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THE FINE STRUCTURE AND FUNCTION OF THE ANTERIOR FOREGUT GLANDS OF *CYMATIUM INTERMEDIUS (CASSOIDEA: RANELLIDAE)*

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ABSTRACT

The fine structure of the salivary glands of *Cymatium intermedius* is described. It is concluded that both the posterior acid secreting and anterior acinous lobes are homologous with the salivary glands of other prosobranchs. At least six different types of secretion have been identified and their possible roles discussed. The anterior lobes secrete mucin and proteins believed to be enzymes, and the posterior lobes are specialized for acid production as well as secreting protein (probably a peptide toxin). The mechanisms of acid secretion and protection from damage to the snail before its release are also considered.

INTRODUCTION

Snails of the genus *Cymatium*, like other Ranellidae (Cymatiidae), Cassidae and Tonnidae (reduced to a subfamily of the latter by Riedel, 1995), are carnivores with a single pair of large salivary glands (Houbrick & Fretter, 1969), which attain relatively massive proportions in cassids (Hughes & Hughes, 1981). In all three groups they are made up of small anterior acinous lobes which secrete mucins and possibly other components, and voluminous posterior lobes, the blind-ending tubules of which secrete sulphuric acid (Wëber, 1927; Houbrick & Fretter, 1969; Fänge & Lidman, 1976: Hughes & Hughes, 1981).

Various names have been assigned to the glands by different authors, resulting in misidentification and doubt as to their homology throughout the superfamily and with those of other gastropods. Beu (1980) clearly defined the relatively small medium brown glands of *Tutufa* (Bursidae) as true salivary glands and the large thin-walled cream glands as accessory glands. Houbrick & Fretter (1969) described the glands in *Cymatium nicobaricum* (Röding, 1789) and *Bursa granularis* (Röding, 1789) as filling a large part of the haemocoel and having a few opaque white lobes anterior to the point of entry of the salivary ducts, but Morton (1990) overlooked their reference to the anterior lobes (the typical salivary glands), and took *Cymatium* and the ranellid *Linatella caudata*, which he examined, to be atypical in lacking additional paired acid-secreting glands. Day (1969) claimed that the anterior lobes in *Argobuccinum argus* open directly into the oesophagus, which is atypical of salivary glands, and that they have high amylase activity. She called the posterior lobes 'proboscis glands'—a term adopted by Hughes & Hughes (1981) in their account of *Cassis*. Wëber (1927) named the glands buccal glands in *Tonna* (as *Dolium*), and referred to the posterior lobes as acid glands and the anterior as accessory glands, but Riedl (1995) applied the term 'accessory salivary glands' to the posterior lobes. In this account the terms anterior (accessory) and posterior (acid) lobes are adopted.

There is little doubt that the secretions of these glands play a major part in feeding, though they may also be used in defence (Houbrick & Fretter, 1969). Bentivegna & Toscano (1991) subscribe to the view held by many that Cassoidea have a strong preference for echinoderms. There is no doubt that cassids use the strongly acid secretion of the glands for rapidly dissolving the spines and tests of echinoids (Hughes & Hughes, 1981), but the diet in different groups of ranellids is more variable (Riedel, 1995; Taylor, 1998, for reviews). Echinoids are included in the diet of some ranellids such as *Fusitriton* (Kohn, 1983) and they may also use acid to attack the skeleton of echinoderms. The shell of molluscan prey is not normally drilled by *Argobuccinum argus* (subfamily Ranellinae), despite evidence that the acid saliva is effective in etching shell (Day,

1969). Molluscs are an important element in the diet of the Cymatiinae (Houbrick & Fretter, 1969), but a report of shell drilling in natural conditions—that of Roughly (1925, cited by Hancock, 1960)—by *Monoplex (Cymatium) australasiae* Perry, which preys on oysters—has been denied by Laxton (1971). *Cymatium nicobaricum* attacks the soft tissues of other gastropods (Houbrick & Fretter, 1969), a method also used by *Linatella caudata* (Morton, 1990).

In other ranellids food may include polychaetes, bivalves, other gastropods or echinoderms (Laxton, 1971), and feeding may involve overcoming prey, gaining access to soft parts, and possibly partial digestion of food before ingestion, depending on the species (Riedel, 1995; Taylor, 1998, for reviews). *Argobuccinum* feeds on sabellid polychaetes and secretion of the 'proboscis glands' (posterior lobes of the salivary glands) is poured over the worm and the soft parts are partially digested in the tubes and pumped into the oesophagus (Day, 1969).

Members of the family Cassidae feed on echinoderms, all but the Tonninae on echinoids. Species of *Tonna* engulf holothurians whole without anaesthetizing (Kropp, 1982; Morton, 1991). Sulphuric acid from the enormous 'accessory salivary or proboscis glands' is believed to initiate digestion after ingestion.

It is therefore clear that the saliva may be used in different ways in different members of the superfamily and has effects not solely attributable to a mixture of sulphuric acid and mucins. A toxic component in the secretion of the posterior lobes of the salivary glands in *Argobuccinum*, was shown to narcotize the trochid *Oxystele variegata*, sabellariid polchaete *Gunnarea capensis* and echinoid *Parechinus angulosus* (Day, 1969), and Houbrick & Fretter (1969) found that *Cymatium nicobaricum* anaesthetized gastropod and bivalve prey with its saliva. Cornman (1963) isolated an unidentified salivary toxin from cassids, effective even at neutral pH, which had a powerful effect on the sea urchin *Diadema antillarum*, possibly by inactivating sensory receptors or their nerves. A highly potent peptide toxin with a molecular weight of ~7000Da has since been partially isolated from the saliva of *Cymatium (Monoplex) echo* by Shiomi, Mizukami, Shimakura & Nagashima (1994), and West, Andrews, McVean, Thorndyke & Taylor (1998) have demonstrated pharmacological activity in extracts of the glands from several species of *Cymatium*. Aqueous extracts of the posterior lobes of the salivary glands of *Charonia rubicunda* have been shown to induce instant paralysis of the starfish *Patiriella regularis* whilst extracts of the anterior lobes caused extension of the tube feet and eversion of the stomach (Endean, 1972, quoting from Laxton's thesis, 1968). However, the only salivary toxin so far identified in a ranellid is tetramine in *Fusitrition oregonensis* (Asano & Itoh, 1960).

No digestive enzymes have been found (or reportedly tested for) in the saliva of *Cymatium* (Houbrick & Fretter, 1969), but Day (1969) reported an amylase in the proboscis glands (posterior lobes of the salivary glands) in *Argobuccinum*. Jenkins (1955) cited by Fretter & Graham (1994) found an amylase in the salivary glands of *Littorina littorea* but the occurrence in a carnivore must be viewed with some scepticism. Houbrick & Fretter (1969) believed that digestive enzymes were more likely to be restricted to oesophageal or digestive glands, as in naticids, a view partially supported by Day's finding (1969) of two proteases in the mid-oesophageal gland of *Argobuccinum*. The presence of proteases in the saliva of *Cymatium* is nevertheless suggested by the fluid or partially digested state of tissues soon after contact with the secretion.

The general anatomy of the gut of ranellids has been described by Houbrick & Fretter (1969) and Riedel (1995), and the account of the gut of *Tonna (Dolium) galea* by Wëber (1927) gives some histological background. Nüske (1973) described the fine structure and cell cycle of the acid glands in the cassid *Galeodea* (as *Cassidaria*) *echinophora* but the ultrastructure of the glands is unknown in other groups. This deficiency, together with the confusion over nomenclature and interpretation, summarized by Hughes & Hughes (1981), prompted the present ultrastructural study of the salivary glands of the tropical Indo-Pacific species *Cymatium intermedius* (Pease, 1889). This species is known to feed on bivalves in the laboratory (Houbrick & Fretter, 1969, as *pileare*). The objectives are also to correlate as far as possible the fine structure of the various cell types with their secretory and protective mechanisms and with the reported properties of the saliva in ranellids.

MATERIALS AND METHODS

Cymatium intermedius was collected from a fringing reef at Fort Kam, Oahu, Hawaii, dissected after anaesthetizing in equal parts of 7.5% magnesium

chloride and sea water, and fixed in 3% glutaraldehyde in 0.1M Sörensen's phosphate buffer at pH 7.2 containing 14% sucrose. Following dehydration in ethanol material for scanning electron microscopy (SEM) was critical-point dried and coated in gold. It was examined in a Hitachi S2400 or S3100 microscope. Specimens for transmission electron microscopy (TEM) were post-fixed in 1% osmium tetroxide in the same buffer as the primary fixative and embedded, after dehydration, in epoxy resin. Sections 0.5μm thick were stained in 1% toluidine blue in 1% borax for optical microscopy, and gold sections were stained in alcoholic uranyl acetate and Reynolds' lead citrate for examination in a Zeiss 109 transmission electron microscope.

RESULTS

Anatomy and histology

The description by Houbrick and Fretter (1969) of the foregut and associated organs in *C. nicobaricum* is applicable to other species of *Cymatium*. The asymmetrical salivary glands lie on either side of the mid-oesophageal gland; the larger left has been removed in Figure 1. The glands are anchored to the body wall and mid-oesophagus by muscles, and receive a blood supply from a branch of the anterior aorta. They are covered by a sheath in which connective tissue fibres are sparse, the most prominent feature being richly innervated muscle fibres, and others form a reticulum around individual tubules. Many nerve fibres in the extensive subepithelial blood spaces cross the basal lamina and make intimate contact with the gland cells. The long salivary ducts (Figure 1A) extend from the antero-ventral faces of the posterior lobes of the glands (Figs 2A and 4A), which lie behind the nerve ring in the cephalic haemocoel, to the buccal cavity at the tip of the pleurembolic proboscis. Each main salivary duct receives two ducts from the anterior lobes close to their origin before passing through the nerve ring (Figs 1C and 2A). The ducts lie parallel to the anterior oesophagus to which they are bound by muscles, and open into the buccal cavity behind the paired serrated jaws dorso-laterally, on either side of the dorsal food groove as is typical for ducts of the acinous salivary glands in prosobranchs. They have a well developed sheath of circular and longitudinal muscle fibres, indicating peristaltic activity.

The massive posterior lobe of each gland (Figure 1A) is composed of slightly curved radially arranged blind-ending branched tubules (Figs 2B, 3A,B and 4D) arising from a main tubule. In glutaraldehyde-fixed specimens the lobe is a lilac-tinted translucent mass covered on its dorsal surface, and to a limited extent ventrally, by a brown-speckled superficial layer which is due to undifferentiated and protein-secreting epithelial cells in the blind ends of the branches (Figure 3A). The branches have a small lumen difficult to distinguish in sections or scanning electron micrographs (Figure 4D, E), but that of the main tubule is broader, and about the same diameter as the salivary duct into which it leads.

