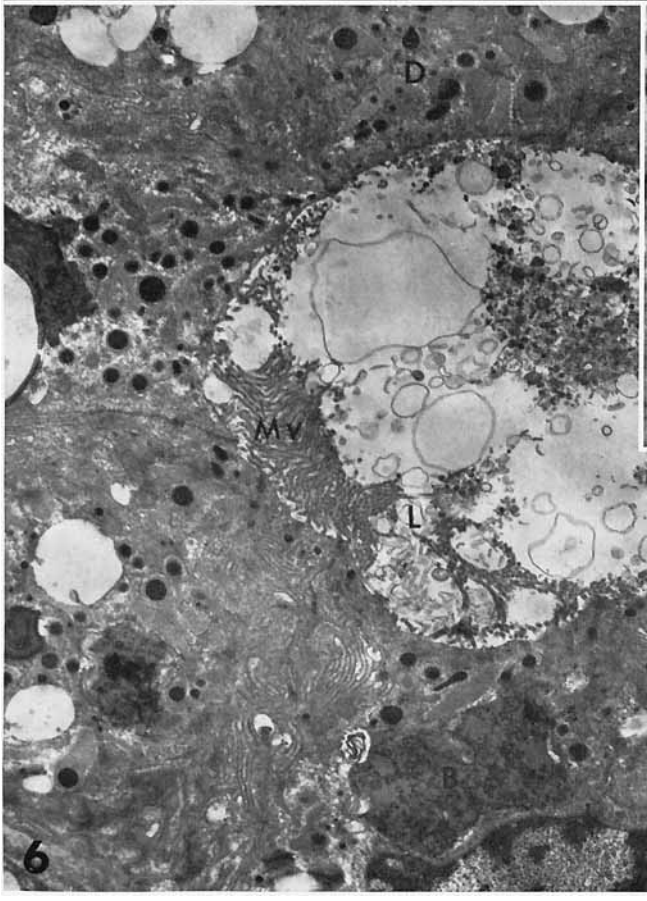
EXPLANATION OF FIGURES

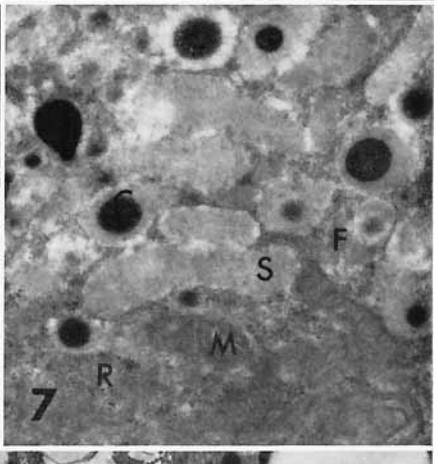
5 Section of apical ends of two adjacent glandular cells of oviduct from female induced to ovulate and sacrificed during oviducal secretion. Secretion body from the cell to the right has just released its contents into the lumen of the gland (arrow). Two large secretory packets (P) within this cell are not yet secreted. In the emptied cell to the left the cytoplasm contains mitochondria (M), an extensive array of vesicles (V) with smooth membranes, and microvilli (Mv.). × 25,750.

6 Cross-section of oviduct from female maintained in laboratory for 2 weeks after induction of ovulation. Glandular cells contain vacuoles (V) and early stages of secretory droplets (D) showing dark, condensed core and electron-lucid shell. Lumen of gland contains debris (L), and microvilli (Mv). Aggregated electron-dense bodies (B) are present, possibly resulting from coalescence of secretory granules undischarged at the time of glandular release. Nuclei (N) are located in the basal halves of these cells. × 7,700.

7 Higher magnification of section of oviduct from female maintained 2 weeks after ovulation. Young secretory granules in this view have condensed electron-dense cores (C) surrounded by electron-lucid shells (S). Mitochondria (M), ribosomes (R), and fine filaments (F) are displaced toward the periphery of the cell. The apical portion of the cell is at the top. × 20,000.


