

# Oral Cleansing in Spiders is Gland Mediated!

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Histochemical and micromorphological investigations of the gnathocoxal gland in spiders brought out its uniquely adaptive cleansing function. The prey particulates, caught in the oral microstructures, are bound with mucopolysaccharides or glyco-muco-proteins from the glandular secretions in *Araneus* and *Theridion* species into a bolus, and are expelled following each food uptake.

In the process of catching prey, paralysing, manipulating and kneading it, or sucking its contents, spiders dirty their mouthparts. After each food uptake, an outflow of clear liquid from a specialized gnathocoxal gland seems to be provisioned to clean the fine oral structures. The gnathocoxal gland was earlier thought to be digestive in function [1] or non-functional [2]. Histochemical and micromorphological investigations of this tiny (100–500 µm in cross-section) gland brought out largely cleansing function of its contents in both, prey kneading (*Araneidae*, etc.) and prey sucking (*Theridiidae*, etc.) [3] species. Oral architecture around the gland openings aids filtration of the predigested prey contents before reaching to the pharyngeal conduit, while contents of the gland seems to bind the remnants of unfiltered and undigested material into a bolus to be expelled with the help of gnathocoxae and chelicerae.

In *Araneus diadematus*, *A. quadratus*, *Theridion sisypium*, *T. impressum*, and several other species (*Theridula opulenta*, *Chiracanthium inclusum* (*Clubionidae*), etc.), the gnathocoxae are covered with bundles of partly or completely fringed hairs that seem to guide the prey contents to the mouth opening by capillarity, in addition to the suction provided by pharyngeal and alimentary muscles. The liquified food passes over an expanded pharyngeal sieve of combed filter structures and pores (Fig. 1). The pharyngeal conduit opens into oesophageal canal toward the midintestine. The contents of the intestinal diverticulae are pumped out and emptied into the prey contents, dissolving its tissue with enzymic activity and digesting extraintestinally prior to ingestion in spiders [4]. The pH of prey

haemolymph may itself contribute to the digestive optimum of the pH-specific proteases, carbohydrases, esterases, phosphatases and nucleases [5]. The excretion of digestive enzymes via oesophageal canal and ingestion of the mixed prey contents in dissolved and emulsified form follow an alternate cycle. The mixing of the two fluids is facilitated by the movement of gnathocoxae, a process that seems to create a haemostatic pressure on the inside gland with movement of the depressor, abductor and median and lateral adductor muscles of gnathocoxae. The gnathocoxal movement continues after food ingestion, to build up enough pressure for emptying the gland chambers through a series of valved openings. The number of chambers vary from 10–32 in *Araneus* and 4–10 in *Theridion*, depending on the species and age of the spider. The chambers open into a chitinous concavity (80 to 112 µm in *Araneus*) in the proximal one-third of gnathocoxa. Each opening is surrounded by fin-like, especially bizarre in *Araneus* species, cuticular foldings that trap kneaded particulates and facilitate uniform flow of the glandular secretion by reducing surface tension (Fig. 2).

The secretory chambers distend to 300 × 120 µm size as their epithelial cells pass through a cycle of, 1) formation of secretory vesicles, 2) their fusion and apical migration for excretion into the lumen by reverse pinocytosis, 3) cell reorganization and membrane retrieval, with the emptying and refilling of the gland. In the individuals, hungered for 10 days, the secretory cells are enlarged to a 50 × 11 µm size and are filled with 3 µm secretory vacuoles, their mitochondria and cytoplasm are dispensed aside, rough endoplasmic reticulum (rER) is collected around the wrinkled nucleus toward the base. The nucleolus is oval and large and the golgi-complex is indiscernible. Immediately after exocytosis, vesicula-