The origin of the duct is marked by a change in epithelium from the tall glandular ciliated cells of the tubule (Figs 3C and 4A, C) to low non-secretory cells bearing prominent cirri (Figs 2A and 4B) and by the openings of the ciliated ducts of the anterior lobes. A group of dilator muscles is anchored on the connective tissue sheath at this point (Figure 1C), where there is a also slight narrowing of the lumen (Figure 2A). Contraction of the muscles would help to prevent possible occlusion of the duct by the action of such other muscles as those of the body wall.

A pair of opaque cream anterior lobes borne on the two branches of the main salivary duct, and enclosed within the same capsule as the posterior lobe, spread over the anterior end of the latter. They are intimately connected to the mid-oesophagus by muscular attachments (Figs 1A,B and 2A). The lobes are branched acinar glands typical of the salivary glands of other prosobranchs (Fretter & Graham, 1994). The acini (Figs 2A and 3D) are small in diameter by comparison with the tubules of the posterior lobe (Figure 4C-E), each with its lumen filled by long cilia (Figure 8A,B). The ducts of these lobes are ensheathed in muscle fibres like the main salivary duct, but differ from it in being lined by columnar protein-secreting cells interspersed with ciliated cells.

Fine structure

The posterior lobes

There is a gradual transition in the appearance of the epithelial cells along the length of the branches of the tubules, and they can be arbitrarily divided into four main groups: undifferentiated, protein-synthesising, protein-secreting, and acid-secreting (Figs 2-5).

The undifferentiated cells (Figs 3A and 5A-C) have large nuclei and scant cytoplasm, and they lie in clusters of two or three in corners of the blind ends of the tubules. They are surrounded by cells synthesizing a secretion rich in proteins, with well developed stacks of granular endoplasmic reticulum and Golgi bodies close to a large nucleus in the basal cytoplasm which also accommodates mitochondria. Nerve fibres are frequently associated with the basal plasma membrane which is slightly infolded. The cytoplasm above the nucleus is filled with secretory vesicles with electron dense contents. The apical cell membrane bears both short microvilli and cilia.

Further along the tubules the secretory vesicles of the cells coalesce, the secretion becomes paler, more diffuse and heterogeneous with lighter patches amongst the dark, suggesting that the secretion may have more than one component. The fused vesicular membranes are reduced to a network of fine strands bathed in secretion in a large central vacuole (Figs 3B, 4E, 6A-C and 7A-C).

Secretion is shed into the tubular lumen in apical blebs which, when shed, leave pores in the membrane (Figure 4G). Release of secretion coincides with signs of degeneration in the organelles of the

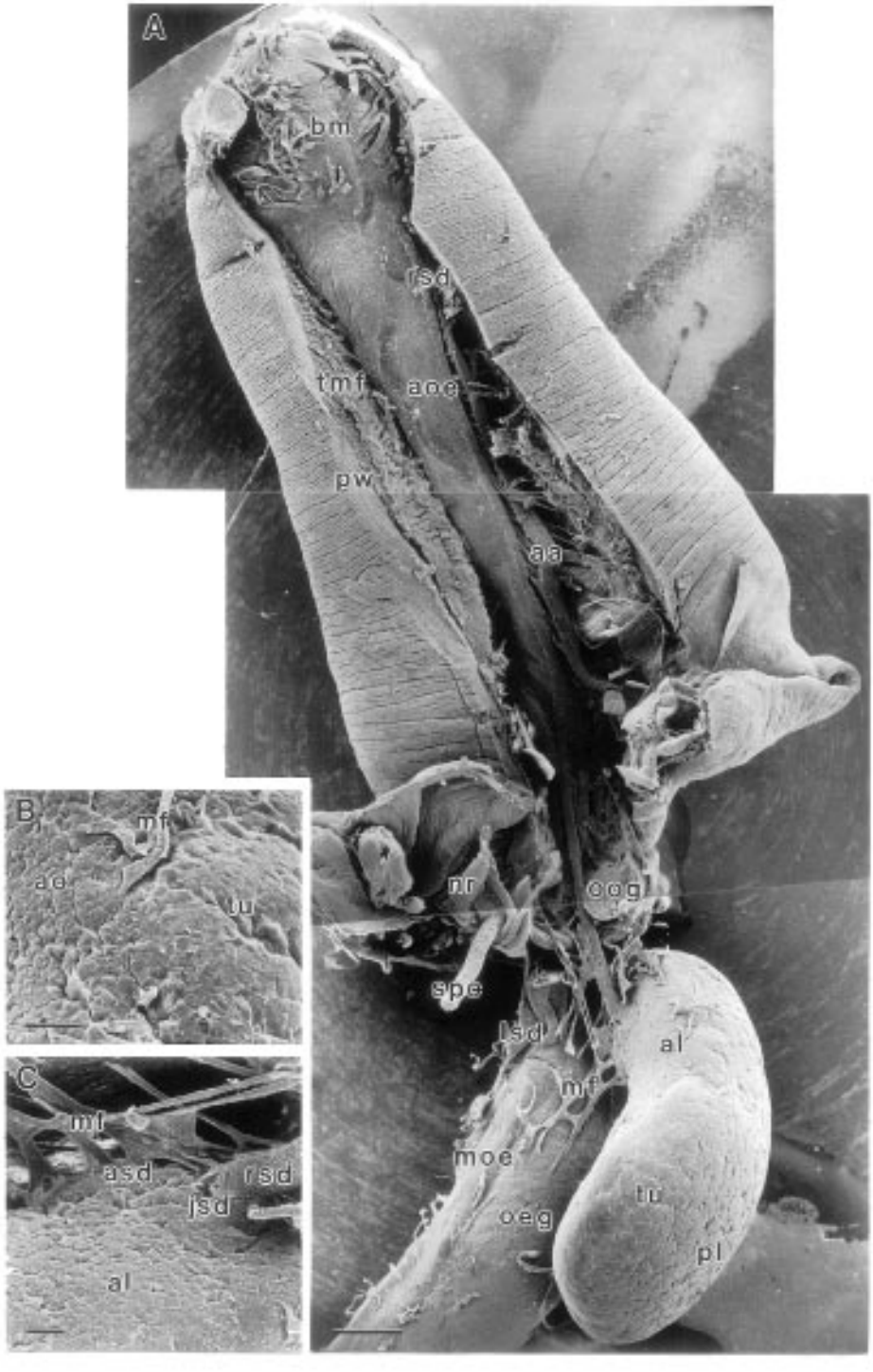
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Figure 1. A. Scanning electron micrograph (SEM) of the extended pleurembolic proboscis and foregut of *Cymatium intermedius* displayed by a mid-dorsal cut. The larger left salivary gland has been removed and the nerve ring cut. Scale bar represents 500μm. **B.** Detail of the surface of the salivary gland showing small acini of an accessory lobe, with attached muscle, and larger tubules of the posterior lobe. Scale bar represents 100μm. **C.** Duct of accessory salivary gland and its junction with the main duct. Scale bar represents 100μm. aa, anterior aorta; ac, acinus; al, anterior lobe (accessory salivary gland); aoe, anterior oesophagus; asd, duct of accessory gland; bm, buccal mass; ccg, cut cerebral ganglion; jsd, junction of salivary ducts; lsd, left salivary duct; mf, muscle fibres; moe, mid-oesophagus; nr, nerve ring; oeg, mid-oesophageal gland; pl, posterior lobe of salivary gland; ps, proboscis sheath; pw, proboscis wall; rsd, right salivary duct; spc, supra-oesophageal connecive; tmf, tensor muscle fibres; tu, tubule.

basal cytoplasm (Figure 6C,D), and lysosome-like vesicles with membranous and finely granular contents lie close to the nucleus.

These changes mark the transition from protein-secreting to acid-secreting cells and are accompanied by an increase in the height of the cells in the larger branches of the tubules and a narrowing of the apical region into a long neck as they are compressed around the small tubular lumen (Figure 4E). In the broader straight main tubule the cells are not contorted and assume a normal columnar form (Figure 4C).

In these cells the basal plasma membrane has deep infoldings (Figure 7C) which extend into a narrow peripheral zone of cytoplasm surrounding a central vacuole with a membranous reticulum like the last stage of the protein secreting cells, but in which the contents of the vacuoles are electron-lucent except for a few small bead-like granules which are not membrane-limited (Figure 7E). Examination of the whole glands confirmed that the vacuoles were fluid-filled, and that the fluid corresponded to the acid secretion of the glands. The only structures in the peripheral cytoplasm are prominent bundles of cytoskeletal fibres parallel to the long axes of the cells (Figure 6C), and channels (Figure 5A inset) and vesicles which in places contain some of the electron-dense granules (Figure 7E and F). Some of this material can be seen in SEM and TEM at the openings of pores in the vacuolar membranes (Figs 4F–H and 7F). Similar granules appear to pass from the vacuole to the lumen of the gland by small channels in the apical cytoplasm and through what may be permanent pores amongst the long microvilli of the apical cell membrane. The electron-lucent fluid escapes from the vacuoles through large ruptures in the apical membranes (Figure 4F). Some secretion (the lilac fluid in whole glands) is free in the lumen of the main tubule and the salivary duct, and in SEM appears flocculent (Figure 4A–C). Apical microvilli are devoid of a glycocalyx and the cilia are unusual in being coiled like a spring, which straighten when extended (Figure 3C). The only conspicuous organelles in both basal and apical cytoplasm are ovoid mitochondria with many cristae and dense particles (Figure 7D–F). The nucleus is small and compressed in the lateral cytoplasm against the plasma membrane (Figure 6B).

In any one cell an area of the membrane lining the central vacuole or apical channels may be covered by an exceptionally electron dense layer on its luminal or vacuolar surface (Figs 6F and 7D), and is often associated with the granular secretion. In mammalian oxyntic cells too, the membranes of the tubulovesicular system are dense when actively secreting acid (Cross & Mercer, 1993).

The most obvious explanation of this sequence of changes in the nature of the epithelium is a migration forwards coupled with change of function of the cells from the inner ends of the tubules. It strongly suggests that there is only one type of cell, continually replaced from undifferentiated cells in the blind ends and passing through an early protein-secreting to a later acid-secreting stage. The oldest cells are those lining the main tubule, which are little more than acid-filled sacs, the apical membranes of which are extensively ruptured during release of secretion. There is no evidence to suggest that they undergo membrane cycling such as that in oxyntic cells (Forte & Machen, 1987), or that they are replaced by young cells in that region. This interpretation of the cell cycle agrees with that of Wëber (1927) in his account of *Tonna* (as *Dolium*), and is based on examination of specimens which were not feeding when fixed. It is therefore impossible to know whether or not the cells undergo several cycles of activity within the two phases (protein secretion and acid production) of their life span, as described by Nüske (1973). He believed that the acid cells of *Galeodea* (*Cassidaria*) undergo repeated cycles, and re-develop protein-synthesizing organelles within a few hours after feeding.

