

Morphology and Ultrastructure of the Venom Glands of the Northern Pacific Rattlesnake *Crotalus viridis oreganus*

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ABSTRACT The venom gland of *Crotalus viridis oreganus* is composed of two discrete secretory regions: a small anterior portion, the accessory gland, and a much larger main gland. These two glands are joined by a short primary duct consisting of simple columnar secretory cells and basal horizontal cells. The main gland has at least four morphologically distinct cell types: secretory cells, the dominant cell of the gland, mitochondria-rich cells, horizontal cells, and “dark” cells. Scanning electron microscopy shows that the mitochondria-rich cells are recessed into pits of varying depth; these cells do not secrete. Horizontal cells may serve as secretory stem cells, and “dark” cells may be myoepithelial cells. The accessory gland contains at least six distinct cell types: mucosecretory cells with large mucous granules, mitochondria-rich cells with apical vesicles, mitochondria-rich cells with electron-dense secretory granules, mitochondria-rich cells with numerous cilia, horizontal cells, and “dark” cells. Mitochondria-rich cells with apical vesicles or cilia cover much of the apical surface of mucosecretory cells and these three cell types are found in the anterior distal tubules of the accessory gland. The posterior regions of the accessory gland lack mucosecretory cells and do not appear to secrete. Ciliated cells have not been noted previously in snake venom glands.

Release of secretory products (venom) into the lumen of the main gland is by exocytosis of granules and by release of intact membrane-bound vesicles. Following venom extraction, main gland secretory and mitochondria-rich cells increase in height, and protein synthesis (as suggested by rough endoplasmic reticulum proliferation) increases dramatically. No new cell types or alterations in morphology were noted among glands taken from either adult or juvenile snakes, even though the venom of each is quite distinct. In general, the glands of *C. v. oreganus* share structural similarities with those of crotalids and viperids previously described.

The venom glands of snakes have been the subject of numerous studies due to the highly toxic nature of their secretions and the evolutionary significance of this specialized trophic and defensive adaptation. Extensive reviews of the biochemistry of the venoms (Lee, '79; Tu, '82; Shier and Mebs, '89) and the morphology/histology of the glands (Taub, '66; Kochva and Gans, '66; Kochva, '78, '87; Gopalakrishnakone and Kochva, '90) exist, but there are few reports describing the ultrastructure of venom glands, particularly those of crotalids (but see Odor, '65; Warshawsky et al., '73). Viperid venom glands, particularly *Vipera palaestinae*, are the most thoroughly studied (Ben-Shaul et al., '71; Brown

et al., '75; Oron and Bdolah, '78; Kochva et al., '80; Lake et al., '82), and past reports provide a significant comparative background for the investigation of the gland structure of other venomous snakes.

An anterior accessory gland is known to be part of the venom gland apparatus of many snakes (Kochva and Gans, '66; Kochva, '78; Gopalakrishnakone and Kochva, '90) but with few exceptions, most workers have studied only the main venom gland. A more recent report gave some details of the fine structure

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of the accessory gland of the viperid *Bitis arietans* (Hattingh et al., '84), but the gland is only partly characterized. The ultrastructure of the accessory gland of crotalids, however, has not been described. Since the accessory gland has been suggested as a possible site for activation of venom toxins and enzymes (Gans and Kochva, '65; Ruzic and Russell, '78), a more complete ultrastructural investigation of this gland might suggest possible mechanisms of storage and activation of venom.

The present study describes the venom glands of the northern Pacific rattlesnake (*Crotalus viridis oreganus*). This species is a widely distributed rattlesnake (Klauber, '72), and many aspects of its predatory behavior (Kardong, '86a,b) and venom composition and variation (Mackessy, '88) have been well characterized. *C. v. oreganus* therefore may be a good representative of the gland morphology to be expected among species of *Crotalus*.

MATERIALS AND METHODS

Four adult (650–800 mm total length) and three juvenile (300–360 mm) northern Pacific rattlesnakes were collected in Whitman County, Washington, and were maintained at ~26°C with a 12L:12D light cycle. Five snakes had not expended venom for at least 2 months (unextracted), and one juvenile and one adult had venom manually extracted 48 hours prior to sacrifice (extracted). Snakes were forced to bite paraffin-covered beakers and the gland was gently massaged to remove venom. All snakes were lightly anesthetized with Halothane, killed via decapitation, and the intact glands were excised under a Wild M5A stereomicroscope. Gland tissue was then carefully sliced into 1 mm blocks while immersed in 0.1 M sodium cacodylate buffer (pH 7.2) containing 5% glutaraldehyde fixative. Three hour fixation was followed by three 10 min washes in cacodylate buffer containing 10% sucrose. After post-fixation in 1% osmium tetroxide (in 0.1 M sodium cacodylate, pH 7.2), specimens were rinsed twice in distilled water and dehydrated stepwise through an ethanolic series (30%, 50%, 70%, 95% ×2, 100% ×3). Specimens for transmission electron microscopy (TEM) and semi-thin-section light microscopy (LM) were dehydrated further in 100% propylene oxide (×2) and then placed in a propylene oxide:Epon 812 resin (1:1) mixture for 12 hr with continuous rotation. After placing the samples in two changes of Epon to aid in penetration of plastic resin, specimens were placed in flat

molds and cured stepwise: room temperature, 10 hr; 50°C, 8 hr; 75°C, 10 hr; 100°C, 1 hr. Following immersion in 100% ethanol, specimens for scanning electron microscopy (SEM) were dehydrated further in a Bomar SPC-1500 critical point drier and then freeze-fractured in liquid nitrogen. Blocks were mounted on stubs and coated with gold (approx. 30 nm) in a Technics hummer sputter coater.

Paraffin-embedded whole gland tissue was sagittally sectioned and stained with hematoxylin/eosin. Epon-embedded tissue blocks were sectioned with glass or diamond knives on a Reichert OM-U2 microtome. Semi-thin sections (0.5–2.0 μm) were stained with either basic fuchsin/methylene blue or with Stevenel's blue (del Cerro et al., '80). Thin sections (straw to silver in color) were lifted onto 100-mesh copper grids (coated with Formvar), stained 18 min in 2% aqueous uranyl acetate, rinsed in a stream of distilled water for 30 sec, stained 3.5 min in lead citrate, and rinsed again for 30 sec.

Micrographs of the semi-thin sections were made with an Olympus CH photomicroscope. Main venom gland cell heights and relative cell frequencies were averages calculated from light micrographs taken of six different tissue samples (two snakes). SEM samples were viewed in a Hitachi S-570 scanning electron microscope at 20 kV. Thin sections (~80–120 nm) were viewed in a Hitachi HS-8 (50 kV), a Zeiss EM 9S-2 (60 kV), or a Hitachi 300 (75 kV) transmission electron microscope. Measurements of various cell parameters (e.g., height, microvilli length) were estimated from micrographs.

RESULTS

In crotalids and viperids, the main and accessory glands are separated by a primary duct (Fig. 1), typically absent in elapids (Kochva, '78; Kochva et al., '82). The glands lie just below the skin and are encased in a capsule of connective tissue. The morphology of the compressor muscle and its relation to the venom gland have not been described in *Crotalus viridis oreganus*, but have been for the crotalid *Agkistrodon piscivorus* (Kardong, '73). As in crotalids generally, the venom gland of *A. piscivorus* is also wrapped in a capsule of connective tissue. The compressor glandulae muscle terminates in a broad aponeurosis which wraps around the lateral and dorsal aspect of the venom gland. Contraction of the muscle, rather than simply pulling on the posterior regions of the

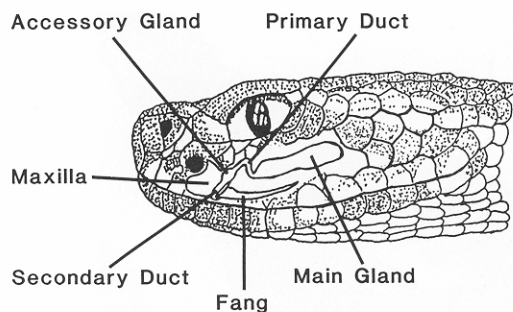


Fig. 1. The venom apparatus of the northern Pacific rattlesnake *Crotalus viridis oreganus* (redrawn from Klauber, '72).

gland, acts on the aponeurosis which effectively "wrings out" the gland via this wound connective tissue sheet (Kardong, '73). It is probable that *C. v. oreganus* utilizes a similar mechanism of gland emptying.

Tubular ductules of the main gland run in a dorso-posterior to ventro-anterior direction, and converge into a large, basally located main gland lumen containing much of the stored venom (Fig. 2). Venom produced by the secretory epithelium flows anteriorly through the primary duct, passing through the "stellate" duct of the accessory gland to the secondary duct and fang. The duct of the accessory gland receives numerous radially oriented ductules from the distal portions of the gland.

Main venom gland

In the secretory epithelium of the main gland (Figs. 3, 4), secretory cells are supported basally by connective tissue layers,

through which pass numerous capillaries. The ductule lumina (seen here in cross-sections) are typically filled with venom in the unextracted state (as in Fig. 2), and condensing and mature secretory granules are present in many cells (Fig. 3A,B). Secretory and mitochondria-rich cells in the unextracted gland are cuboidal, and secretory cells average $11.3 \pm 1.5 \mu\text{m}$ in height. Secretory cells of milked main gland tissue (48 hr post-extraction) are columnar, averaging $16.4 \pm 1.1 \mu\text{m}$ in height; they lack mature granules, and lumina do not contain venom (Fig. 4A,B).

Surface features of the secretory epithelium are revealed by SEM. Most regions of the unextracted gland (Fig. 5A) are covered with precipitated venom, even though tissues were gently agitated in the fixative. Washing tissue blocks with an iso-osmotic buffer might prevent this problem, but due to venom viscosity, clearance of ductules is unlikely. Unextracted secretory cells appear convex with venom adhering to the cell surface (Fig. 5A). Exposed cell surfaces of secretory cells in the extracted gland are somewhat flattened, and short (approx. $0.1\text{--}0.4 \mu\text{m}$), abundant microvilli are apparent (Fig. 5B). Scanning electron microscopy also reveals the presence of pores between adjacent secretory cells (Fig. 6A), as well as recessed cells (Fig. 6B). Sunken cells in the pores are surrounded by secretory cells and are characterized by relatively long microvilli (approx. $0.8\text{--}1.0 \mu\text{m}$).