Mv
V
M
P
5


D
Mv
L
B
N
6


S
F
M
R
7

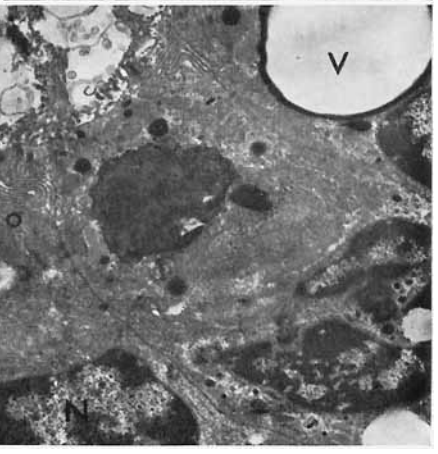

V

PLATE 3

EXPLANATION OF FIGURE

8 Section of oviducal gland from female one month after ovulation. Secretory cells show stages of growth from young droplet (D) with condensed central body to more advanced packets (P) with a branching electron-dense component (DC). A lumen (L) and intracellular space (I) can be seen. These cells are difficult to fix and embed as is evidenced here by artifacts. × 22,300.

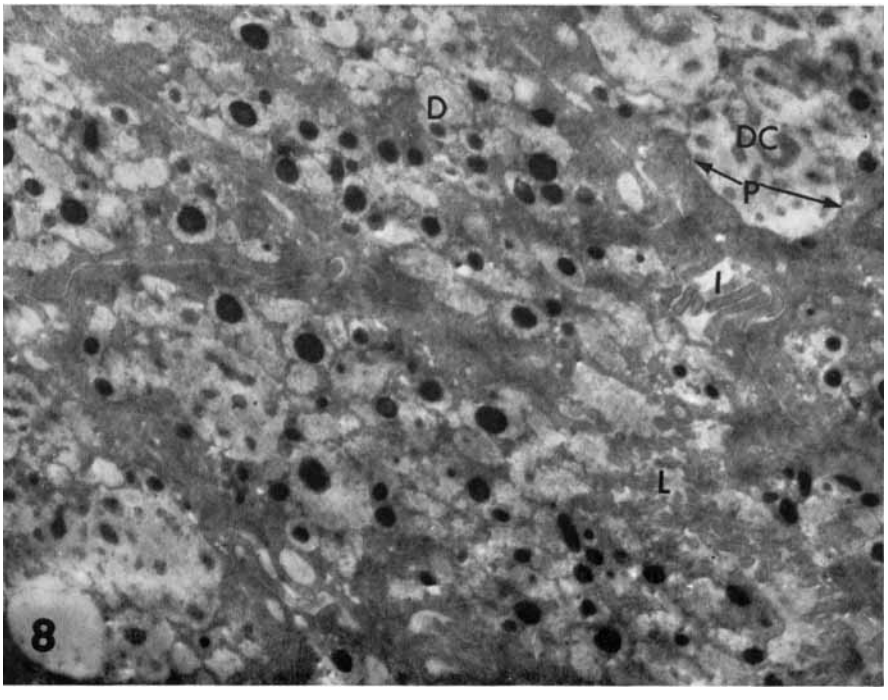
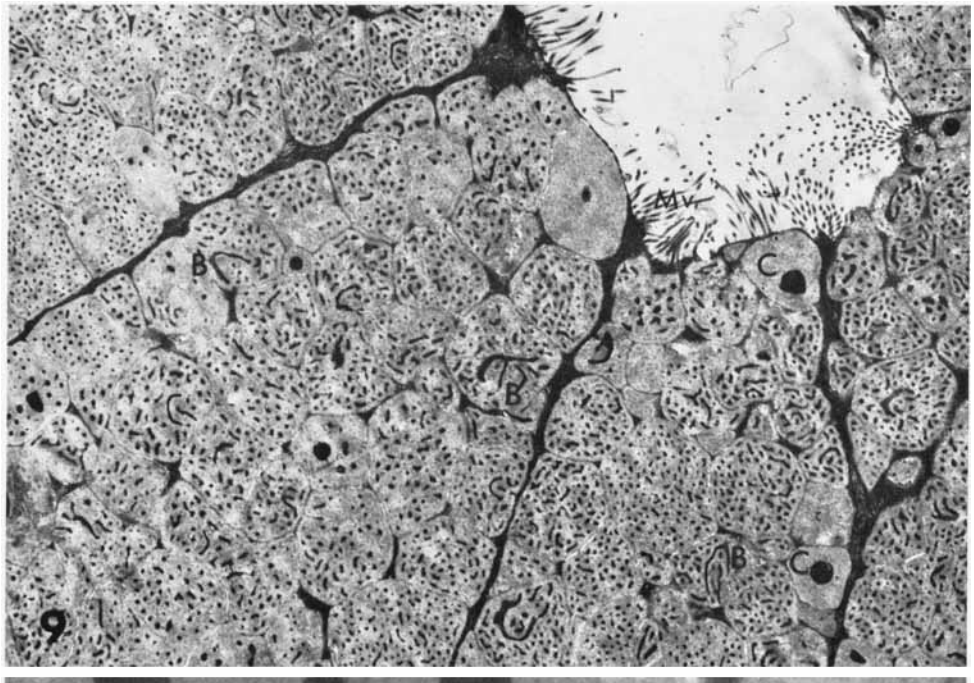