The shallow epithelium of the main salivary duct appears to be highly specialised in two respects: possession of large strap-like cirri (Figs 2A and 4B), and the infolding of the basal membranes associated with mitochondria, indicative of ion transport.

Anterior lobes

The acini of these lobes are lined by three types of cell, one being protein-secreting, a second mucous, and a third ciliated (Figure 8). In thick resin sections the mucous cells clustered in the acini are the most conspicuous, being metachromatic in toluidine blue, as is characteristic for acid mucopolysaccharide (glycosaminoglycan). The cells have basal nuclei and are filled with secretory vesicles in which polymerized chains of macromolecules are visible at high magnification (Figure 8E). The most abundant cell type in the necks of the acini is protein secreting, characterized by the great density of the granular endoplasmic reticulum which ramifies throughout the cytoplasm amongst secretory vesicles in which

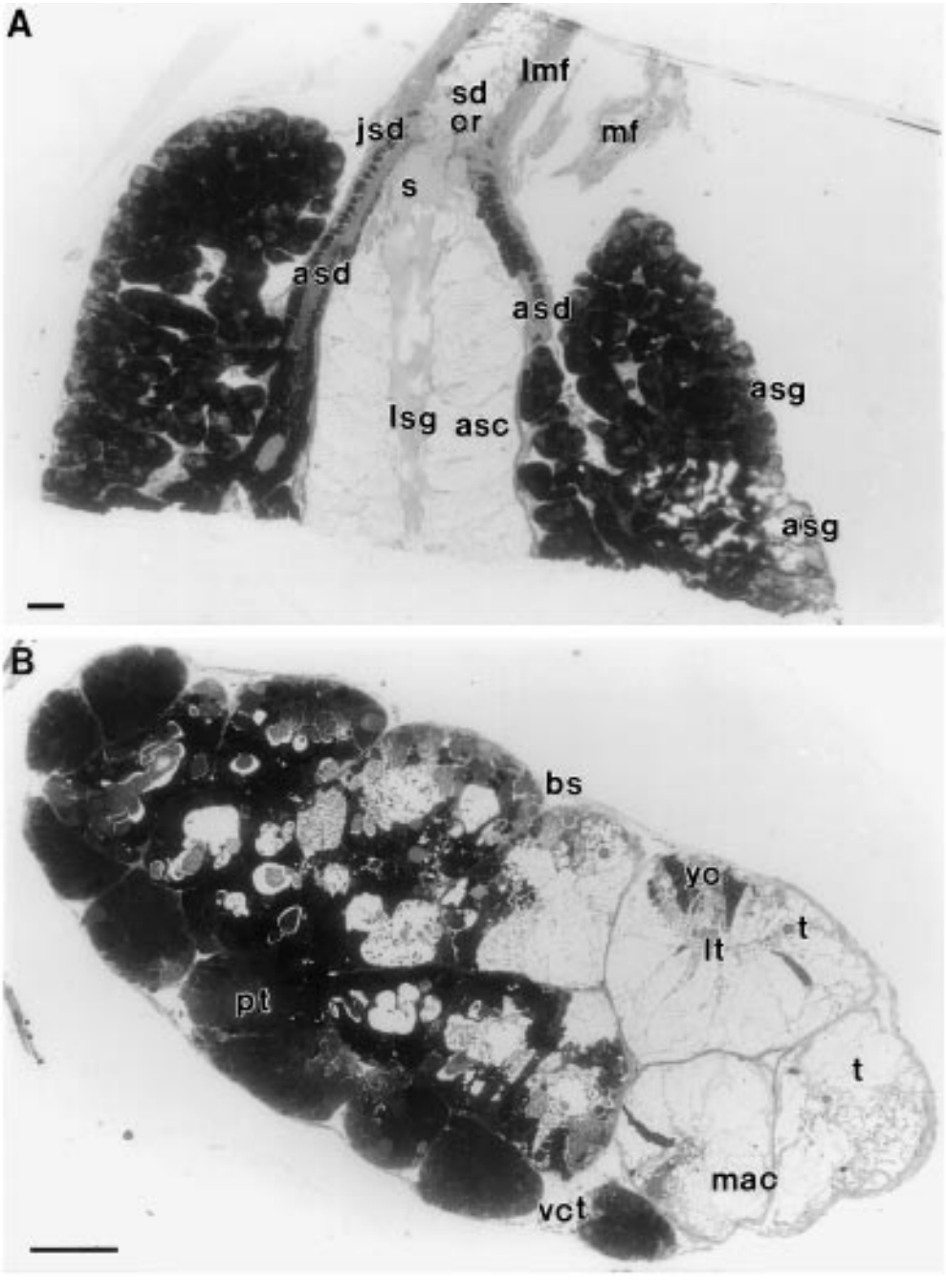
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Figure 2. 0.5μm-thick resin sections of the left salivary gland of *C. intermedius*, stained in toludine blue. **A.** L.S. of part of the main tubule of the posterior lobe surrounded by two anterior (accessory) lobes, the ducts of which open into the main salivary duct (just out of plane of section). Scale bar represents 100μm. **B.** Oblique section of the posterior lobe of the salivary gland showing darkly stained blind ends of tubules lined by protein-secreting cells, and light acid-secreting cells further along the tubules. Scale bar represents 100μm. asc, acid-secreting cells; asd, ducts of accessory lobes; asg, acinous accessory lobes; bs, blood space; cr, cirri; jsd, junction of salivary ducts of anterior and posterior lobes; lmf, longitudunal muscle fibres; lsf, lumen of main tubule of posterior lobe of salivary gland; lt, lumen of tubule; mac, mature acid cells; mf, muscle fibres; pt, protein secretion; s, secretion; sd, salivary ducts; t, tubule; vct, vascular connective tissue; yc, young cells.

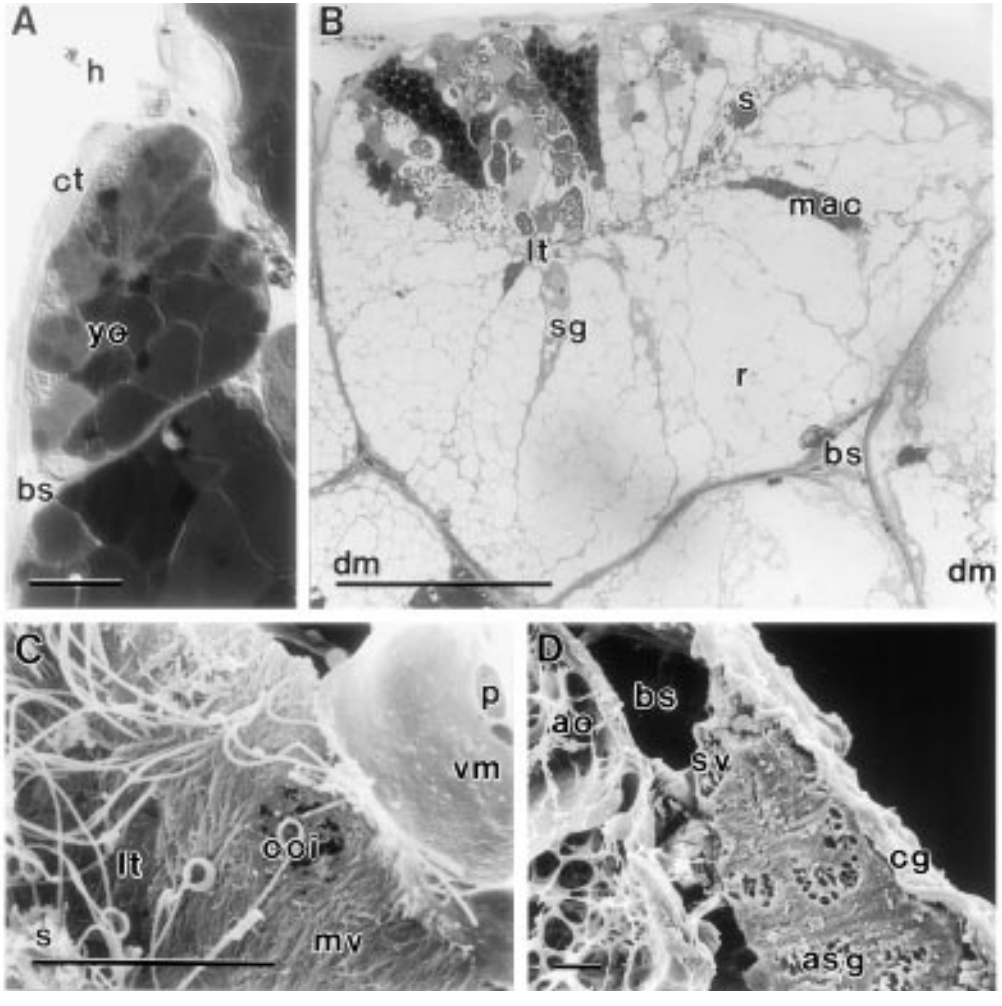
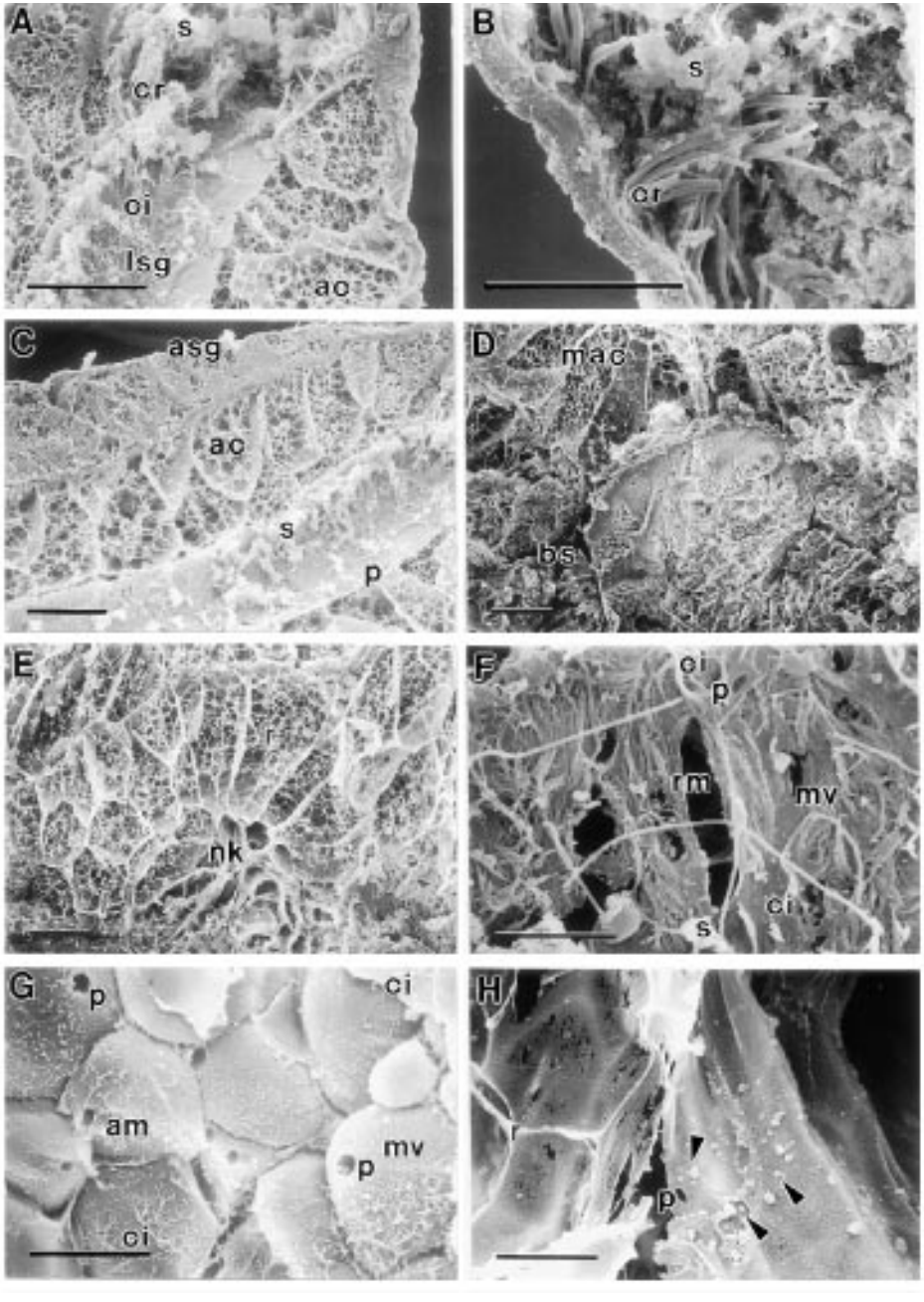


