
WONG PAT SHUN, PATSY

Dedicated to those who appreciate
the wonderful works of the Creator
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REFERENCES
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The marine pulmonate limpet *Siphonaria zelandica* is commonly found in the mid-eulittoral zone in shallow pans or rock pools with dense algal growth. Though it resembles a true limpet externally, it is active when emersed during the ebbing of the tide browsing on small succulent algae, rather than grazing the surface film. The structure and function of the digestive and respiratory systems of *Siphonaria* were studied, particularly in relation to its adaptations to the intertidal mode of life.

The digestive system is relatively simple consisting of a large buccal mass with paired salivary glands, a spacious esophageal crop, a slightly muscular stomach with two digestive diverticula and a simple intestine-rectum.

The arrangement of the 28 muscles of the buccal mass and the odontophore is typical of patelliform pulmonates. The radula is a broad sheet with 144 ± 31 rows of teeth, each row having a tooth formula of (18±4) + (18±3) + (1) + (18±3) + (18±4). The radular teeth are continuously produced at a rate of 4.9 rows per day similar to those of active feeding herbivorous gastropods. The characteristics of the teeth and the movement of the mouth and the feeding traces showed that the feeding processes do not involve rasping of a hard substratum but browsing and scooping of soft lush algal growth.

The whole alimentary tract is lined by ciliated columnar cells of a basic form but with slight structural variations in different region. Those in the oesophagus and stomach contain apical vesicles
and lysosome-like bodies indicating involvement in intracellular digestion, while those in the intestine contain various amounts of lipid and glycogen, functioning in active absorption and storage. The ciliated cells of the post-intestine, having a much folded basal plasma membrane are apparently involved in osmoregulation. The proteinaceous secretory cells in the crop and mid-intestine and the glycoprotein cells in the pro-intestine are either producing enzymes for extracellular digestion or secreting the faecal-binding sheath. Mucous cells interspersed among the ciliated cells of the oesophagus intestine and rectum produce mucoid material for lubrication and transport of food particles. The cuticular strip in the anterior chamber of the stomach, probably a vestigial gastric shield consists of tall non-ciliated cells with thick microvilli embedded in a dense fibrous matrix.

The salivary gland contains six cell types: grain cells, duct mucocytes, mucocytes I and mucocytes II, duct ciliated cells and undifferentiated cells. The first four are secretory, the grain cell being serous secretory and the other three muciparous, with varying amounts of mucopolysaccharides.

The tubules of the digestive gland are made up of five cell types, acinous digestive cells, neck digestive cells, crypt cells, vacuolated cells and undifferentiated cells. Both types of digestive cells participate in absorption and digestion. The high lipofuscin content in the neck digestive cell suggests its possible role in lipid digestion. The crypt cell with elaborate GER whorls and proteinaceous globules is apparently secretory, producing enzymes for extracellular
digestion. Apocrinal secretion of the globules from the crypt cells was observed for the first time. The vacuolated cells appear to be degenerated crypt cells with an excretory function. The digestive gland tubule was found to undergo cyclic changes, correlated with the feeding activity which is related with the tides.

Enzyme assays showed the presence of various carbohydrases, proteolytic and lipolytic enzymes in the different region of the digestive system. The digestive gland is the main site of enzyme production. The pH optima of amylase, laminarinase and sucrase from various regions of the digestive tract were found to be within the pH range of the alimentary tract. The activities of various enzymes in the salivary gland and the oesophagus but not the digestive gland were synchronous and correlated with feeding.

When emersed, *Siphonaria* respires atmospherically with the pneumostome wide open, and the mantle cavity functioning as a lung without any active ventilatory movements. When submerged, the mantle cavity is filled with water, a strong water current through the gill being created by the ciliated dorsal and ventral raphe behind the gill. The available respiratory surfaces include the sides of the foot, the anterior mantle roof and the single plicate gill. All these are lined by a thin epidermis with large blood spaces beneath it. The non-ciliated epidermal cells in different regions carry different lengths of microvilli, apparently related to the degree of their protective role.

The respiratory physiology of *Siphonaria* was also studied. No tidal or diurnal rhythm in oxygen consumption was revealed. In unagitated conditions the aerial respiratory rate was much higher than
the aquatic rate but with agitation, the aquatic rate increased to near the aerial rate. Cutaneous respiration constituted about 25% of the total. The respiratory rate varied with body weight, the coefficient b being temperature-independent (0.791 ± 0.122). Both aerial and aquatic respiratory rates increased with temperature. The highest temperature-sensitive range was 10-25°C in winter and 15-30°C in summer which coincided with the normal environmental temperature. No seasonal temperature acclimation was found within the normal thermal range.