Cryoprotection by sucrose caused disruption of intracellular organelles; hence visualization of intracellular structures by SEM of freeze-fractured specimens was precluded. A typical fracture face of a ductule wall and the

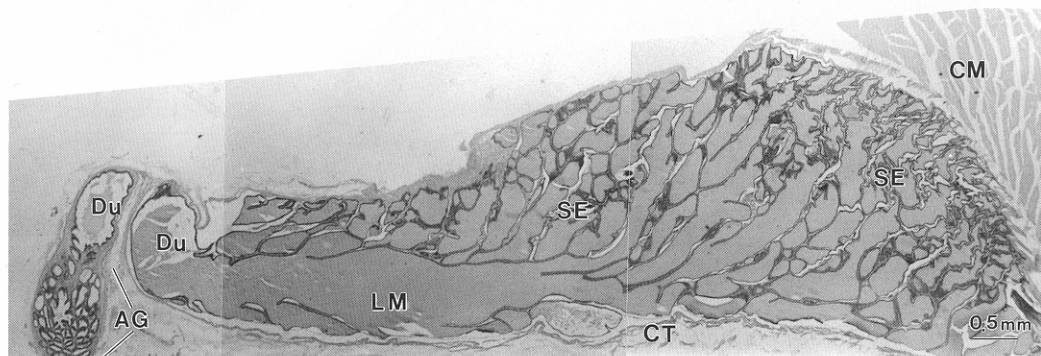


Fig. 2. Montage of photomicrographs of a mid-sagittal section of an unextracted main venom gland and primary duct (Du), and the associated accessory gland

(AG). CM, compressor glandulae muscle; CT, connective tissue; LM, lumen of main gland; SE, secretory epithelium of main gland.

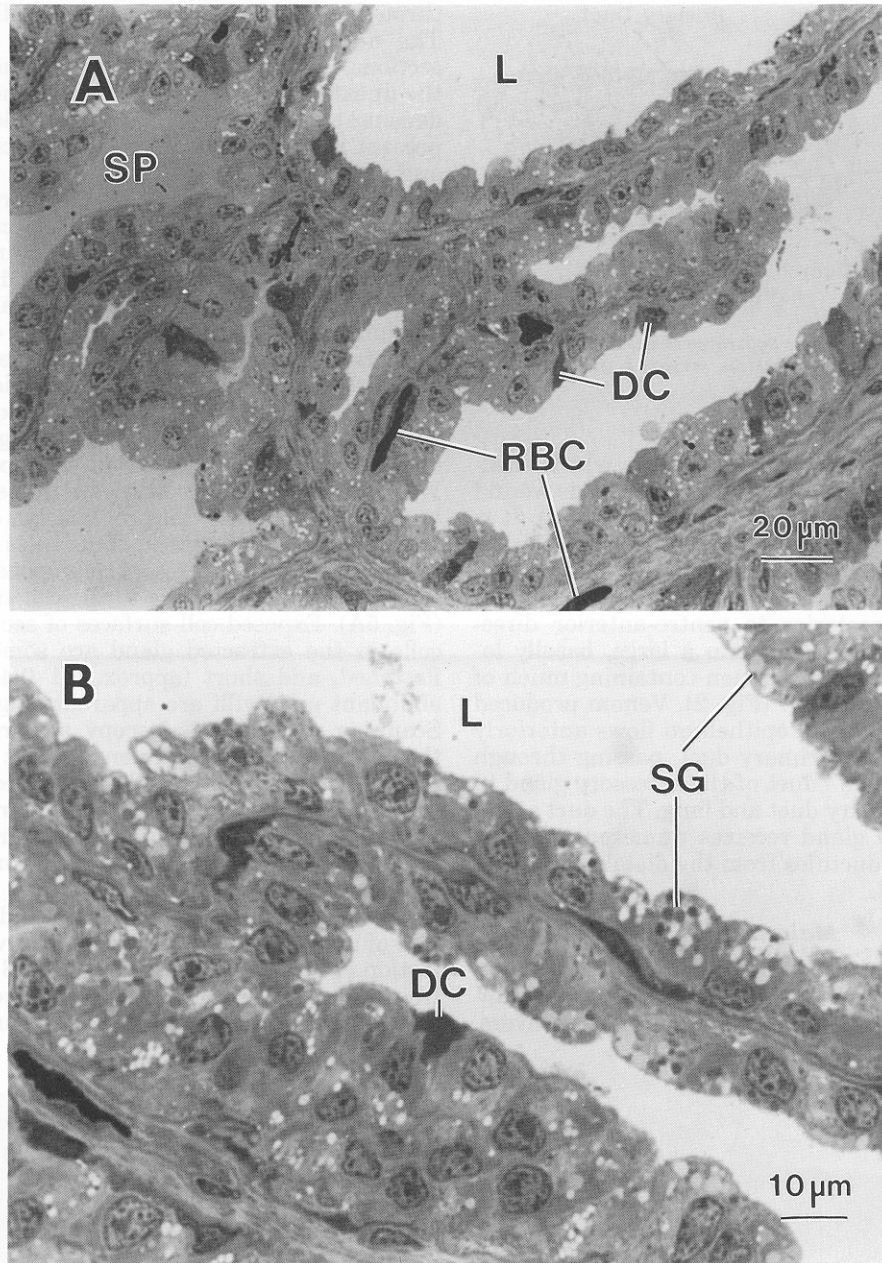


Fig. 3. **A,B:** Unextracted main venom gland, showing the secretory epithelium. Cells are typically cuboidal, and secretory cells average $11.3 \mu\text{m}$ in height. Stevenel's

blue. DC, dark cell; L, lumen; RBC, red blood cells; SG, secretory granules; SP, secretory product (venom).

relation of the secretory epithelium to capillaries and connective tissue are shown in Figure 7. The cuboidal secretory epithelium is underlain by a layer of connective tissue containing fibrocytes, capillaries, and nerve processes.

The secretory epithelium of the main gland as observed by TEM contains at least four types of morphologically distinct cells: secretory, horizontal, "dark," and mitochondria-rich cells (Fig. 9). Secretory cells (Figs. 8, 9A), which account for approximately 79% of

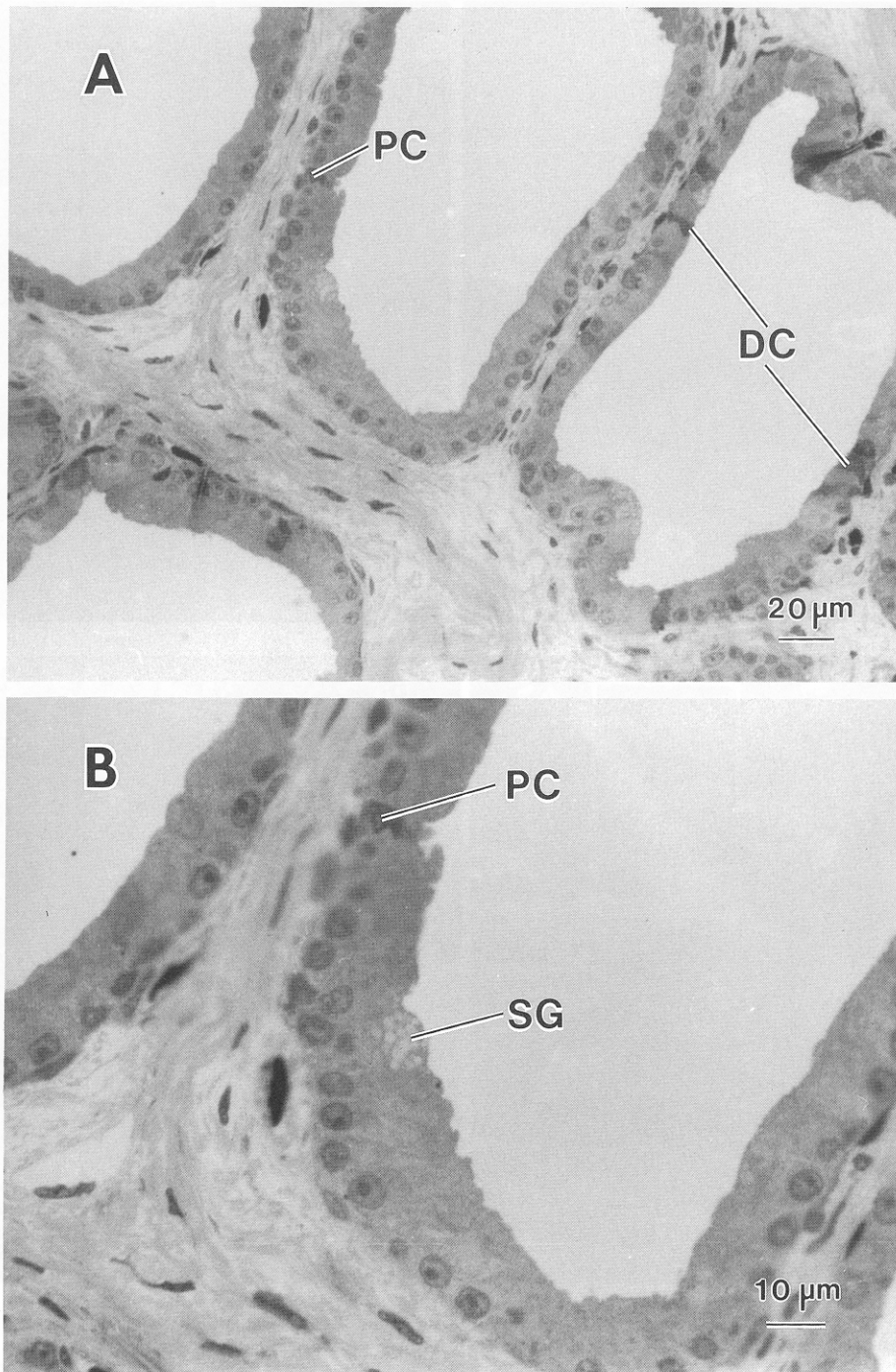


Fig. 4. **A,B:** Extracted main venom gland, showing the secretory epithelium. Cells are typically columnar, and secretory cells average 16.4 μm in height. Stevenel's blue. DC, dark cell; PC, pore cell; SG, secretory granules.