Figure 3. **A** and **B:** Detail of tubules of posterior lobe of salivary gland in 0.5μm thick resin section. **A:** blind end of tubule with undifferentiated and protein-secreting cells, photographed using Nomarski optics; scale bar represents 10μm. **B:** Larger branch of tubule showing transition from protein-secreting to acid-secreting cells; scale bar represents 100μm. **C:** SEM of mature acid cell in main tubule of gland showing long microvilli and coiled cilia on apical membrane; scale bar represents 10μm. **D:** part of accessory gland overlying acid cells of posterior lobe; scale bar represents 10μm. ac, acid cell; asg, accessory salivary gland; bs, blood space; cci, coiled cilium; cg, capsule of gland; ct, connective tissue; dm, dense membrane of acid cell; h, haemocoel; lt, lumen of tubule; mac, mature acid cell; mv, microvilli; p, pore; r, membranous reticulum; s, secretion; sf, secretory granule; sv, secretory vesicle; vm, vacuolar membrane; yc, young cell.



the dense contents clump to one side, giving them a patchy appearance often seen in glycoprotein secretions (Figure 8A,D). T-shaped ciliated cells are interspersed between the gland cells, their long cilia filling the small lumen of an acinus.

The ducts from the anterior lobes are lined by a columnar epithelium with another type of protein-secreting cell in which the secretory granules are uniformly electron-dense (Figure 9A,C). These cells are interspersed with ciliated cells (Figure 9B). Cells in different phases of the cell cycle occur simultaneously in both the acini and duct cells, the vesicles becoming larger and uniformaly denser until they obscure other organelles.

Figure 4. SEM parasagittal section of posterior lobe of salivary gland. **A.** Junction of main tubule of posterior lobe and main salivary duct; scale bar represents 50μm. **B.** Part of main salivary duct showing stout cirri and secretion in lumen; scale bar represents 50μm. **C.** Part of main tubule of posterior lobe and anterior lobe (accessory gland); scale bar represents 50μm. **D.** Branches of tubules of posterior lobe, with acid cells top left and protein-secreting cells bottom right; scale bar represents 50μm. **E.** Branch of tubule cut across showing membranous reticulum in central vacuoles and narrow necks; scale bar represents 50μm. **F.** Apical membranes of acid cells in main tubule showing long microvilli, pores and ruptures; scale bar represents 5μm. **G.** Apical membranes of younger acid cells in branch of tubule from same specimen as F, with short microvilli and pores; scale bar represents 5μm. **H.** Vacuolar membrane of acid cell showing, left, disintegration of separate vesicular membranes, and right, lateral membrane with bead-like granules (arrowheads); scale bar represents 5μm. ac, acid cell; am, apical membrane; asg, acinous lobe of gland; bs, blood space; cci, coiled cilium; ci, cilium; cr, cirri; lsg, lumen of main tubule of posterior lobe; mac, mature acid cell; mv, microvilli; nk, neck; p, pore; r, reticulum of vacuolar membrane; rm, ruptured apical membrane; s, secretion.

DISCUSSION

The ability to produce a strongly acid secretion imposes on animals a series of problems concerning its safe handling and storage. These are most severe where the acid is secreted from acid glands into an internal cavity where it is active, as in the stomach of vertebrates. The problems are considerably simpler in *Cymatium* and related genera, where the acid is forcibly discharged externally and only the gland and ducts have to be protected. Less rigorous precautions may be permissible in naticids and muricids, where parts of the body are still in contact with a site where a much weaker external acid is active. They are least acute in such prosobranchs as cypraeids and many lamellariids, and in some opisthobranchs where the acid is stored in epidermal cells as defence, and may never be used, as predators quickly learn to avoid such animals.

Nevertheless, in all these animals the ultrastructural features associated with acid secretion suggests that they use similar cellular mechanisms in acid production. Interpretation is complicated by the fact that the cells are often also involved in synthesis of other secretions, and in *Cymatium* saliva they interact with those from other types of cells in a potent cocktail. The observations described here lend support to earlier behavioural and experimental evidence as to the complexity of this secretion in ranellids.

The composition of the saliva

There is evidence from the work of Houbrick & Fretter (1969), Day (1969), Shiomi *et al.* (1994), and West *et al.* (1998), on *Cymatium* and other ranellids that the secretion of the salivary glands is a mixture of at least six constituents of which the major is sulphuric acid, others being a chelating agent contributing to dissolution of a calcareous test, at least one paralytic toxin, enzymes for external digestion of prey tissues, and mucus which may help to protect the epithelium of the salivary ducts and stop rapid diffusion of toxin from the target site in the prey.

There is no doubt that the posterior lobes are the site of acid secretion, and the anterior lobes of mucus secretion, but only tentative proposals can be made about the origin and uses of the other constituents. The possibilities are as follows.

(1) Chelating agent

Day (1969) showed that the acid secretion of *Argobuccinum argus* was 33% more effective in dissolving $CaCO_3$ than 0.47N H_2SO_4 alone (the calculated concentration in the saliva), and whilst both secretion of the accessory salivary glands and 0.4–0.6N acid eroded shells of *Macoma* 1N acid did not, because the precipitation of $CaSO_4$ created a barrier (which would also be so with echnoderm tests). She concluded that the greater efficiency of the saliva might be attributable to a chelating agent. Such an agent has since been found in the secretion of the accessory boring organ (ABO) of muricids (Carriker, 1981). In *Urosalpinx cinerea* the pH of the ABO secretion is 3.8–4.1 (Carriker, Van Zandt & Grant, 1972, cited by Carriker & Williams, 1978), but the saliva of *Cymatium* is pH2 (Houbrick & Fretter, 1969), and the rapid formation of an insoluble salt may make a chelating agent even more important. A non-protein chelating agent would not be packaged in membrane-bound vesicles resulting from normal secretory processes, and is more likely to be synthesized by specialized membranes. The only product of the salivary glands which fulfils these criteria is the bead-like granular material produced by the acid cells.