Respiratory pigments haemocyanin and myoglobin were detected in the blood and buccal mass respectively. Their characteristics were studied and their role in oxygen transfer system was postulated. The reverse Bohr shift of the haemocyanin may facilitate oxygen uptake in the lung during burses of activity at low tide. A high oxygen-combining capacity of the buccal mass myoglobin (21.2 vol%) indicated a role of oxygen storage during bursts of feeding activity. The distribution of carbonic anhydrase in various tissues was consistent with a transfer system facilitating the release of metabolic CO₂ from the buccal mass.

Finally the phylogenetic relationships of Siphonariidae with other groups of marine pulmonates were discussed. The unspecialised digestive system, the advanced form of nervous system and the possession of a secondary gill suggest that Siphonariidae, as a family, may not be an advanced derivative of any of the more primitive living basommatophoran groups, but could represent, along with the Gadiniidae, a specialised group which has originated directly from a primitive pulmonate stock and are adapted to life on exposed rocky shores.
CHAPTER 1

GENERAL INTRODUCTION

The limpet-like gastropods belonging to the family Siphonariidae are located in the order Basommatophora of the subclass Pulmonata. Species of *Siphonaria* are the dominant limpet form on many marine shorelines especially in the warm temperate and tropical Indo-Pacific region (Hubendick, 1945). Their distribution is almost world-wide except for the Northern Atlantic (Hubendick, 1978). Siphonariids parallel true limpets (Patellacea) in habit and ecology and in some areas have replaced them completely as the dominant limpet in the mid to upper eulittoral zone (Stephenson & Stephenson, 1972; Fretter, 1975).

The great majority of the pulmonates live on land or in fresh-water and the most detailed work on their morphology or physiology has been concentrated on forms from these habitats. For marine pulmonates a full description of the form and function of the most evidently primitive family, the Ellobiidae, has been given by J.E. Morton (1955a, b, c). The morphology of *Amphibola arenata*, belonging to an adjacent marine pulmonate family, the Amphibolidae, has been described by Farnie (1919) and the digestive gland structure with its cyclic activity has been studied by B.S. Morton (1975). Work has also been done on the ecology and morphology of the Trimusculidae (syn. Gadiniidae) which is closely related to the Siphonariidae (Yonge, 1958; Walsby *et al.*, 1973; Haven, 1973; Walsby, 1975).

For the Siphonariidae, research on animal structure and function has so far been limited in scope. The principal monograph on the family,
by Hubendick (1945), is anatomical rather than functional in its approach, and contains much information on the gross structure of the digestive, respiratory and reproductive systems within different sections of the family. Yonge (1952) gave a detailed description of the form and function of the pallial cavity of *Siphonaria alternata*. Brief references to some aspects of the functional morphology of various siphonariids have also been given by Hubendick (1955), Marcus & Marcus (1960) and Yonge (1960).

In the New Zealand region, Powell (1976) recognises seven species of Siphonariidae. Four of these, *Siphonaria zelandica* Quoy & Gaimard, 1883, *S. australis* Quoy & Gaimard, 1883, *S. cookiana* Suter, 1909 and *Benhamina obliquata* Sowerby, 1825 are found in the two main islands and the other three, *Kerguelenella innominata* (Iredale, 1915), *K. lateralis* (Gould, 1846) and *K. stewartiana* (Powell, 1939) are located in small islands north and south of the main islands. Of these New Zealand siphonariids, brief anatomical studies have been made on *S. australis* (Hutton, 1882), *B. obliquata* (Cottrell, 1910, 1911) and *K. stewartiana* (Knox, 1955). Although *S. zelandica* is by far the commonest species throughout New Zealand (Morton & Miller, 1968), no detailed work on this limpet has been carried out. Bedford (1967) briefly described some aspects of the digestive and excretory systems of this species in her study of the biochemical physiology of its nitrogen metabolism.

The present study has therefore been directed towards an integrated investigation into the various aspects of morphology and physiology of the digestive and respiratory systems of *Siphonaria zelandica*. It is hoped that from this work more complete information on the form
and function, including ultrastructure and histochemistry, of these systems in pulmonates will be obtained, together with some insights into the adaptations of the pulmonate limpets, both anatomical and physiological to the intertidal environment. Finally it is also hoped to throw some light on the phylogenetic relationships of the Siphonariidae with other groups of pulmonates.
CHAPTER 2