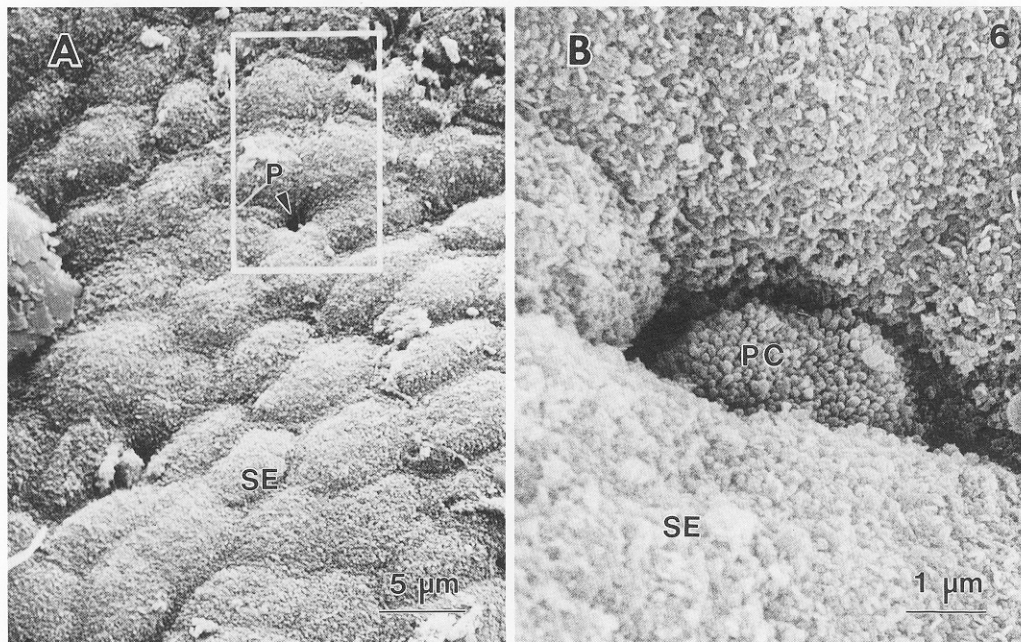
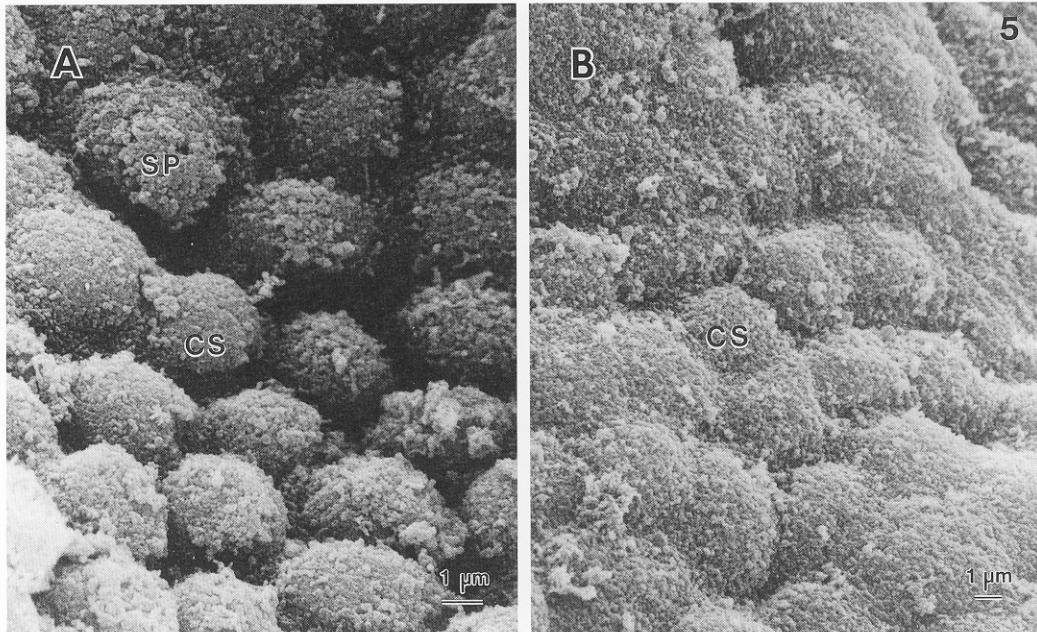


Fig. 5. (Top) **A,B**: Scanning electron micrographs of apical surfaces of secretory epithelial cells lining a ductule of main venom gland. **A**: Unextracted gland. Cell surfaces are convex and most are coated with adherent secretory product. **B**: Extracted gland. Cell surfaces are more flattened, coating of exudate is reduced, and short microvilli are apparent. CS, cell surface; SP, secretory product.

Fig. 6. SEM of cell surfaces (CS) of secretory epithelium in an extracted main venom gland. **A**: Pores (P) are seen as depressed areas between adjacent secretory cells. **B**: Within the pores, surrounded by secretory epithelial cells (SE), are mitochondria-rich cells (PC) which bear elongate microvilli.

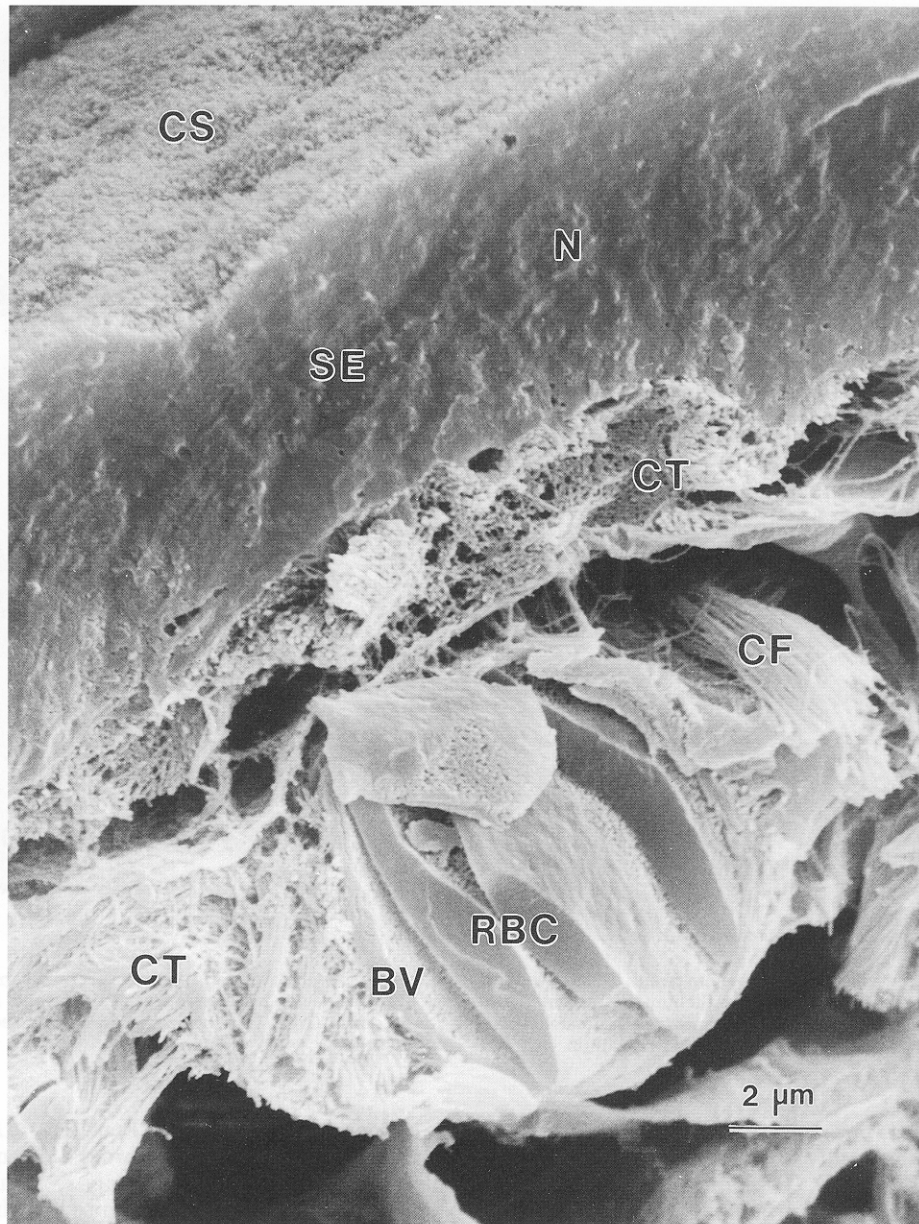


Fig. 7. SEM of wall of freeze-fractured ductule of an extracted main venom gland, showing secretory epithelium (SE) and underlying connective tissue (CT) contain-

ing collagen fibrils (CF) and blood vessel (BV). CS, surface of secretory cell; N, nucleus; RBC, red blood cell.

all cells, have short microvilli, numerous secretory granules (unextracted), and prominent rough endoplasmic reticulum (RER). Several different types of granules are seen in the secretory cells. Cells from unextracted

glands contain numerous electron-dense granules, often near the apex, and condensing granules are usually located more basally (Fig. 10A). In the extracted state (48 hr post-extraction), mature secretory granules are