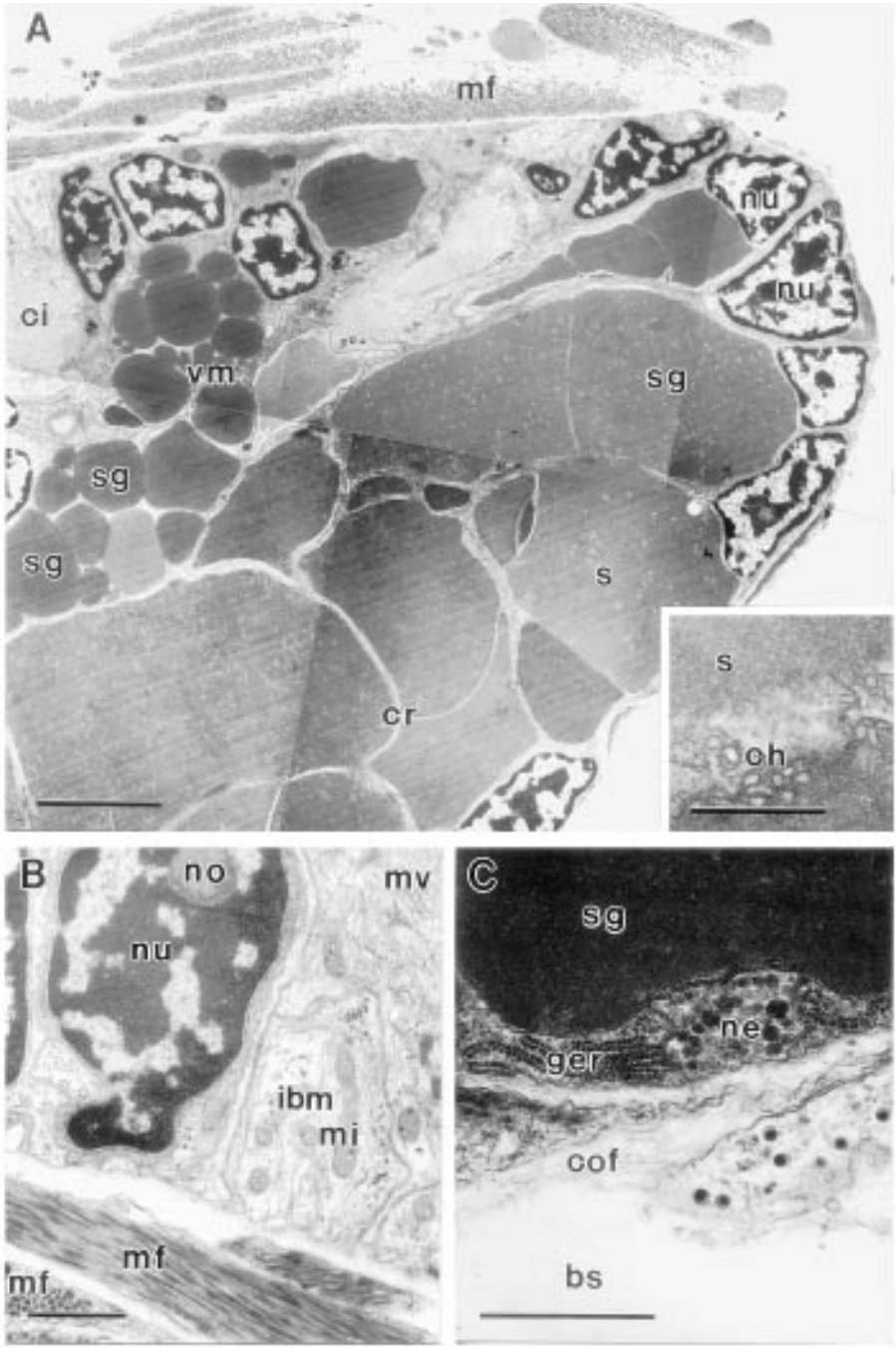

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Figure 5. Transmission electron micrographs (TEM) of blind end of tubule of posterior lobe of salivary gland; scale bar represents 5μm. **Inset:** part of lateral vacuolar membrane showing channels in peripheral cytoplasm; scale bar represents 0.5μm. **B.** Detail of undifferentiated cell (left) with large nucleus and little cytoplasm; scale bar represents 1μm. **C.** Detail of basal region of young protein-secreting cell with adjacent nerve ending; scale bar represents 1μm. bs, blood space; ci, cilia; ch, channels in peripheral cytoplasm; cof, collagen fibres; cr, membranes coalescing to form reticulum; ger, granular endoplasmic reticulum; ibm, infolded basal membrane; mf, muscle fibres; mi, mitochondrion; mv, microvilli; ne, nerve ending; no, nucleolus; nu, nucleus; s, secretion; sg, secretory granules; vm, membrane of secretory vesicle.

(2) Protein secretions

Three different types of serous cells have been identified in the salivary glands of *Cymatium*, one in the posterior lobes, and two in the anterior, the probable functions of which are secretion of toxins and enzymes.

(a) A peptide toxin

The isolation by Laxton (1971) of a paralytic toxin from the posterior lobes of the salivary glands of the triton *Charonia rubicunda* suggests that the protein-secreting cell type in the blind ends of the posterior lobes may be the source of a similar, suspected toxin, in *Cymatium*. In *Conus* the source of the peptide toxins is the venom gland, probably a homologue of mid-oesophageal glands of other prosobranchs (Ponder, 1970, 1973; Fretter & Graham, 1994, and Taylor, 1998 for reviews). The source of a salivary toxin in *Nucella lapillus* is the pair of tubular glands which, like the salivary glands of cymatiids and venom gland of cones, are ectodermal in origin (Ball, Taylor & Andrews, 1997). The main component in *Nucella* is serotonin, but there remains a biologically active constituent yet to be identified.

Laxton (1968, cited by Endean, 1972) isolated another biologically active agent from the anterior lobes of the glands in *Charonia*. The most likely source of a comparable component in *C. intermedius* is the serous cell type of the salivary ducts. Protein-secreting cells are a secondary feature in the epithelium of salivary ducts, and their relatively superficial position would favour almost immediate contact with prey tissues when the snail strikes, before the secretions of the acini are released.

(b) Enzymes

This leaves the protein-secreting cells of the anterior acinar lobes as the probable source of enzymes. Reports that *Argobuccinum* and *Cymatium* partially digest prey externally (Day, 1969; Houbrick & Fretter, 1969) is a strong indication that the saliva contains a proteinase, though Day's report of an amylase is surprising, especially as the very low pH of the saliva (~2) is optimal for pepsins but not for other enzymes. The mid-oesophageal proteases Day identified are unlikely to be involved in external digestion as there is no evidence that they could be regurgitated. Furthermore, the acinous salivary glands of the neogastropod *Murex* are known to secrete proteolytic enzymes (Mansour-Bek, 1934, cited by Fretter & Graham, 1994).

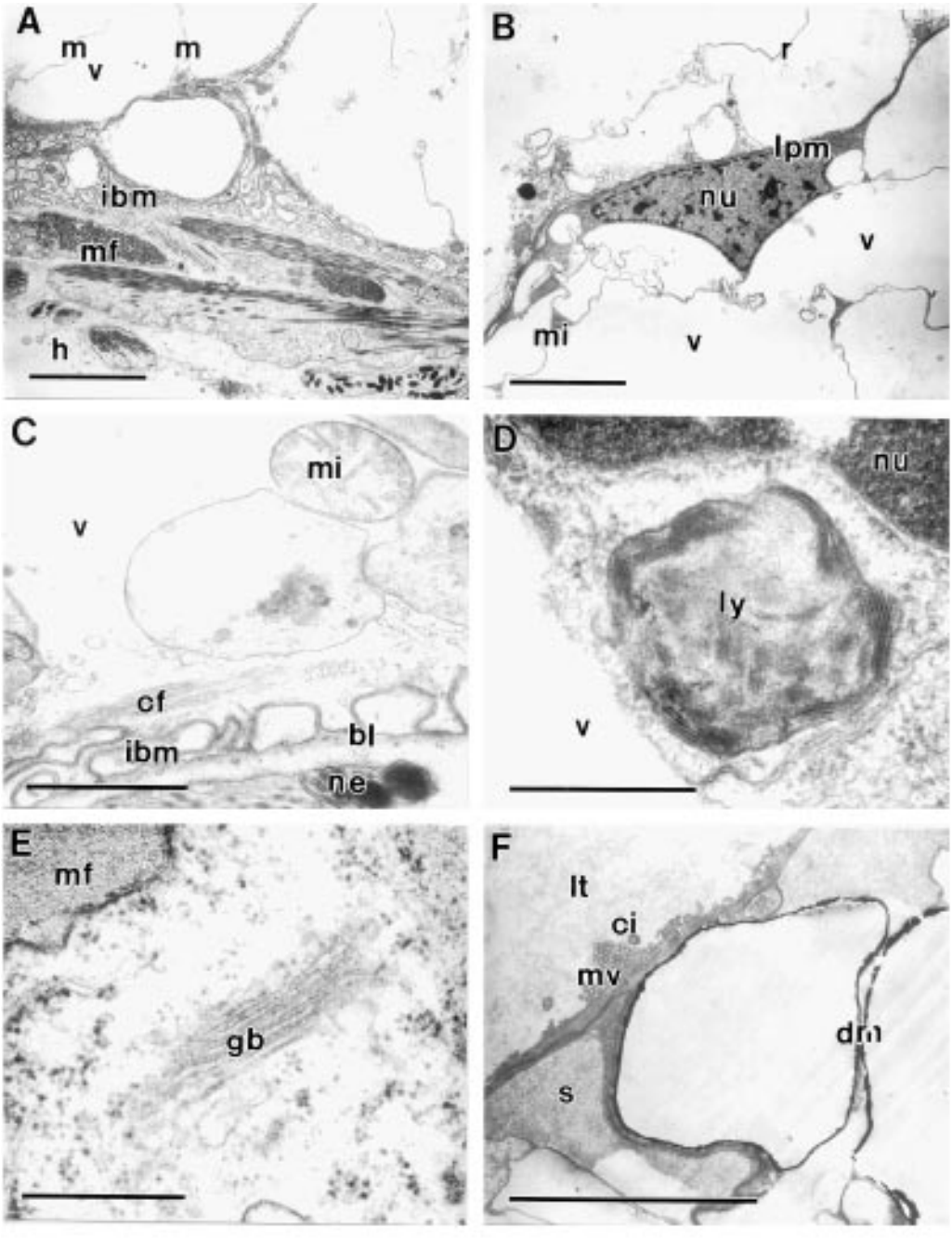
Since the cells of the gastric glands of lower vertebrates simultaneously secrete acid apically and protein (pepsinogen) basally (Noaillac & Depeyre, 1978; Fänge & Grove, 1979), the possibility remains that a component of the serous cells of the posterior lobes is a pepsinogen. Since a pepsinogen released into acid in the lumen of the gland could become an active pepsin and attack its tissues this seems unlikely. By contrast, in the cells of the anterior lobes the secretory granules are sequestered and remain in membrane-bound vesicles until the saliva is expelled, as digestive enzymes are.

Enzymes attacking the shell matrix have already been implicated in boring in naticids and muricids (Carriker & Williams, 1978; Carriker, 1981), but Day's experiments (1969), and strong evidence that cassoids are not normally shell drillers makes it improbable that any exist in ranellids.

Evolution and feeding strategies

On the basis of the arguments given above it would appear that the salivary glands of ranellids form two discrete functional units which reflect their structural subdivision, the anterior lobes representing unmodified acinar salivary glands, secreting mucus and enzymes, and the posterior lobes being specialized for offence: paralysis of prey, dissolution of the purely calcareous and delicate skeleton of echinoderms, and provision of a medium for the first stage in protein digestion of prey externally. Their increasing specialization for acid production in other members of the superfamily suggests a trend towards specialization for feeding on echinoids.

Acid secretion by skin glands (presumably



modified mucous cells) for defence has evolved independently in several different lines of gastropods. In naticids and muricids, the cells of the ABO are also specializations of the skin. The cassoideans are unique in that all but the Personidae produce and store large quantities of strong acid internally. Whilst salivary glands, like skin glands, are ectodermal, it is highly unlikely that an arrangement so specialized would have evolved primarily for defence.

Creation of an acid medium necessary for a peptidase seems to be the most likely primitive

Figure 6. TEM of acid cells in different stages. **A.** Subepithelial connective tissue and basal cytoplasm showing infolding of basal plasma membrane and strands of reticulum in central vacuole; scale bar represents 5μm. **B.** The small nucleus of an acid cell in the lateral cytoplasm; scale bar represents 5μm. **C.** The basal region of degenerating acid-secreting cell showing cytoplasmic fibrils; scale bar represents 1μm. **D.** A lysosome-like vesicle close to the nucleus of a cell losing its protein-synthetizing organelles and developing the features of an acid-secreting cell; scale bar represents 5μm. **E.** Golgi body in late stage of protein-secreting cell; scale bar represents 1μm. **F.** Apical region of an acid cell showing (active) dense membrane forming part of the reticulum lining central vacuole; adjacent areas surrounding secretion lack dense membrane; scale bar represents 5μm. bl, basal lamina; cf, cytoplasmic filaments; ci, cilium; dm, dense membrane; gb, Golgi body; h, haemocoel; ibm, infolded basal plasma membrane; 1pm, lateral plasma membrane; lt, lumen of tubule; ly, lysosome; m, part of membrane; mf, muscle fibre; mi, mitochondrion; mv, microvilli; ne, nerve fibre; nu, nucleus; s, secretion in vacuole; v, vacuole.

role of the acid glands in Cassoidea, with their later specialization and hypertrophy in cassids for dissolution of echinoid tests. The ranellid foregut lacks the complexity of that of *Tonna* which possess a 'Delle Chiaje' organ in the anterior oesophagus (Wëber, 1927), a probable specialization for the digestion of holothurians swallowed whole (Morton, 1991). All these facts support the idea that ranellids are the most primitive of the three groups, and that *Tonna* is further removed from the cassids than Riedel's (1995) classification implies.