D
DC
P
I
L
8

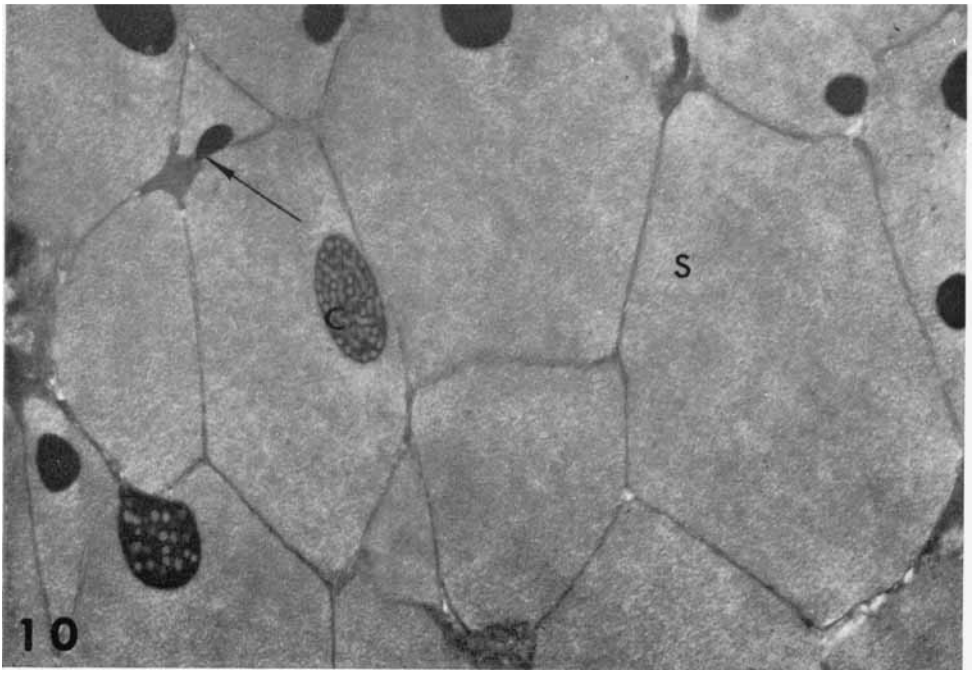
PLATE 4

EXPLANATION OF FIGURES

9 Cross-section of gland from an animal maintained four months after induced ovulation, showing apical portions of five jelly-secreting cells. Secretory droplets show accumulation of electron-lucid material around sections of condensed (C) or branching (B) electron-dense material. Microvilli (Mv) project from the apical surfaces. Note that the appearance of the secretion in these cells resembles that of the mature oviduct illustrated in figure 2. × 4,800.

10 Secretory droplets from a gravid female with mature oviducts collected from nature prior to spawning. The condensed pattern of the electron-dense core (C) enclosed within the light shell (S) is evident. Note that the dense material is toward one side of a granule and sometimes appears to be in contact with the outer membrane (arrow). Net-like pattern of dense component shows in some granules. × 11,250.


B
B
B
C
C
C
Mv
9


C
S
10