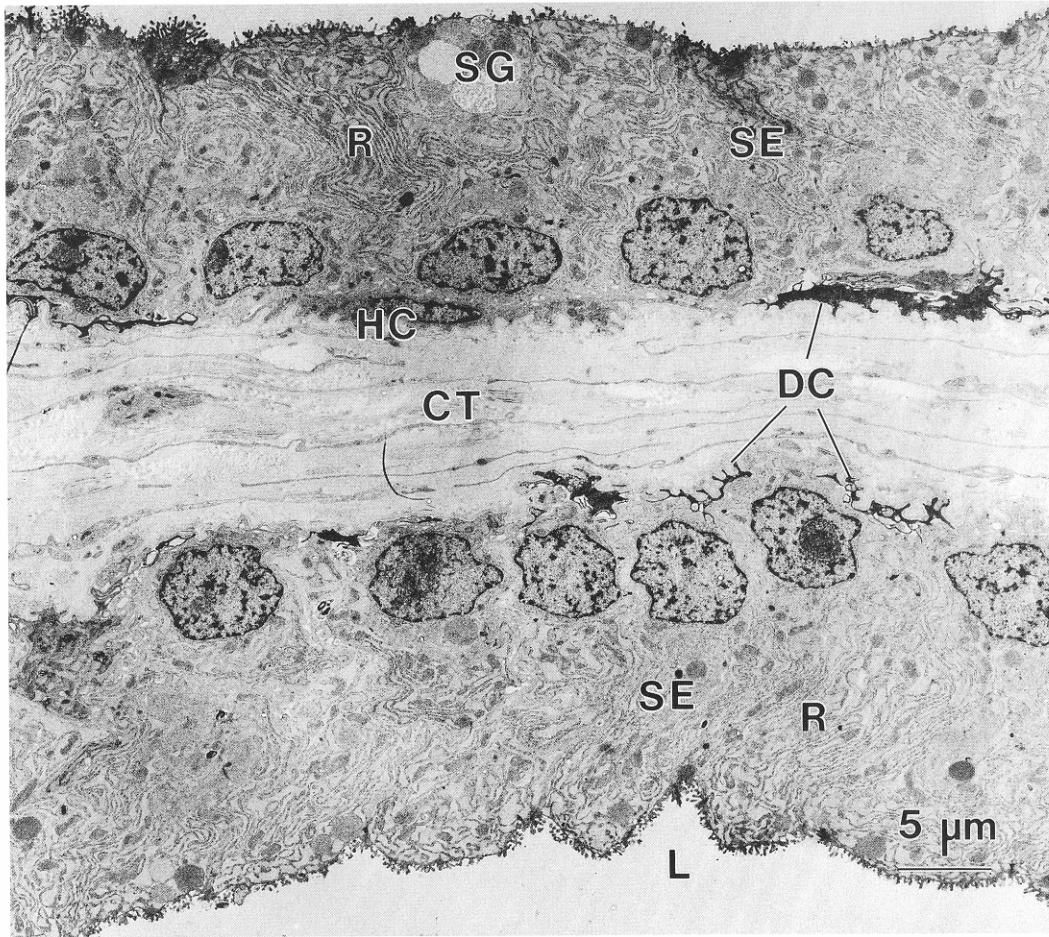


Fig. 8. Extracted main venom gland; TEM of walls of ductules, showing secretory epithelium (SE) and intervening connective tissue (CT). Secretory cells are columnar, with distended rough endoplasmic reticulum (R), and

relatively few electron-dense mature secretory granules (SG). Note the dendritic processes of the basally located "dark" cells (DC). HC, horizontal cell; SE, secretory epithelium (secretory cells); L, lumen.

not seen but electron-translucent granules are occasionally observed at or near the cell apex (Fig. 10B). Cisternae of the rough endoplasmic reticulum appear greatly elongated and widened at this stage (Figs. 8, 9A).

Horizontal cells (Figs. 8, 9D) account for 10% of the cell population. They are elongated along the basal border of the epithelium and underlie the basal ends of some secretory cells.

"Dark" cells (Figs. 8, 9C) comprise approximately 9% of the cells and are distinguished by an extremely electron-dense cytoplasm, a lack of secretory granules, and (usually) long dendritic processes extending below the bases of many cells.

Mitochondria-rich cells (Figs. 9B, 11A) occur with low frequency (approx. 2%) and are distinguished by a conical apex, relatively long microvilli, numerous mitochondria, a lack of secretory granules, and a basal nucleus. Their apical cell borders are recessed below the surface of surrounding secretory cells.

Intercellular associations in the main gland include interdigitations of the lateral plasmalemmae of adjacent cells and of the basal border of secretory cells in contact with the apical border of "dark" cells; prominent trilaminar junctional complexes at the apical ends of columnar cells (Fig. 11A); and desmosomes between the basal ends of secretory

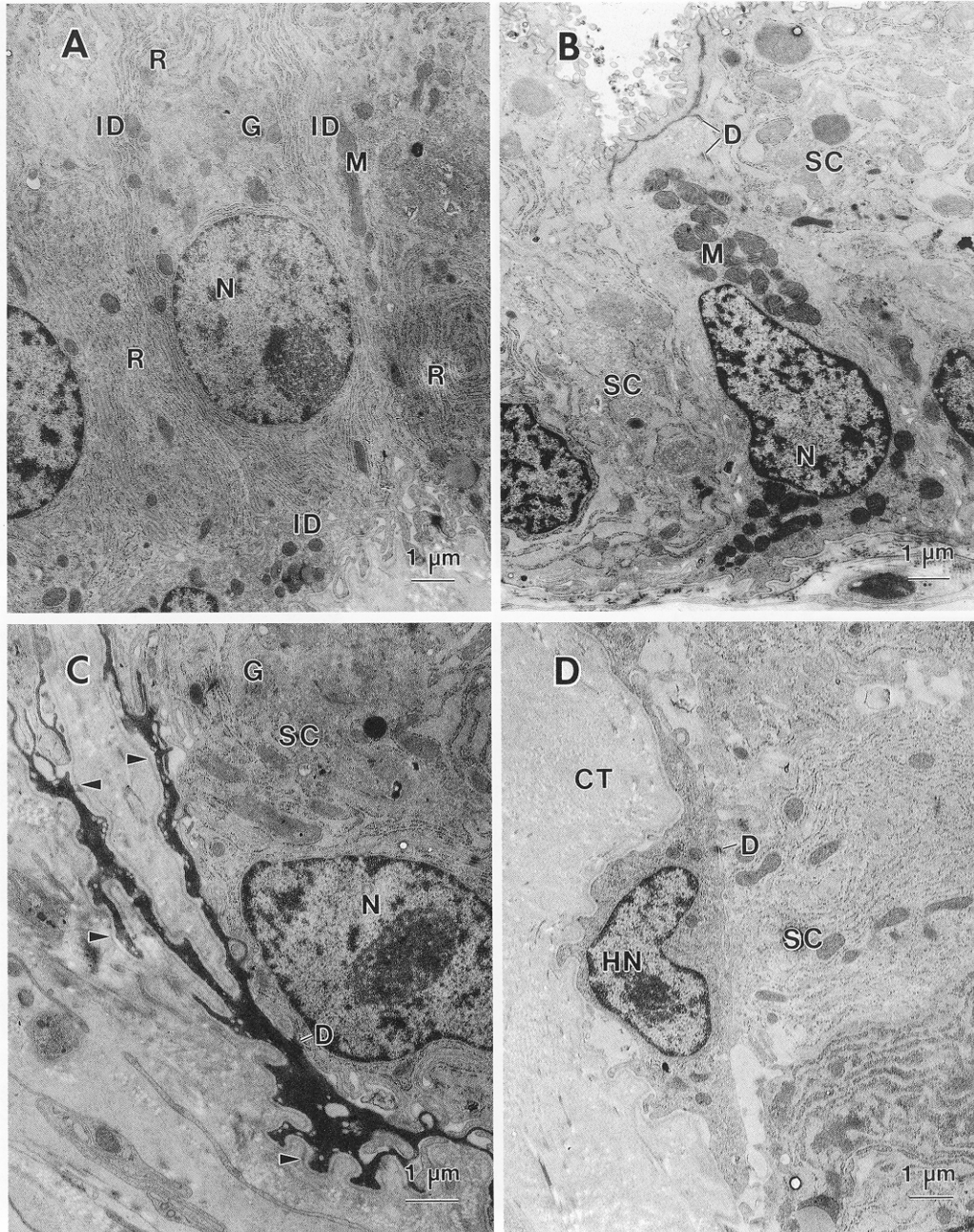


Fig. 9. Extracted main venom gland; TEM of the different cell types in epithelium. **A:** Secretory cells (SC) are characterized by columnar shape, prolific rough endoplasmic reticulum (R), and relatively few mitochondria (M). **B:** Mitochondria-rich cells are characterized by a basal nucleus (N), a large number of mitochondria, and a lack of secretory granules. This cell is usually recessed below the apices of surrounding secretory cells. **C:** Dendritic dark cells are basal, have long, thin extensions,

electron-dense cytoplasm, and peripheral vesicles (micro pinocytotic vesicles?). The basal lamina closely follows cell borders (arrowheads). **D:** Horizontal cells have flattened nuclei and are basally located. The apical surface interdigitates with and has desmosomal connections (D) to the bases of secretory cells. CT, connective tissue; G, Golgi apparatus; ID, interdigitations; HN, nucleus of horizontal cell.