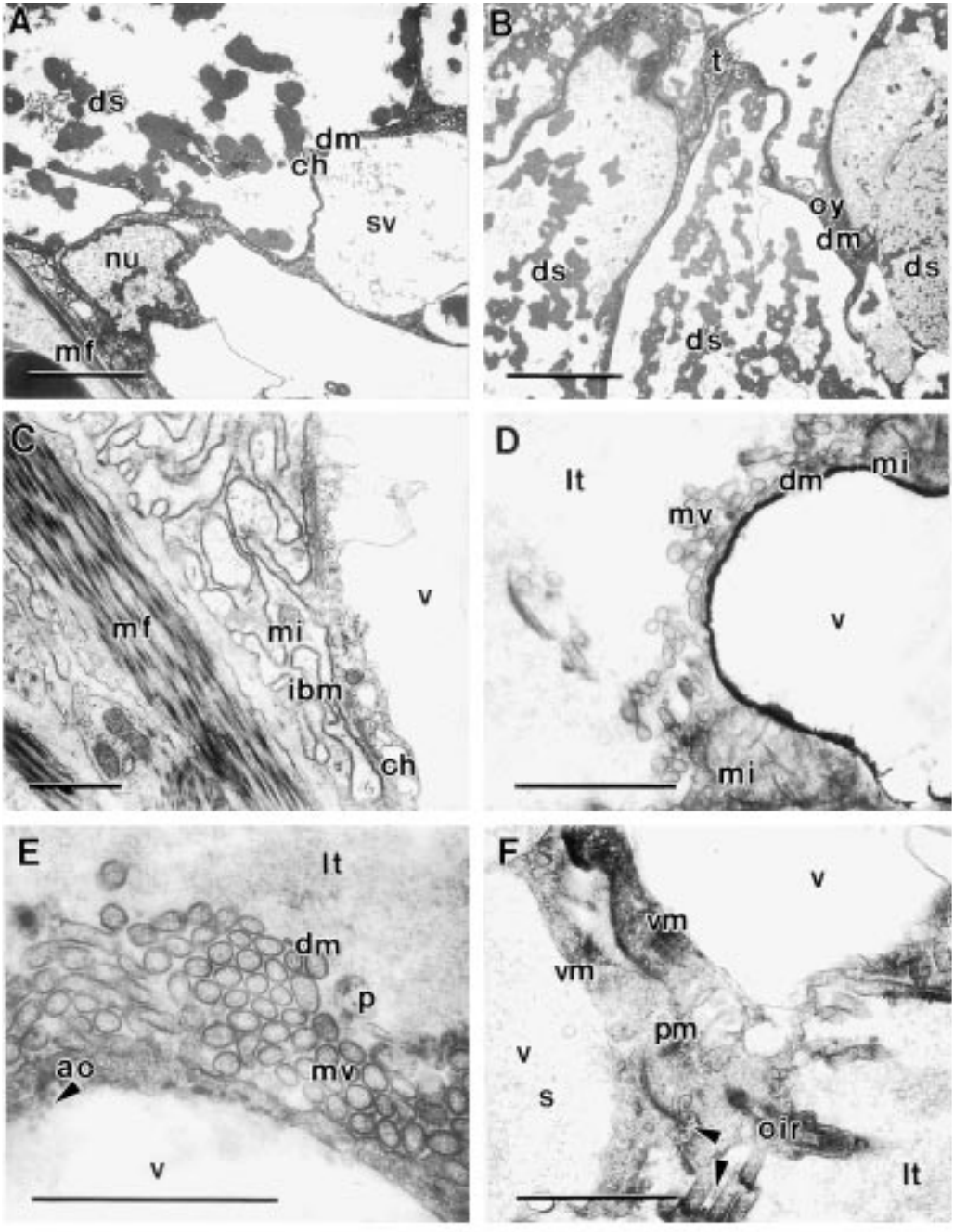
The size of the acid glands in different groups of Cassoidea is clearly correlated with diet, since they are best developed in the specialist echinoid feeders among the Cassidae. In the Personidae their absence reflects a diet of chaetopterid worms. They are nevertheless so well developed in the Ranellidae, which are not all echinoderm-feeders, that the acid must have another important (more primitive) role in this family. The Ranellinae, which seem to be the least specialized of the Ranellidae, take a variety of prey, though echinoderms are included among them (Taylor, 1998). Echinoderms feature more prominently in the diet of other ranellids except the Cymatiinae which take ascidians as well as molluscs, although *Cymatium intermedius* and *C. muricinum* feed only on bivalves and *C. nicobaricum* eats only gastropods. The Bursinae eat polychaetes as well as ophiuroids, but *Charonia*/*Sassia* show increasing conservatism for a diet of echinoderms.

In the Cassidae the acid glands reach massive proportions, and according to Nüske (1973) in *Galeodea* (as *Cassidaria*) *echinophora* (Oocornythinae), the cells show repeated secretory cycles and regeneration of protein-synthesizing organelles in the acid cells, which show synchronous secretion of protein and acid. This was not found in *Cymatium* (this paper) or *Tonna* (Wëber, 1927), in which the glands show close similarity at the histological level, with two consecutive phases, protein synthesis and acid secretion, in one cell type, the phases spatially separated in the gland. Each phase may repeat secretory cycles. The difference could be linked to a greater demand for toxin in cassids in overcoming their echinoid prey. Echinoids such as *Diadema* are themselves armed with toxic spines and their predators must therefore be capable of immobilizing them rapidly before attacking the test.

It is striking that shell drilling is not the common strategy in those Cassoidea that take molluscan prey (Riedel, 1995, and Taylor, 1998, for reviews), although their strongly acid saliva is effective in attacking the shell. This was demonstrated experimentally by Day (1969) using the acid secretion of *Argobuccinum*. Nevertheless Morton & Taylor watched a specimen of the Hong Kong rocky shore species *Gyrineum natator* (Ranellinae) in an aquarium dissolve a large hole in the shell of a small oyster *Saccostrea cucullata* (Taylor, 1998). Whether or not this is common practice for this species in natural conditions remains to be established.

Shell drilling is known in only four families of prosobranchs, the most notable being the Naticidae and Muricidae (including Rapanidae). Hydrochloric acid is a minor constituent of the weakly acid complex secretion secretion of the ABO (Carriker & Williams, 1978, Carriker, 1981). Two species of marginellid have been shown to corrode and drill bivalve shells, but the source and chemical composition of the secretions responsible are not known (Ponder & Taylor, 1992). Similarly, shell drilling has recently been reported in *Cominella* species (Buccinidae) (Peterson & Black, 1995), but no details are available.

Muricids have a more complex feeding mechanism than cassoideans, summarized by Fretter & Graham (1994). The possession of a pedal ABO (Nylen, Provenza & Carriker, 1969) reflects their ability to drill molluscan



shells, though at a much slower rate (72h for an oyster shell) than that achieved by *Octopus* (2~2h) (Nixon & Macconachie, 1988), and a second pair of (tubular) salivary glands secretes a paralytic toxin. This gives flexibility in the method of prey capture, and although drilling is their normal practice, in contrast to that in the Cymatiinae, they can paralyse prey without drilling (Fretter & Graham, 1994 for review). Use of separate secretions (of tubular salivary glands and ABO respectively) for paralysing prey and eroding shell allows a

Figure 7. TEM detail of cells of posterior lobe of salivary gland in different stages. **A.** Basal region, late stage of protein-secreting cell, secretory vesicles ruptured and secretion dispersing; scale bar represents 5μm. **B.** Apices of same cells clustered around small lumen of tubule showing two phases of secretory material: dense, and finely granular; scale bar represents 5μm. **C.** Basal region of acid cell with elaborate infolding and loss of organelles except mitochondria; scale bar represents 1μm. **D.** Apical region of acid cell showing scant cytoplasm and large mitochondria; scale bar represents 1μm. **E.** Grazing section of apical region of acid cell showing channel from central vacuole in apical cytoplasm and bead-like granules (arrowhead); scale bar represents 1μm. **F.** Detail of apical region of acid cell showing channel containing bead-like granules (arrowhead), opening to cell surface; scale bar represents 1μm. ac, apical cytoplasm; ch, channel in cytoplasm; cir, ciliary rootlet; cy, cytoplasm; dm, dense vacuolar membrane; ds, dispersing protein secretion; ibm, infolded basal cell membrane; mf, muscle fibres; mi, mitochondrion; mv, microvilli; nu, nucleus; lt, lumen of tubule; p, pore; s, secretion; sv, secretory vesicle; t, tubule; v, central vacuole; vm, vacuolar membrane.

muricid to resort to drilling immediately if it fails to get access to soft parts.

Cassoideans cannot release individual constituents of the saliva separately but, depending on the species, the one secretion can paralyse prey, dissolve purely calcareous material and partially digest tissues rapidly (in minutes rather than hours). The reticulate calcareous ossicles of which echinoid tests are composed area far less formidable barrier than molluscan shell in which dense calcite and aragonite crystals are laid down in protein matrix, which is energetically more expensive and slower to erode. If an attack on a prey fails some time must elapse before the secretion is replenished, which may have influenced the feeding strategy of those ranellids which feed on molluscs. Paralysis of prey is quicker and less costly if unsuccessful than drilling.

The secretory mechanism of acid cells

The secretory mechanism of acid cells of invertebrates is relatively little known, studies of gastropod acid cells are largely restricted to the histological and ultrastructural observations of Thompson (1969, 1983) on skin glands of Cypraeoidea, a superfamily closely related to the Cassoidea, and Edmunds (1968) on skin glands of opisthobranchs. However, the ultrastructural features of these cells, where known, suggest that the cellular pathways concerned in production of sulphuric acid in gastropods is essentially the same as that in secretion of hydrochloric acid by mammalian oxyntic cells (Forte & Machen, 1987; Cross & Mercer, 1993).

The density of the apical and tubulo-vesicular system membranes of oxyntic cells, a character shared with the vacuolar and apical membranes of the acid cells in *Cymatium*, is attributed to the H^+ K^+ ATPases which reside in them, providing the pumps that release H^+ ions into the canaliculi when stimulated by a combination of neurotransmitters and hormones. The narrow canaliculi allow the concentration of ions which create the osmotic gradient to draw water across the cells; in *Cymatium* the narrowness of the cell necks and tubule lumen provide similar confined spaces.