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tion from the enlarged golgi elements takes place. The provesicles appear to be pinched off along the concave side of golgi elements, enlarged into meso- and metavesicles through the accumulation of more secretion, and provided with membrane with their own fusion. Such vesicles are transported toward the supranuclear part. The appearance of parallel rER, large oval nucleus (electrooptically visible, heterochromatin-rich nucleus), movement of mitochondria away from the cell wall and visible golgi-complex are characteristic of the cells after 1 h of exocytosis (Fig. 3). The lumen of secretory chambers expands with the emptying of vesicles. As the gland is emptied, the membrane is recovered by pinocytosis. A similar sequence of processes has been observed in the exocrine cells of invertebrates (insects [6], snails [7]) and vertebrates [8]. The secretory cells are innervated with large, gliated (about 1 µm) basal and small glialess intercellular efferent neurons. The smaller axons enclose 50–100 nm electron-dense granular and 30–40 nm agranular vesicles, the former apparently neurosecretory, similar to those in the poison gland [9]. Each secretory cell in *Araneus* spp. is anchored against collapsing with a collaring accessory cell, rich in microtubuli and with its upper third connected through septate desmosomes to the secretory cell. Collection of the secretion from gnathocoxal gland for biochemical testing invariably contained the secretions from midintestine and labral glands, the function of which also poses a controversy. Histochemically, the gnathocoxal gland seems to provide substances, chiefly cleansing in function. The secretory vesicles in *A. diadematus* and *quadratus* appear to be full of mucopolysaccharides in an acidic aqueous medium (positive to Hale, gamma-metachromatic with toluidine-blue [10], blue with Specht and Mallory stains [11]), ultrastructurally similar to goblet cells (osmiophobic faint-grey, faint magenta to periodic acid Schiff reagent). Those in *T. sisyprium* and *impressum* appear to be glyco- or muco-proteinous and osmiophilic, similar to pan-

Fig. 1. Pharyngeal sieve in *Araneus diadematus* (X 35) with apical long bristles, fringed at the base (inset X 3960) (a), and combed pore borders (b, X 14436) associated with the filtering mechanism. The predigested food, reaching the food conduit is clear and the unfiltered particulates larger than 1 µm are trapped in the mesh, as shown in the cross-section of the covered food conduit (c). Scanning electron micrographs were taken with Cambridge Mark II SEM after double charcoal and single gold coating. Scale 50 µm.