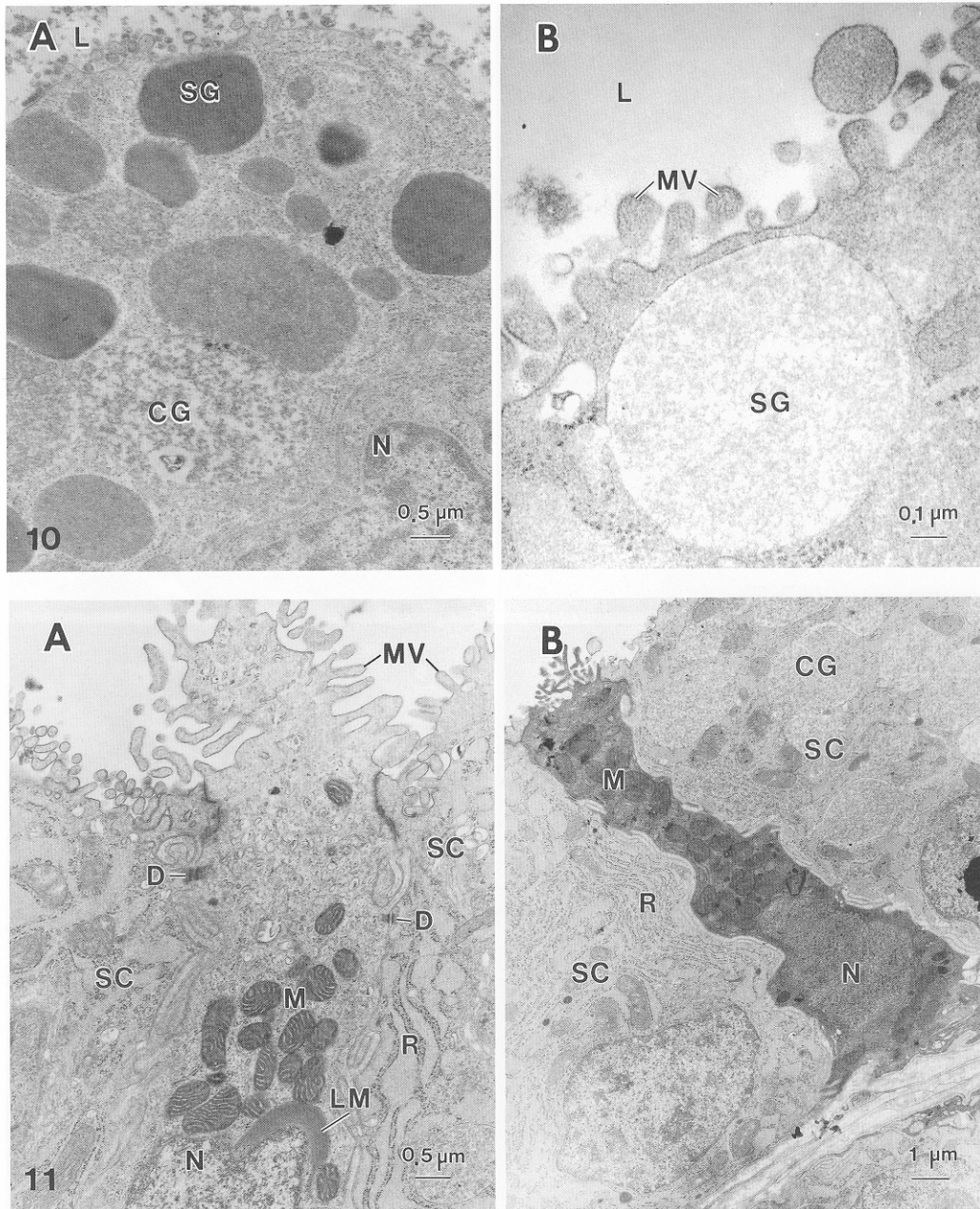


Fig. 10. Secretory granules in the main venom gland. TEM. **A:** Unextracted state: condensing granules (CG) become progressively more electron-dense as they approach the cell apex. **B:** Extracted state: mature granules are uncommon, but electron-opaque granules are seen at the cell apex. L, lumen; MV, microvilli; N, nucleus; SG, secretory granule.

Fig. 11. Extracted main venom gland; TEM of mitochondria-rich cell and a dark cell. **A:** Tall mitochondria-

rich cell showing conical apical protrusion and relatively large microvilli (MV), numerous mitochondria (M), and lack of secretory granules. In this section, an electron-dense lamellar membranous (LM) structure was noted at the nuclear apex. **B:** Dark cell extending from the basal lamina to the lumen; most are basal only. This cell may represent a particular functional state of a recessed cell (note numerous mitochondria). CG, condensing granule; D, desmosome; L, lumen; N, nucleus; R, rough endoplasmic reticulum; SC, secretory cell.

cells and horizontal cells, and between secretory cells and mitochondria-rich cells (Figs. 9D, 11A).

In fixed tissues, venom appears as flocculent precipitated particles within secretory granules of unextracted secretory cells and in the lumina of ductules (Fig. 12). Venom is likely released by exocytosis of secretory granule contents through the plasma membrane (Fig. 12C). In addition, vesicles containing venom are apparently released into the lumen (Fig. 12A); venom is then secondarily secreted from the primary released vesicles (Fig. 12B). Dead cells are occasionally seen in contact with the ductule lumen (Fig. 12D) and may account for some of the cellular debris seen in extracted venom.

Primary duct

The primary duct of the main gland is lined by a simple epithelium with two apparent cell types: basal, flattened horizontal cells and columnar secretory cells (Fig. 13). Blood vessels and nerve fibers run parallel to the duct in the connective tissue underlying the epithelium. Secretory cells contain secretory granules of varying electron density, prominent rough endoplasmic reticulum and mitochondria, and a supranuclear Golgi apparatus. Lateral interdigitations and apical trilaminar junctions join most cells. The horizontal cells appear to be basal stem cells.

Accessory gland

The principal duct of the accessory gland receives numerous radially arranged tubules (Fig. 2). The anterior portion of the gland can be distinguished from posterior regions by the presence of large mucosecretory cells. However, adjacent tubules in the anterior region do not necessarily have the same cell types (Fig. 14A). The mucosecretory cells contain electron-lucent granules localized in the cytoplasm above the basal, often flattened nucleus. In the granular region of swollen, "mature" mucous cells the cytoplasm is reduced to a thin layer surrounding the mucous granules (Fig. 15A) and the ductule lumen may be very nearly occluded. As cells fill with mucous granules, the nucleus becomes flattened basally.

In addition to the mucosecretory cells, the epithelium contains mitochondria-rich cells, basal horizontal cells, and dendritic "dark" cells (Fig. 14). The latter two types occur less frequently than in the main gland. Mitochondria-rich cells vary in morphology, depending

on their location. One type (Fig. 16A) appears to lack secretory granules but has apical vesicles. These cells overlap the apices of mucosecretory cells and nearly cover them. A second type of mitochondria-rich cell, perhaps a different functional state of the first, contains electron-dense secretory granules and a large number of mitochondria but does not directly contact mucosecretory cells (Fig. 15). A prominent supranuclear Golgi apparatus is associated with budding vesicles. A third type of mitochondria-rich cell, limited to peripheral anterior regions of the accessory gland, is characterized by 50–70 cilia, a more apically located nucleus, numerous mitochondria, and an apparent lack of secretory granules (Fig. 16). These mitochondria-rich cells also cover much of the surface of adjacent mucosecretory cells. Cilia are in dense fields of approximately 50–70/cell with the typical 9 + 2 configuration of microtubules.

Release of mucus from the mucosecretory cells is exocytotic (Fig. 17), similar to the secretion of venom from secretory granules contacting the surface in the main gland. Intact vesicles are never observed in the lumina of the accessory gland.

DISCUSSION

Main venom gland

Cell types and functions

The ultrastructure of the main gland of *Crotalus viridis oreganus* is similar in several respects to that described for *Crotalus durissus* (Warshawsky et al., '73). "Dark" cells found in *C. v. oreganus* have not been previously noted in *C. durissus* but may be present in other species. Secretory cells, the dominant cell type, cycle from cuboidal (unextracted) to columnar (48 hr post-extraction), an increase in height of 45%. They occur with approximately equal frequency (~80%) in the main glands of *Vipera palaestinae* (Ben-Shaul et al., '71) and *C. v. oreganus*. Evacuation of the gland lumina appears to stimulate synthesis of venom, and by 48 hr post-extraction most secretory cells are partially through the cycle from cuboidal to columnar, lack mature granules, and are largely filled by the expanded rough endoplasmic reticulum. In both *V. palaestinae* (Ben-Shaul et al., '71) and *C. durissus* (De Lucca et al., '74), maximum cell height was reached by day 4, and it is probable that *C. v. oreganus* follows a similar pattern.

Mitochondria-rich cells have been previously noted in crotalid (Odor, '65; Warshawsky et al., '73) and viperid (Ben-Shaul et al.,

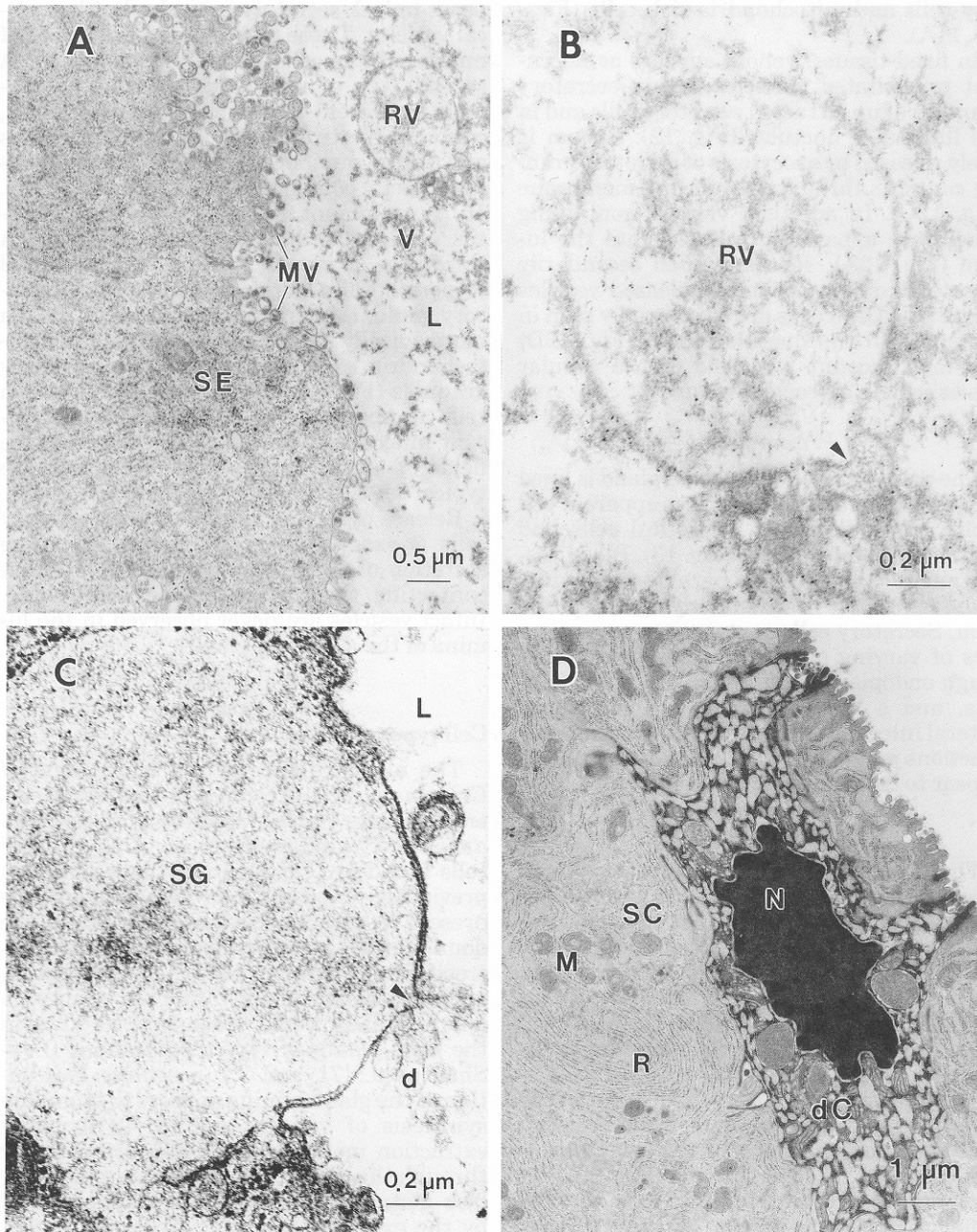


Fig. 12. Unextracted main venom gland; TEM of venom release. Precipitated venom (V) and released vesicles (RV) are seen in the ductule lumen (L). **A:** Released vesicle close to secretory epithelium ($\sim 1 \mu\text{m}$). MV, microvilli. **B:** Enlarged view of the vesicle in A. Note apparent secondary release of contents (arrowhead). **C:** Secretory granule at the cell apex releasing contents into the

lumen. Note dissolution of the conjoined granule and plasma membranes (arrowhead) and expelled droplets (d). **D:** Extracted main venom gland. A dead secretory cell, which is electron-dense and vacuolated, appears to be ready for sloughing into the ductule lumen. dC, dead cell; L, lumen; M, mitochondria; N, nucleus; R, rough endoplasmic reticulum; SC, secretory cell.