Carbonic anhydrase is the enzyme responsible in oxyntic cells for compensating for the resulting alkalinity of the cytoplasm by hydrolysis of one molecule of CO_2 (from the blood) to HCO_3^-. This enzyme, though not yet identified in the acid cells of *Cymatium*, is known to be involved in shell formation in molluscs, and plays an important part in shell dissolution by the ABO of muricids (Carriker & Williams, 1978; Carriker, 1981). Infolding of the basal plasma membranes, which house Cl^- HCO_3^- carriers in oxyntic cells, is more extensive in the acid cells of *Cymatium*. If a similar transport mechanism exists in the two cell types, a greater surface area of membrane may be expected in a system involving exchange of HCO_{3-} with a divalent than a monovalent anion. In *Cymatium* the process must involve exchange of two HCO_3^- ions from the cytoplasm for one SO_42^- from the blood, rather than one-to-one exchange for Cl^- in oxyntic cells.

Storage of acid and protective devices

The indications are that free sulphuric acid is stored in the cells that secrete it in *Cymatium* as demonstrated histochemically in *Linatella* (Morton, 1990), and that there is some storage in the lumen of the main tubule of the gland, when acid will be in contact with the epithelium of the tubule and main salivary duct. This epithelium, and that of the buccal cavity must be protected from damage. Thompson (1983) also demonstrated free sulphuric acid in vacuoles of the acid glands of the opisthobranch *Philine* using barium salts. In this respect the acid cells of molluscs appear to differ from

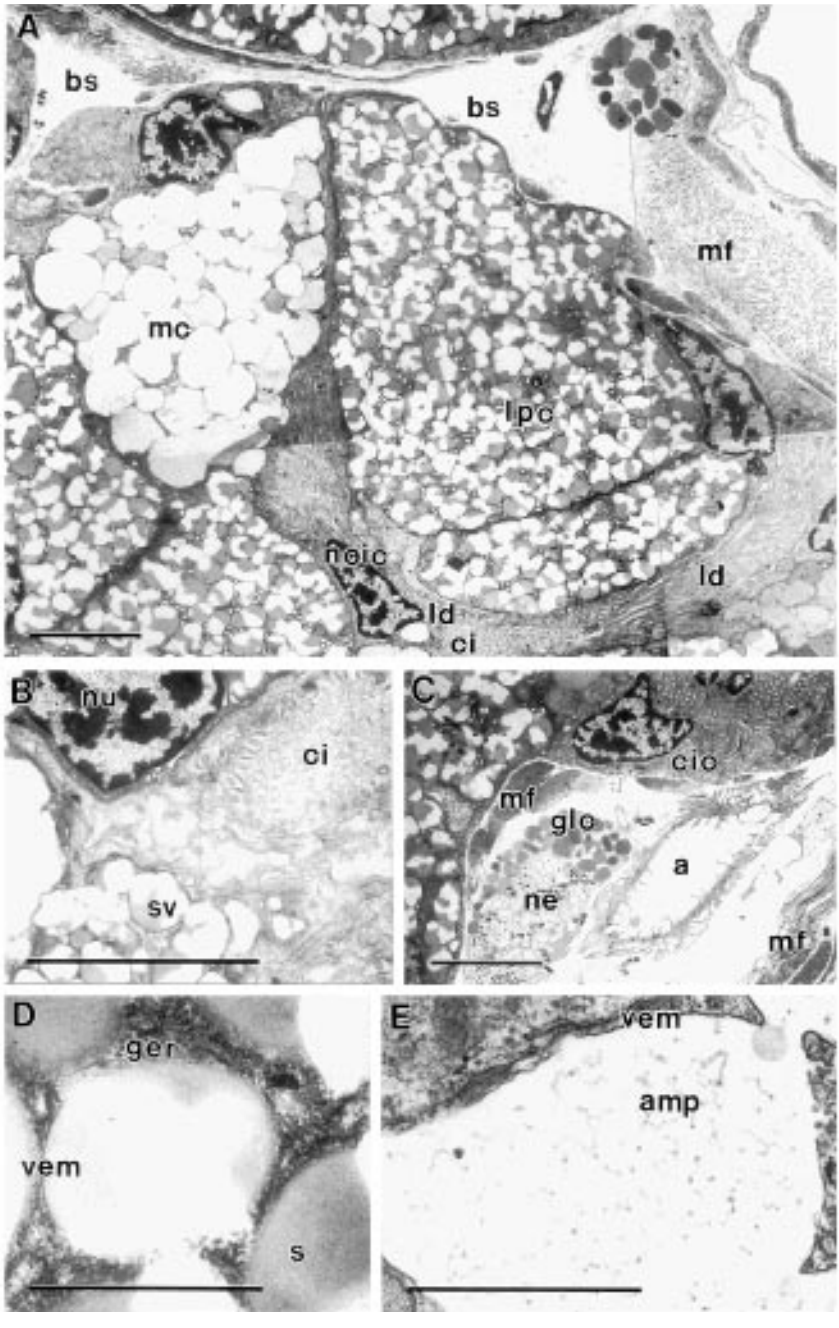


Figure 8. TEM acinus of anterior lobe (accessory salivary gland). **A.** Montage showing mucous, serous, and ciliated cells; scale bar represents 5μm. **B.** Detail of apical cytoplasm of ciliated and serous cells; scale bar represents 5μm. **C.** Subepithelial connective tissue showing nerve and muscle fibres and artery; scale bar represents 5μm. **D.** Detail of secretory vesicle of serious cell; scale bar represents 1μm. **E.** Detail of mucous secretory vesicle; scale bar represents 1μm. **E.** Detail of mucous secretory vesicle; scale bar represents 1μm. a, artery; amp, acid mucopolysaccharide; bs, blood space; ci, cilia; cic, ciliated cell; ger, granular endoplasmic reticulum; glc, neuroglial cell; ld, lumen of duct; lpc, 'light' protein-secreting (serous) cell; mc, mucous cell; mf, muscle fibre; ne, nerve; nu, nucleus; ncic, nucleus of ciliated cell; s, secretion; sv, secretory vesicle; vem, vesicular membrane.

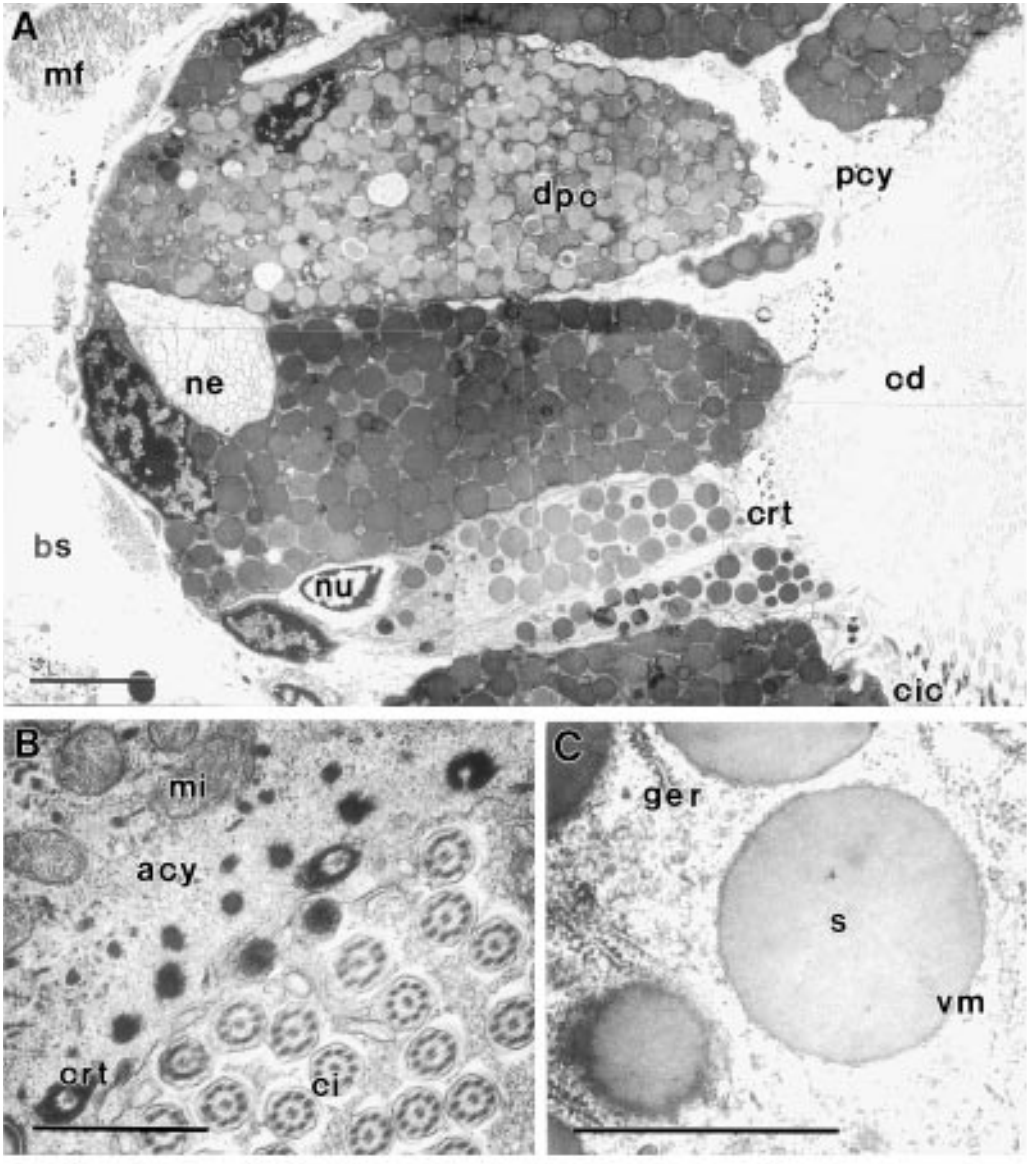


Figure 9. TEM duct of anterior lobe (accessory salivary gland). **A.** Epithelium of duct showing protein-secreting and ciliated cells; scale bar represents 5μm. **B.** Apical cytoplasm of ciliated cell; scale bar represents 1μm. **C.** Secretory vesicle of gland cell; scale bar represents 1μm. acy, apical cytoplasm; bs, blood space; cd, cilia in duct; ci, cilia; cic, ciliated cell; crt, ciliary rootlet; dpc, 'dark' protein-secreting cell; ger, granular endoplasmic reticulum; mf, muscle fibre, mi, mitochondrion; ne, nerve ending; nu, nucleus; pcy, pale cytoplasm; s, secretion; vm, vesicular membrane.

oxyntic cells, and consequently may require additional protective mechanisms. No evidence of this was found at the ultrastructural level which therefore suggests that the protective devices are at the molecular level.