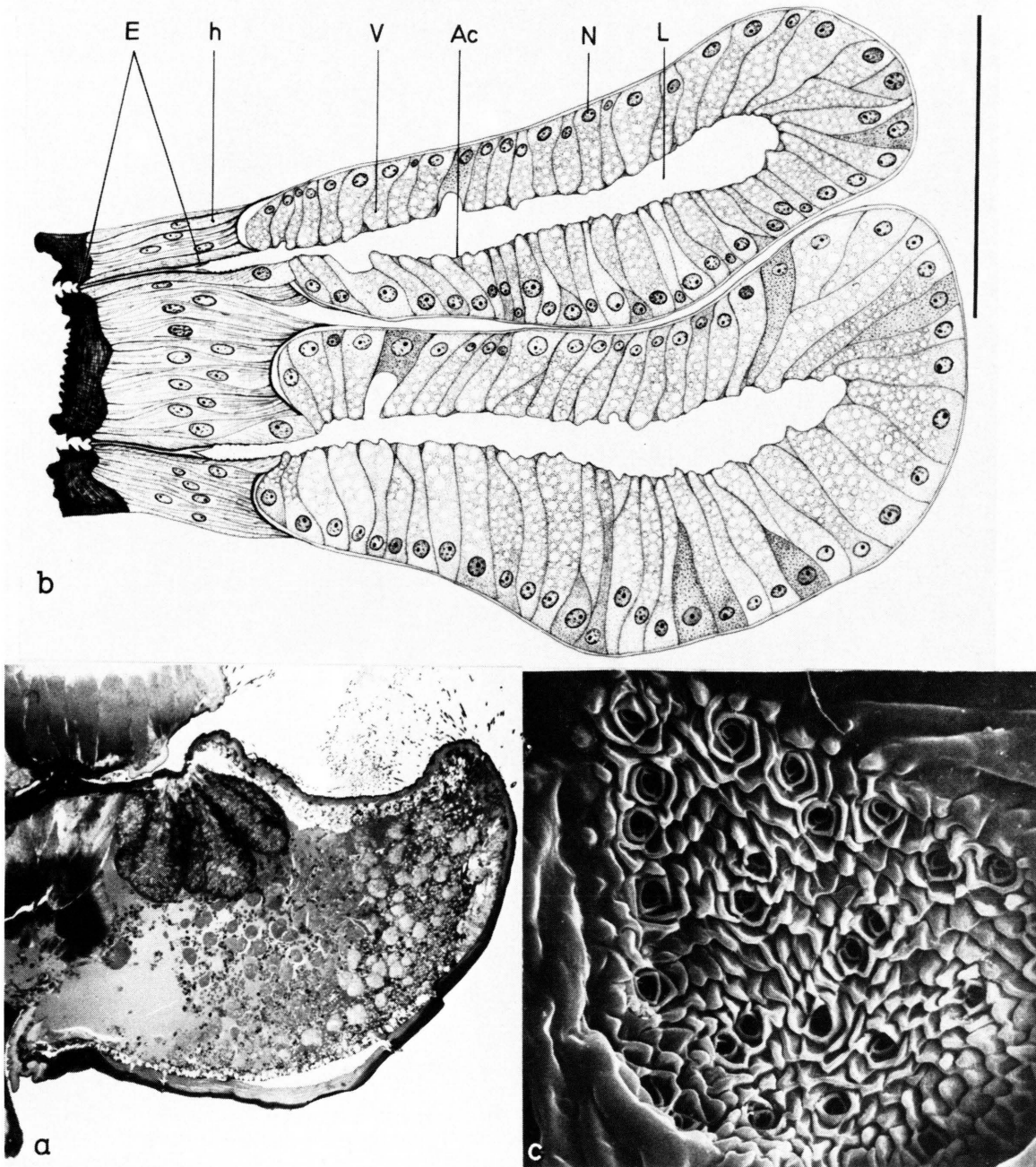


Fig. 2. (a) Long. section of *Araneus quadratus* gnathocoxa (X 73), showing the freely suspending gland chambers opening into a cuticular concavity in the oral opening. Median adductor muscles connecting the gnathocoxa to cephalothorax above the gland administer the movement of gnathocoxa and the flow of haemolymph toward the gland. Other musculature (not shown) is below the level of this gland. Note a large number of nephrocytes basally touching the gland chambers. (b) A serial section reconstruction of two secretory chambers of *A. quadratus* gland. Each chamber is enclosed in a basal-membrane and connected to the cuticle with hypodermal cells (h). The lumen opens through a bivalve canal in each case. The secretory cells are collared with accessory cells towards the lumen (Ac) and are interconnected with desmosomes; efferent innervations between the cells and at their bases are not shown. L-lumen, E-valve, N-nucleus, V-secretory vesicles. (c) Bizarre openings surrounded by fins in *A. diadematus* (X 756). As the gland is pressed, the secretion spreads between the fins and rises as a single droplet. Scale 100  $\mu$ m.