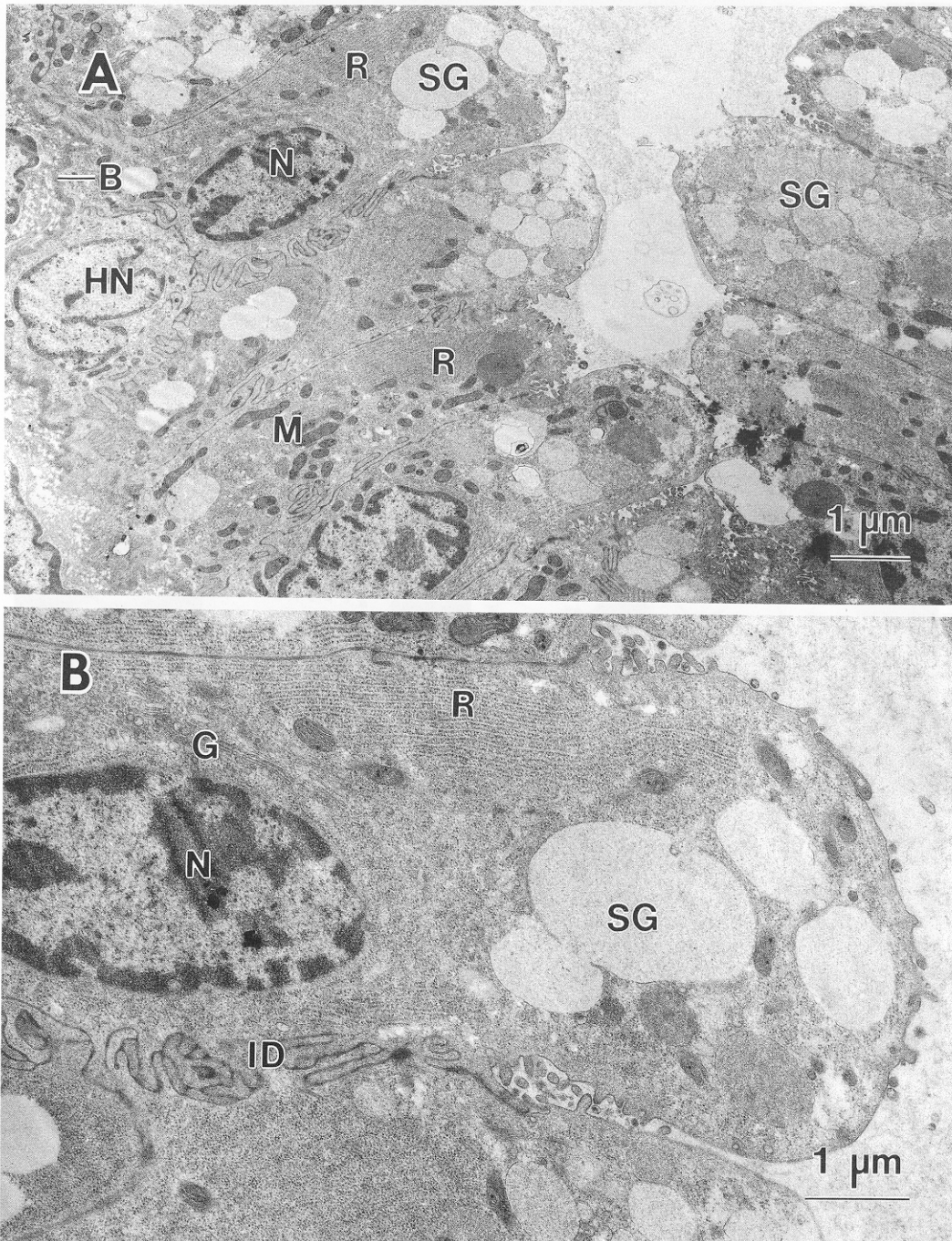


Fig. 13. Unextracted primary duct. TEM. **A:** Secretory cells are columnar and form a single layer joined laterally by interdigitations and desmosomes. Basal horizontal cells (HN) are also present. **B:** Enlargement of a

secretory cell in A. Rough endoplasmic reticulum (R), Golgi apparatus (G), mitochondria (M), and electron-opaque secretory granules (SG) are prominent. B, basal lamina; ID, interdigitations; N, nucleus.

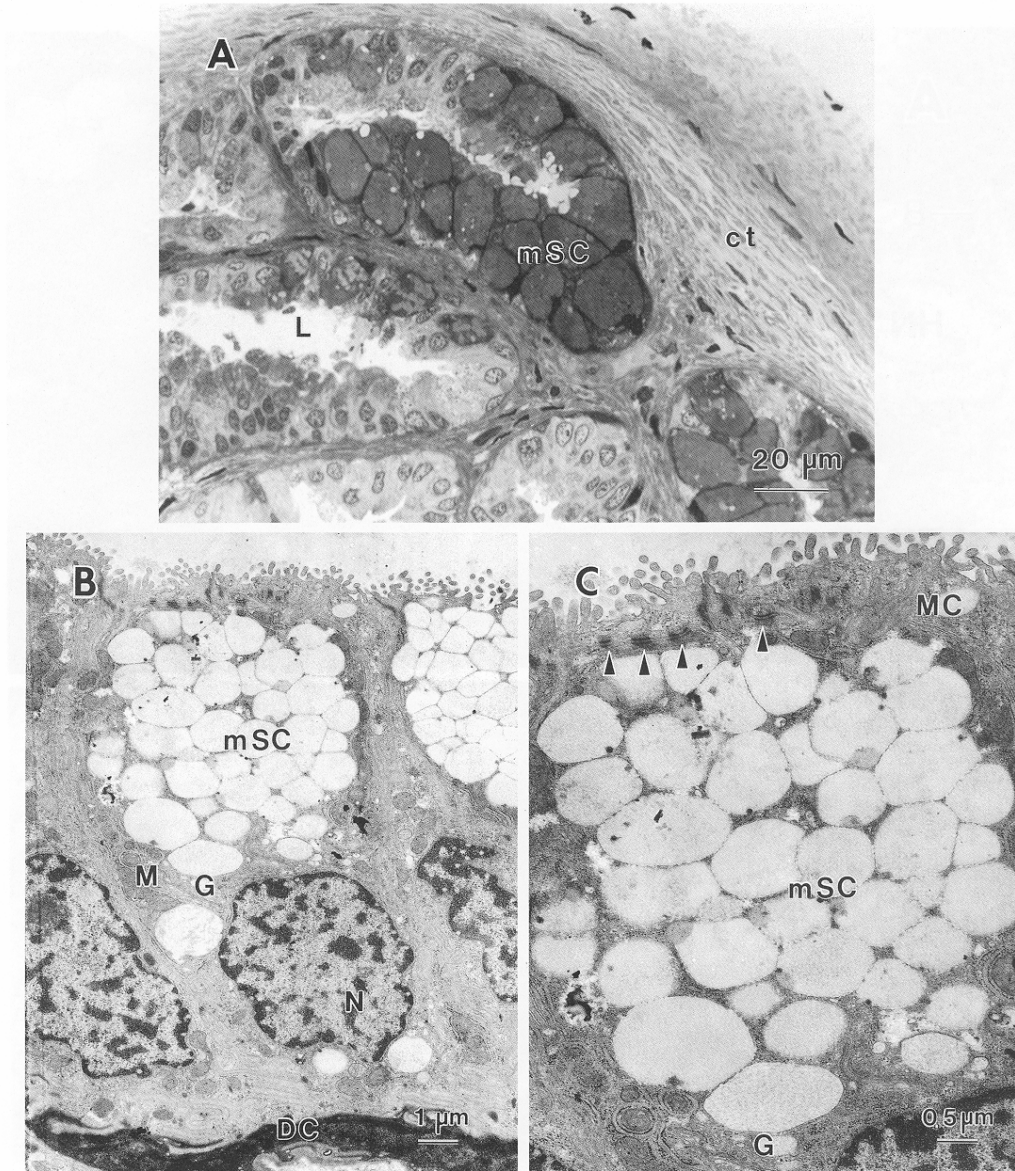


Fig. 14. **A:** Semithin section of unextracted accessory gland. Light micrograph. **A:** Ductules contain mucosecretory cells (mSC) but note that adjacent ductules do not always contain them. ct, connective tissue; L, lumen. **B,C:** Unextracted accessory gland showing relation of secretory cells and mitochondria-rich cells. TEM. **B:** Mucosecretory cells (mSC) are typically bordered by mito-

chondria-rich cells. In "immature" mucosecretory cells, the nucleus is rounded, and mitochondria (M) and Golgi apparatus (G) are apparent. Note dark cell (DC) at base. **C:** Detail of cell apex in B. Mitochondria-rich cells (MC) nearly cover the apical end of the mucosecretory cell and are connected via desmosomes (arrowheads).