The membranes of the undifferentiated and protein-secreting cells in the end branches of the tubules never display the density exhibited by some of the acid cell membranes, and at this phase in their life cycle have not acquired the specialized features of the acid-producing phase.

They would therefore probably be vulner-

able to damage if exposed to acid in the event of its reflux into the branches of the tubules. The narrowness of the branches and the ciliation of their walls would, however, probably protect against reflux.

This leaves the epithelium distal to the site of acid release as the most vulnerable to acid attack, as in the stomach of vertebrates. In *Cymatium* this is the lining of the salivary ducts. There is no ultrastructural evidence in the ducts of a mucous coating such as protects the gastric mucosa, they lack mucous cells and surprisingly few occur in the acini of the anterior lobes. The ducts are unusual, however, in their possession of powerful cirri and the apparent specialization of the epithelium for ion transport. The cirri, combined with the action of the long, unusually coiled cilia in the main tubule of the gland are likely to be important in the prevention of unstirred layers in the contents of the lumen, which suggests that an active transport mechanism prevents the build up of acid close to the cell membranes. Furthermore, expulsion of secretion is so rapid that contact with this epithelium or that of the buccal cavity is brief.

The salivary ducts may be devoid of a protective mucous coating but the buccal epithelium is typically well endowed in prosobranchs. In ranellids this may protect against acid attack in the same way as the mucous gel lining the gastric mucosa of mammals, the $HCO3^-$ ions in the gel neutralizing the acid by the time it reaches the cell surface (Cross & Mercer, 1993). Denny (1983) has taken the sulphated mucin of the pig gastric mucosa as a possible model for the sulphated and carboxylated glycosaminoglycans of molluscs. They, like gastric mucin, become more viscous with increase in acidity. The importance of mucus in protecting any surface of the snail exposed to its own acid secretion cannot therefore be underestimated.

There remains the question as to how such apparently delicate organs as the salivary glands, with a relatively thin connective tissue sheath, can be so forcefully squeezed by muscular contraction during ejection of saliva without rupture. It is suggested here that suction created by eversion of the proboscis might contribute to this, reducing the muscular force required—a possibility so far not examined.

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REFERENCES

ANDREWS, E.B. (1991). The fine structure and function of the salivary glands of *Nucella lapillus* (Gastropoda: Muricidae). *Journal of Molluscan Studies*, **57**: 111-126.

ASANO, M. & ITOH, M. 1960. Salivary poison of a marine gastropod, *Neptunea arthritica* Bernadi and the seasonal variation in its toxicity. *Annals of the New York Academy of Science*, **90**: 674-688.

BALL, A.D., TAYLOR, J.D. & ANDREWS, E.B. 1997. Development of the acinous and accessory salivary glands in *Nucella lapillus* (Neogastropoda: Muricoidea). *Journal of Molluscan Studies*, **63**: 245-260.

BENTIVEGNA, F. & TOSCANO, A. 1991. Observations au laboratoire sur le comportement alimentaire de trois espèces de la superfamille Tonnoidea (Mollusca, Gastropoda). *Revue français d'Aquariologie et d'Herpetologie*, **18**: 33-38.

BEU, A.G. 1980. Australian gastropods of the family Bursidae. Part 1. The families of Tonnacea, the genera of Bursidae, and revision of species previously assigned to *Tutufa* Jousseaume, 1881. *Records of The Australian Museum*, **33**: 248-324.

CARRIKER, M.R. 1981. Shell penetration and feeding by naticacean and muricacean predatory gastropods: a synthesis. *Malacologia*, **20**: 403-422.

CARRIKER, M.R. & WILLIAMS, L.G. 1978. The chemical mechanism of shell dissolution by predatory boring gastropods: a review and an hypothesis. *Malacologia*: **17**: 143-156.

CORNMAN, I. 1962. Toxic properties of the saliva of *Cassis*. *Nature*, **200**: 88-89.

CROSS, P.C. & MERCER, K.L. 1993. *Cell and tissue. A functional perspective*. Freeman, New York.

DAY, J.A. 1969. Feeding of the cymatiid gastropod *Argobuccinum argus*, in relation to the structure of the proboscis and secretions of the proboscis glands. *American Zoologist*, **9**: 909-916.

DENNY, M. 1983. Molecular biomechanics of molluscan mucous secretions. In: *The Mollusca*, **1**: *Metabolic biochemistry and molecular biomechanics* (P.W. Hochachka, ed.), 431-466. Academic Press, New York.

EDMUNDS, M. 1968. Acid secretion in some species of Doridacea (Mollusca, Nudibranchia). *Proceedings*

of the Malacological Society of London, **38**: 121-133.

ENDEAN, R. 1972. Aspects of molluscan pharmacology. In: *Chemical Zoology*, **7**: *Mollusca* (M. Florkin & B.T. Scheer, eds), 421-466. Academic Press, New York.

FÄNGE, R. & GROVE, D. 1979. Digestion. In: *Fish physiology*, **8**: *Bioenergetics and growth* (W.S. Hoar, D.J. Randall, & J.R. Brett, eds), 162-260. Academic Press, Orlando.

FÄNGE, R. & LIDMAN, U. 1976. Secretion of sulphuric acid in *Cassidaria echinophora* Lamarck (Mollusca: Mesogastropoda, marine carnivorous snail). *Comparative Biochemistry and Physiology*, **53A**: 101-103.

FORTE, J.G. & MACHEN, T.E. 1987. Ion transport by gastric mucosa. In: *Membrane transport processes in organized systems* (T.E. Andreoli, J.F. Hoffman, D.D. Fanestil, & S.G. Schultz, eds), 535-558. Plenum, London.

FRETTER, V. & GRAHAM, A. 1994. *British prosobranch molluscs*. Revised and updated edition. Ray Society, London.

HANCOCK, D.A. 1960. The ecology of the molluscan enemies of the edible mollusc. *Proceedings of the Malacological Society of London*, **34**: 123-143.

HOUBRICK, J.R. & FRETTER, V. 1969. Some aspects of the functional anatomy and biology of *Cymatium* and *Bursa*. *Proceedings of the Malacological Society of London*, **38**: 415-429.

HUGHES, R.N. & HUGHES, H.P.I. 1981. Morphological and behavioural aspects of feeding in the Cassidae (Tonnacea, Mesogastropoda). *Malacologia*, **20**: 385–402.

KOHN, A.J. 1983. Feeding biology of gastropods. In: *The Mollusca*, **5**: *Physiology* part 2 (A.S.M. Saleuddin & K.M. Wilbur, eds), 1-63.

KROPP, R.K. 1982. Response of five holothurian species to attack by a predatory gastropod, *Tonna perdix*. *Pacific Science*, **36**: 445-452.

LAXTON, J.H. 1971. Feeding in some Australasian Cymatiidae (Gastropoda: Prosobranchia). *Zoological Journal of the Linnaean Society*, **50**: 1-9.

MORTON, B. 1990. Prey capture, preference and consumption by *Linatella caudata* (Gastropoda: Tonnoidea: Ranellidae) in Hong Kong. *Journal of Molluscan Studies*, **56**: 477-486.

MORTON, B. 1991. Aspects of predation by *Tonna zonatum* (Prosobranchia: Tonnoidea) feeding on holothurians in Hong Kong. *Journal of Molluscan Studies*, **57**: 11-20.

NIXON, M. & MACCONACHIE, E. 1988. Drilling by *Octopus vulgaris* (Mollusca: Cephalopoda) in the Mediterranean. *Journal of Zoology*, London, **216**: 687-716.

NOAILLAC, J. & DEPEYRE, N. 1978. Ultrastructural and cytochemical study of the gastric epithelium in a fresh water telesotean fish (*Perca fluviatilis*). *Tissue and Cell*, **10**: 23-37.

NÜSKE, H. 1973. Cytologische untersuchungen an der Säuerdrüse der Meeresschnecke *Cassidaria echinophora. Cytobiologie*, **7**: 164-180.

NYLEN, M.U., PROVENZA, D.V. & CARRIKER, M.R. 1969. Fine structure of the accessory boring organ of the gastropod *Urosalpinx*. *American Zoologist*, **9**: 935-965.

PETERSON, C.H. & BLACK, R. 1995. Drilling by buccinid gastropods of the genus *Cominella* in Australia. *Veliger*, **38**: 37-42.

PONDER, W.F. 1970. Some aspects of the morphology of the neogastropod family Marginellidae with a discussion of the evolution of the toxoglossan poison gland. *Journal of the Malacological Society of Australia*, **2**: 55-81.

PONDER, W.F. 1973. The origin and evolution of the Neogastropoda. *Malacologia*, **12**: 295-338.

PONDER, W.F. & TAYLOR, J.D. 1992. Predatory drilling by two species of *Austroginella* (Gastropoda: Marginellidae). *Journal of Zoology, London*, **228**: 317-328.

RIEDEL, F. 1995. An outline of cassoidean phylogeny (Mollusca, Gastropoda). *Contributions to Tertiary and Quaternary Geology*, **32**: 97-132.

SHIOMI, K., MIKUKAMI, M., SHIMAKURA, K. & NAGASHIMI, Y. 1994. Toxins in the salivary gland of some marine carnivorous gastropods. *Comparative Biochemistry and Physiology*, **74a**: 615-621.

TAYLOR, J.D. 1998. Understanding biodiversity: adaptive radiations of predatory marine gastropods. In: *The Marine Biology of the South China Sea* (B. Morton, ed.), 187-206. *Proceedings of the Third International Conference on the Marine Biology of Hong Kong and the South China Sea*. Hong Kong University Press.

THOMPSON, T.E. 1969. Acid secretion in Pacific Ocean gastropods. *Australian Journal of Zoology*, **17**: 755-764.

THOMPSON, T.E. 1983. Detection of epithelial acid secretions in marine molluscs: review of techniques and new analytical methods. *Comparative Biochemistry and Physiology*, **74A**: 615-621.

WËBER, H. 1927. Der Darm von *Tonna galea* L., eine vergleichend anatomische Untersuchungen unter besonderer Berücksichtigung der *Tritonium*-Arten. *Zeitschrift für Morphologie und Ökologie der Tiere*, **8**: 663-804.

WEST, D.J., ANDREWS, E.B., BOWMAN, D., MCVEAN, A.R. & THORNDYKE, M.C. 1996. Toxins from some poisonous and venomous marine snails. *Comparative Biochemistry and Physiology*, **113C**: 1-10.

WEST, D.J., ANDREWS, E.B., MCVEAN, A.R., THORNDYKE, M.C., & TAYLOR, J.D. 1998. Pharmacologically active secretion from the salivary glands of the predatory marine snail *Cymatium intermedia* (Gastropoda: Ranellidae). *Toxicon*, **36**: 25-29.