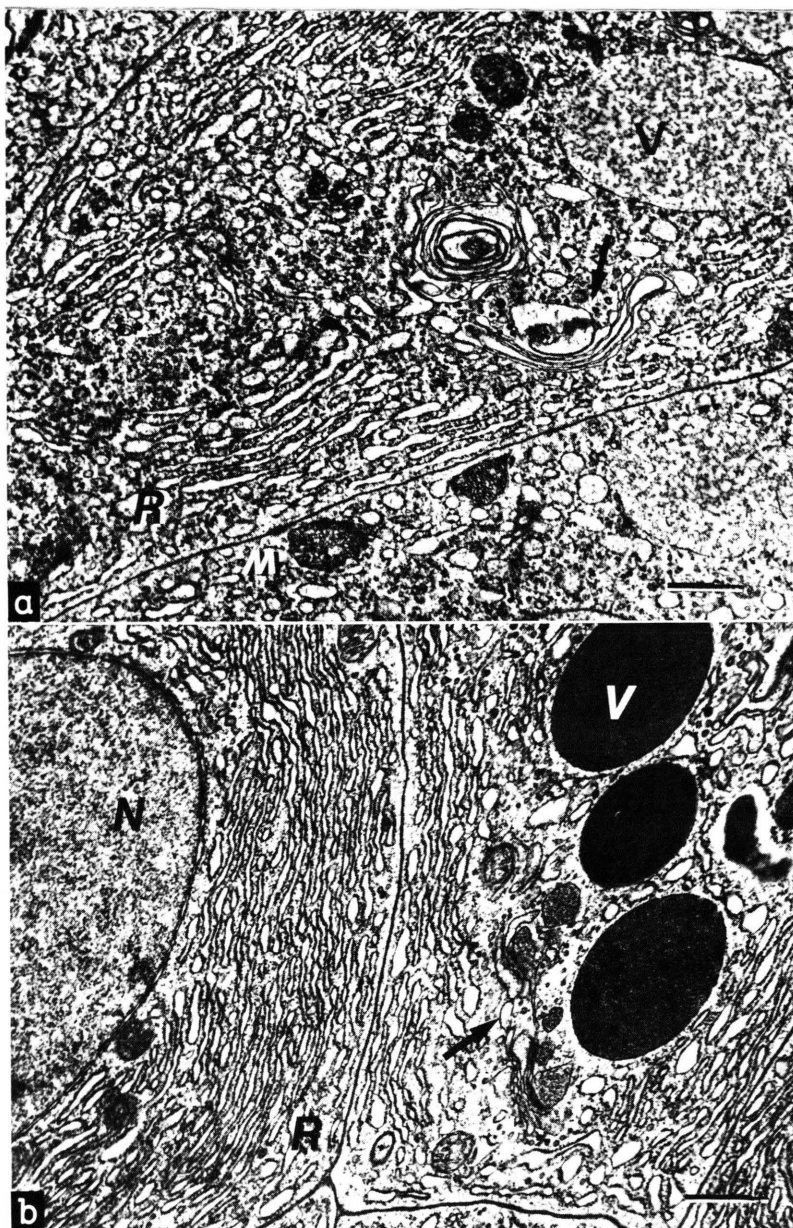


Fig. 3. Secretory cells in *Araneus diadematus* (a) and *Theridion sisypium* (b) (scale 1  $\mu$ m) after 1 h of exocytosis, prepared and stained identically for transmission electron microscopy with Philips TEM type UM 200. The formation of provesicles at the concave side of golgi elements (arrow), their fusion into meso- and meta-vesicles, parallel rER, dispersed mitochondria and oval heterochromatic nucleus; two distinct forms and ultrastructures are shown. V-vesicle, M-mitochondria, N-nucleus, R-rER. The gnathocoxa was dissected and fixed in 2%  $\text{OsO}_4$  and 0.1 M sodium cacodylate (pH 7.2) under 250 torr vacuum at 20 °C for 1 h, then at 4 °C under 760 torr for 2 h; it was dehydrated and block-contrasted in 0.1% phosphotungstic acid and 0.5% uranylacetate in 70% ethanol and embedded in styrol methacrylate. Polymerization was under 250 torr at 32 °C, 40 °C and 50 °C for 24 h each. Double staining with uranylacetate and lead citrate was done according to Mollenhauer [16]. Photographed at 60 KV and 30  $\mu$ m aperture blend. Semithin serial sections were fixed in  $\text{OsO}_4$  as above and stained with 1% toluidine blue in 1% borax soln. for light microscopy with Zeiss Ortholux photomicroscope (Fig. 1 c, 2 a).

creatic cells in ultrastructure [12]. A large number of nephrocytes accumulate at the base of the glandular chambers in the gnathocoxal haemolymph. The precursors may probably be obtained from the exchange of material from the nephrocytes, the proteins may be built at the ribosomes of rER and the sugars may be added immediately or during their transfer from rER to golgi elements [13]; golgi complex that appears immediately following exo-

cytosis may be actively involved in the build up of secretory vesicles.

The mode of feeding in spiders appears to be directly related to the morphology and function of their gnathocoxal gland and auxiliary oral architecture surrounding it. Those kneading their prey with enlarged chelicerae and serrulate gnathocoxae have a many-chambered gland; those sucking their prey contents through a single opening have a few-cham-

bered gland. The bristles, guiding the liquified food from outside to the oral cavity in araneids are long and coarse, the pharyngeal conduit is completely closed with the pharyngeal sieve and the marginal gnathocoxal (scopular) hairs are fringed for filtration. The glandular canals are provided with inner and outer valves against any reverse flow of oral fluids. In theridiids, the external setae are reduced, scopular hairs are smooth and adhered together in groups with their terminal grasping fringes, apparently providing an uniform flow of fluid to the pharyngeal conduit. The glandular canals are narrowed and curved to avoid the reversal of fluid flow. The outflow of glandular secretions may release the unfiltered chitinous tissue with their mucoproteinous or glycoproteinous and acidic activity, dissolve lipid particulates and facilitate the formation of expellable bolus, thus help cleansing the micro- and macrostructures in and around the oral opening. Such a bolus is worked for a prolonged period between the gnathocoxae and chelicerae, before it is finally dislodged by the spiders with the help of pedipalpal claws. Eventhough protease, peptidase, alfa-amylase,

chitinase/chitobiase, esterase/lipase, beta-N-acetylglucosaminidase activity of the digestive enzymes from midintestinal gland is sufficient for extraintestinal digestion, insufficient alfa-glucosidase, insignificant hyaluronidase and sulphate-breaking activity [5] may allow the mucopolysaccharide and glycoprotein to play their cleansing role. Part of the components (N-acetylglucosamine) may be further recovered during the next prey catch. The midintestinal epithelium in spiders, unlike insects, is devoid of a protective peritrophic membrane, thus the gnathocoxal gland serves an adaptive function. A supposition by Schimkewitsch [14], that the gnathocoxal gland may be "glandes muqueuses" seems to be appropriate in the light of present investigations. Whether the glandular contents may also improve thermoelectret properties of the spider's oral and tarsal sensilla during grooming [15] remains to be seen.

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