'71) venom glands, and they are present in *C. v. oreganus* as well. In *C. v. oreganus* these cells are recessed below the secretory cells, and often appear to be in pits. The large number of mitochondria present suggests a

high energy capacity of these cells, but a role limited to water-resorption (Warshawsky et al., '73) seems unlikely because venom in mature secretory granules appears more electron-dense than released vesicles or free

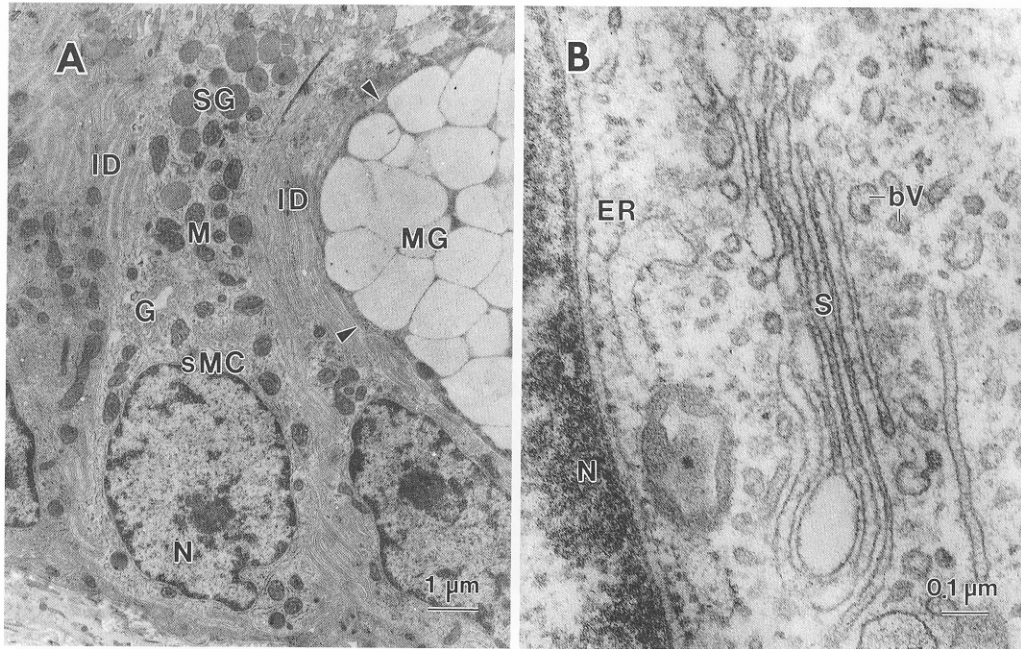


Fig. 15. Unextracted accessory gland in a region of "mature" mucosecretory cells. TEM. **A:** A mitochondria-rich cell containing secretory granules (SG) is seen immediately adjacent to a mucosecretory cell. Note the elaborate interdigitations (ID) between cells and the extremely thin layer of cytoplasm (arrowheads) of the mucosecre-

tory cell. **B:** Detail of Golgi apparatus in a mitochondria-rich cell. bV, budding vesicles stemming from Golgi cisternae; ER, endoplasmic reticulum; G, Golgi apparatus; M, mitochondria; MG, mucous granules; N, nucleus; S, saccules of Golgi membrane.

venom in the lumen. This suggests that dilution rather than concentration initially occurs in the lumen, followed by an increase in protein concentration of venom with time (as has been observed in *V. palaestinae*, Kochva, '78). Resorption of autolytically degraded venom constituents could be carried out by mitochondria-rich cells, but protein composition of venom contained in the gland appears to be stable in this species (Johnson, '87). Mitochondria-rich cell height also increases with the secretory cells during protein synthesis, yet they lack secretory granules. Since mitochondria-rich cells cycle with the secretory cells but do not produce an exported product (to the lumen), a supportive role in secretion seems probable.

Horizontal cells, as described by previous investigators (e.g., Warshawsky et al., '73), are also found in the venom gland of *C. v. oreganus*, but unlike those of the gland of *C. durissus*, the horizontal cells of *C. v. oreganus* lack phagosomes, microfilaments, and microtubules. It seems unlikely that these cells are similar to myoepithelial cells, since they lack contractile structures. Instead, they may serve as stem cells to replace dead secre-

tory cells. Dead secretory cells are sloughed into the ductule lumen (Fig. 12D), which may account for the turbid cellular debris present in venom (Gennaro et al., '68) which is most prevalent in adult snake venoms (Mackessy, '88). However, rough handling during extraction, mechanical damage during tissue preparation and incomplete fixation will greatly increase the extent to which cell fragments occur in venom and ductule lumina (pers. obs.).

A fourth cell type, the "dark" cell, has not been previously described in snake venom glands. On rare occasion, a dark cell has been seen extending from the basal lamina to the lumen (Fig. 11B), but such cells probably represent an altered functional state of a mitochondria-rich pore cell (based on the electron-dense cytoplasm), since long microvilli and numerous mitochondria were also present in the cell. Most frequently, dark cells are located in basal regions of the epithelium, just above the basal lamina, and have extremely long dendritic processes (Figs. 8, 9C) which interdigitate with several secretory cells. Intracellular filaments have not been observed, but small vesicles similar to the

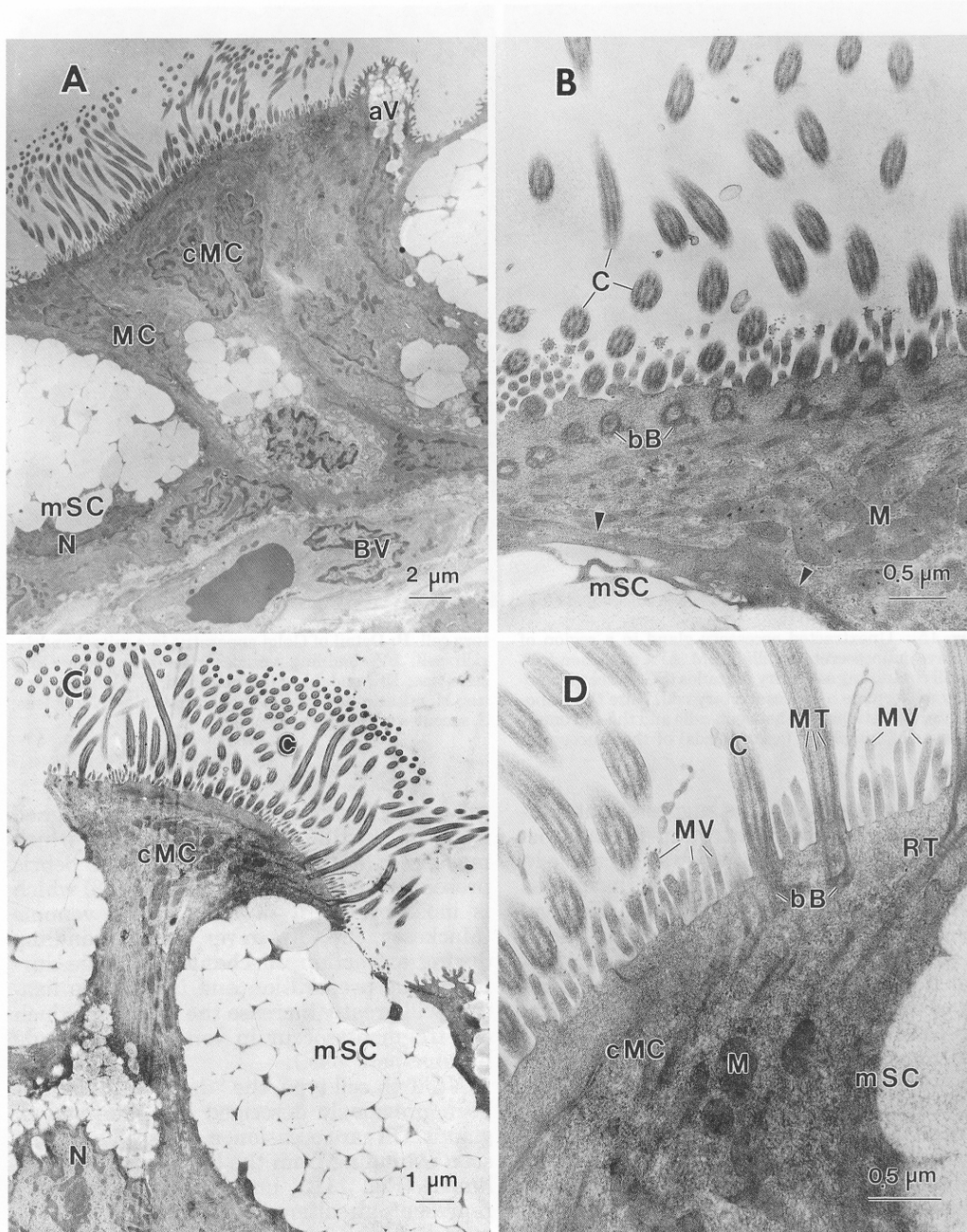


Fig. 16. Unextracted accessory gland showing ciliated mitochondria-rich cells found in some distal areas of anterior tubules. TEM. **A:** Relation of ciliated mitochondria-rich cells (cMC), non-ciliated mitochondria-rich cells (MC), and mucosecretory cells (mSC). **B:** Cross-section of cilia (C) at apical cell border. Basal bodies (bB) and 9 + 2 microtubule (MT) arrangement are apparent. The bound-

ary with mucosecretory cell cytoplasm is indicated by arrowheads. **C:** Ciliated mitochondria-rich cells (cMC) directly contacting mucosecretory cells. **D:** Details of the cell apex as in C. Note ciliary structures and the size relation between cilia and microvilli (MV). aV, apical vesicle; BV, blood vessel in connective tissue; M, mitochondria; N, nucleus; RT, rootlet of cilium.

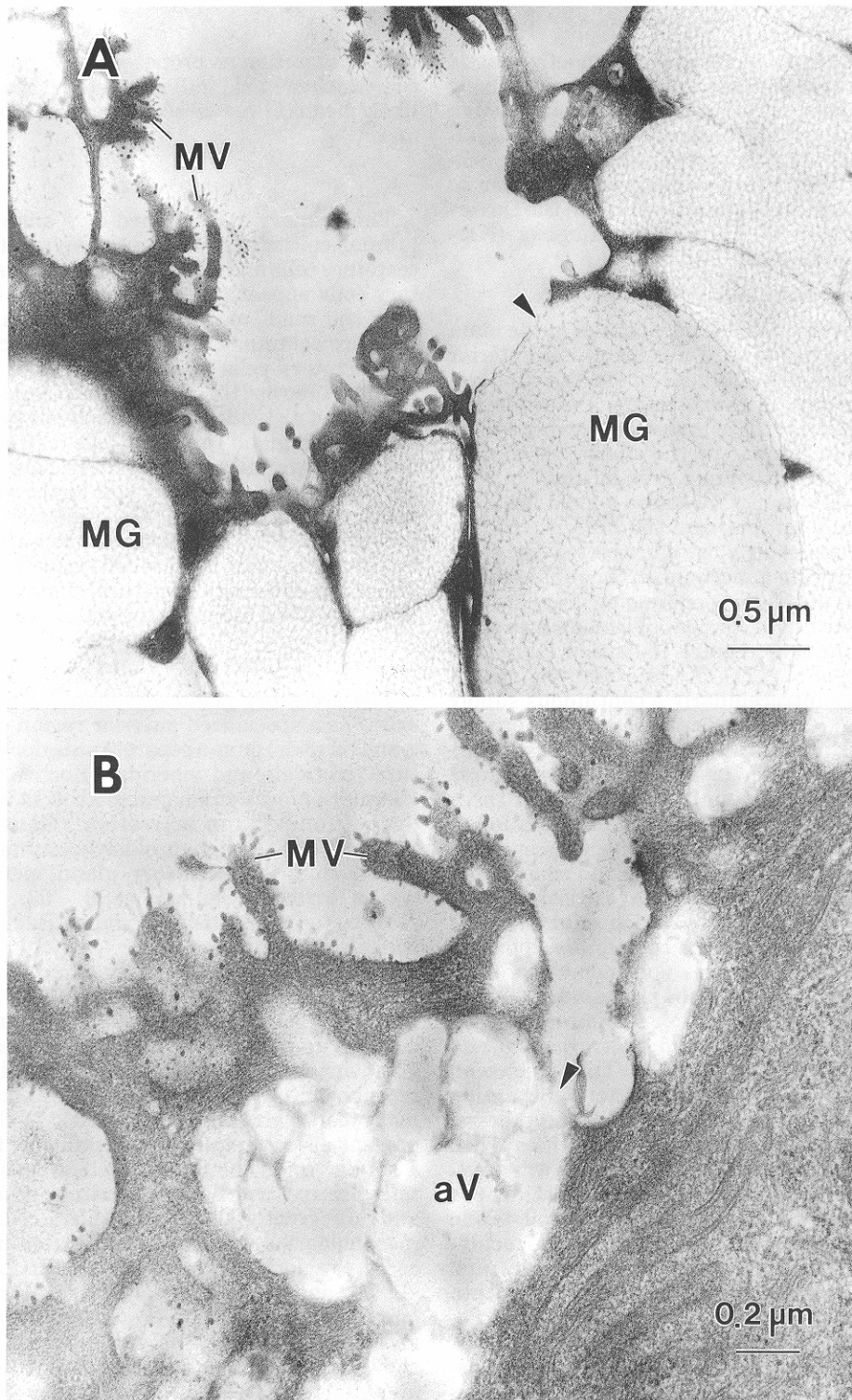


Fig. 17. Unextracted accessory gland showing release of secretory products. TEM. **A:** Release of secretory product from mucous granules (MG) occurs via disruption of the granule membrane contacting the plasma membrane

(arrowhead). **B:** Release of secretory product from the apical vesicles (aV) of the mitochondria-rich cells containing them appears to occur by the same process of membrane disruption (arrowhead). MV, microvilli.

micropinocytotic vesicles of smooth muscle cells are present. Dark cells seem more likely to be analogous to myoepithelial cells, though the dark cytoplasm precludes viewing of microfilaments or microtubules, if present; otherwise, they are morphologically similar to the myoepithelial cells observed in the Duvernoy's gland of *Thamnophis elegans* (Kardong and Luchtel, '86).

Secretory mechanism

The orientation of the ductules of the main venom gland toward a ventral and anterior main lumen facilitates venom injection via transduction of hydrostatic pressure during contraction of the compressor glandulae muscle. Most lumina in the unextracted state contain venom, while extracted gland lumina are usually empty. Released venom is stored in ductule lumina, and the presence of mature granules in most unextracted secretory cells suggests a mechanism for rapid replenishment of venom spent during prey capture. Complete replenishment of venom in an emptied gland may require 16 days or more (Rotenberg et al., '71), but by retaining mature granules intracellularly a continuous supply of venom can be maintained under normal circumstances. It would be of interest to determine whether or not small expenditures of venom (for example, bites to two or three prey) result in as dramatic a proliferation of the machinery for protein synthesis in secretory cells as does complete gland evacuation.

Differences in opinion have arisen regarding the mechanism by which secretory cells release venom into the lumen. Based on the presence of organelles and membrane fragments in venom within the lumen, an apocrine or holocrine type of secretion has been suggested for the crotalid *Aghistrodon piscivorous* (Gennaro et al., '68). This conclusion may have resulted from osmotic disruption of secretory cells during the fixation process, which produces much larger quantities of cell debris in the lumen. If 10% sucrose was omitted from buffer rinse after glutaraldehyde fixation, *C. v. oreganus* main gland tissue showed numerous fixation artifacts, including cell disintegration, with mitochondria and nuclei present in the lumen. Venom within ductules of unmilking main glands also inhibited penetration of fixative; some areas of a single piece of tissue showed evidence of osmotic shock (vacuolation, etc.), while others were sufficiently fixed to preserve fine details of the Golgi apparatus and the rough endoplasmic reticulum. Presently, an exocrine

type of secretion as proposed for *V. palaestinae* (Kochva et al., '80) seems to be the most likely means of venom secretion in *C. v. oreganus*.

Primary duct

The primary duct is lined by a simple columnar epithelium with only two cell types, secretory cells and horizontal cells. The secretory cells appear different from those in either the main or accessory glands in that they are columnar, in contrast to the cuboidal secretory cells of the unextracted main gland. Moreover, the rough endoplasmic reticula are not distended as in the actively synthesizing main gland secretory cells (extracted). Secretory granules are present in most cells, suggesting that they may add to the venom bolus during injection. The secretion may protect cells from hydrolytic venom enzymes, but a specific role for these cells is not known, other than forming a structural channel from the main gland lumen to the accessory gland.

Accessory gland

The accessory gland of venomous snakes exists as a specialized anterior region of the gland (elapids) or as a separate anterior structure (crotalids and viperids) (Kochva, '78). Its anterior location suggested a role in venom "conditioning" or activation (Gans and Kochva, '65). Although older accounts suggested that the accessory gland increases venom toxicity (Gennaro et al., '63), more recent studies refute this claim (Ruzic and Russell, '78).

Structurally, the accessory gland of *C. v. oreganus* shows numerous differences from the main gland epithelium. Though much smaller than the main gland, the accessory gland appears to contain a greater number of morphologically distinct cell types. Like the main gland, horizontal and "dark" cells are present in low frequency and probably fulfill a similar role. The mucosecretory cells contain electron-translucent secretory granules and are greatly distended; these cells are morphologically similar to those seen in the accessory gland of *Bitis arietans* (Hattingh et al., '84) and to the duct epithelial cells of the opisthoglyph colubrid *Rhabdophis tigrinus* (Yoshie et al., '82). This cell type has been shown to be PAS positive (Kochva, '78) and it is assumed that the cells in *C. v. oreganus* also synthesize mucopolysaccharides.

Mitochondria-rich cells are common in the regions containing mucosecretory cells, and

none resemble the recessed mitochondria-rich cells of the main gland. Three types are distinguished. One type contains electron-dense secretory granules, a basal nucleus, and does not appear to overlap the apical ends of mucosecretory cells. A second mitochondria-rich cell lacks granules, has an apically located nucleus, and nearly covers the apical surface of mucosecretory cells. This cell also contains apical vesicles which apparently release contents into the ductule lumen. Their close association with mucosecretory cells suggests that they may protect mucosecretory cells, perhaps from lytic venom components or from the secretory products of the first type of mitochondria-rich cell. These two mitochondria-rich cells may also represent different functional states of the same cell, because they are primarily distinguished by the presence or absence of electron-dense secretory granules. A third mitochondria-rich cell has an apical nucleus, is closely associated with and may cover mucosecretory cells, and has a dense cover of cilia.

Ciliated cells have not been described from snake venom glands. These cells are uncommon (< 1%) and are limited to distal portions of anterior tubules, and they may help distal secretions flow toward the central duct or mix locally released components. A structurally similar association of ciliated cells and mucosecretory cells occurs in mammalian lung bronchi (Frasca et al., '68).

The accessory gland of *Crotalus viridis oreganus* is structurally more complex than the main gland (in terms of cell types present), but its contribution to the bolus of venom injected must be relatively small compared to the contribution from the main gland. It appears to be adding some specialized component to the main gland product, but based on several different chemical analyses (Mackessy, unpub. data) there are no readily detectable biochemical contributions to venom. The structural complexity of the gland argues against the possibility that it exists as an "evolutionary remnant," but its biological role in venom production and deployment is unresolved.

The accessory and main venom glands of *Crotalus viridis oreganus* are structurally similar to those found in previously described viperid and crotalid glands. In the present study, several new cell types are described: dark cells in the main and accessory glands, which are possibly myoepithelial cells, and ciliated mitochondria-rich cells in the

accessory gland, which may mix/expel secretory components. No cytological differences were noted between glands from juvenile and adult snakes, although the chemistry of the venoms differs markedly (Mackessy, '88). Histochemical localization of several enzyme activities in the venom glands of the related subspecies *C. v. helleri* also did not distinguish specific subtypes of secretory cells (Schaeffer et al., '72a,b). Similarly, the glands of *C. v. oreganus* and *C. durissus* are structurally similar, but the chemistry and pharmacology of their venoms are quite different (Brazil, '66; Lomonte et al., '83; Mackessy, '88, unpub. data). The similarities between the dominant cell type, the secretory cells, in these two species demonstrate that morphological distinctions alone are not an accurate indicator of functional differences.

ACKNOWLEDGMENTS

I would like to thank Dr. Christine Davitt for assistance and advice on many aspects of this study. Dr. Kenneth V. Kardong performed gland removal surgeries, for which I am indebted. Dr. K.V. Kardong, Dr. John H. Larsen, and Dr. Jennifer A. Clarke provided many helpful comments concerning the manuscript. The Washington State University School of Veterinary Medicine prepared paraffin-embedded slides of glands, and their help is gratefully acknowledged. Comments of two anonymous reviewers are also appreciated.

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