THE ECOLOGY AND HABITS OF *SIPHONARIA ZELANDICA*

An introductory account of the ecology and habits of *Siphonaria zelandica* is a necessary prelude to the study of its morphology and the functioning of the several systems in the living animal under field conditions. Abe (1939) gave the first detailed contribution to siphonariid ecology with a study of mode of life and homing behaviour of *S. atra* and later (1940) of *S. japonica*. Allanson (1958) discussed the tolerances to heat, desiccation and salinity in relation to the position on the shore for three African species of *Siphonaria*: *S. capensis*, *S. deflexa* and *S. aspersa*. For *S. pectinata* on the coast of Florida, North America, the ecology was studied by Voss (1959) and the homing behaviour and activity rhythm in relation to tides and shore conditions were observed by Thomas (1973). The same species was also mentioned as one of the dominant zoning organisms in the littoral ecology of West Africa (Lawson, 1966). The homing behaviour and activity with relation to the tidal cycle of *S. normalis* and *S. alternata* were studied in detail (Cook, 1969, 1971, 1976; Cook & Cook, 1977, 1978). It has been suggested that the feeding habits, with radula abrasion, of *S. pectinata* are influential in the erosion of beach rock (Craig et al., 1969) while algal cleaning by the Argentinian species *S. lessoni* was considered to promote barnacle settlement on wooden piles (Bastida et al., 1971).

The ecological study of New Zealand siphonariiids was initiated by Borland’s (1950) work on *Benhamina obliquata*, in which a brief comparative account of ecology of *Siphonaria zelandica* was also added.
Batham (1956), describing the littoral ecology of rocky shores in southern New Zealand, discussed the relationships of the *Siphonaria* species to plants and other animals. The general ecology of *S. zelandica* and the other New Zealand species, their shore position in relation to the tides, and their New Zealand distributions have been well introduced by Morton and Miller (1968). Bedford (1967), in her primarily physiological study of *S. zelandica* had already briefly described the habitat with its characteristic algal species. Beckett (1969) dealt with the movement pattern and homing behaviour of *S. zelandica* in his study of the ecology and movements of upper and middle shore gastropods at Auckland, New Zealand. In the following study, the nature of the shore habitat, the substratum requirements, the associated flora and fauna and the behaviour of *Siphonaria* with reference to the cyclic effects of night and day and of the tides have been further investigated.

2.1 The habitat

*Siphonaria zelandica* is very common in open shores throughout New Zealand and is increasingly represented on sheltered shores towards the south (Morton & Miller, 1968). A brief survey of North and South Island shores has confirmed this. A more detailed study of the habitat of *S. zelandica* was carried out at Goat Island Bay, Leigh (Fig. 1). Five sites of different characteristics with respect to substrate hardness, profile of the shore, degree of exposure and abrasion were chosen (Fig. 2) and the distribution of *S. zelandica* and the associated species was recorded. The results are shown in Fig. 3.

Shores A, B, C and D are all built of a similar substrate of
Fig. 1

A. Map of New Zealand showing the position of Auckland.

B. Map of Auckland showing the different locations where specimens of *Siphonaria zelandica* were collected for this study.
Fig. 2

Map of Goat Island Bay, Leigh showing the position of study sites A, B, C, D, E.

- intertidal rock platforms.
Fig. 3

Shore profiles and patterns of zonation, degree of exposure and rock types of the shore at sites A, B, C, D, E at Goat Island Bay, Leigh selected for habitat study of *Siphonaria zelandica*.

- *Carpophyllum maschalocarpum*
- *Carpophyllum plumosum*
- *Cystophora torulosa*
- *Xiphophora chondrophylla*
- *Hormosira banksii*
- *Splachnidiurn rugosum*
- *Gigartina oawellae*
- *Nemastoma feredayae*
- *Laurencia*
- *Lygnia*
- *Ralfisia*
- *Liagora harveyana*
- *Chaetangium corneum*
- *Jania micrarthrodia*
- *Colpomenia*
- *Corallina officinalis*
- *Coralline turf*
- *Hildenbrandtiia*
- *Gelidium pusillum*
- *Chamaesipho brunnea*
- *Chamaesipho columbia*  
  ![sand]
- *Elminius plicatus*  
  ![rock pool]

For distribution of the five limpet-shaped snails on the shore:

- **Sz** - *Siphonaria zelandica*
- **Co** - *Cellana ornata*
- **Cr** - *Cellana radians*
- **Pc** - *Patelloidea corticata*
- **Sp** - *Sypharochiton pelliserpentis*

Scale of kite diagrams: 

- **——** = 100 animals/m$^2$
- **—** = 1000 animals/m$^2$
greywacke pebble conglomerate. Shore E is a wave-cut platform of soft, very erosible, greyish 'papa' mudstone of the Waitemata series. The degree of wave exposure was assessed according to the biologically-defined exposure scale for rocky shores (Ballantine, 1961; Ballantine et al., 1973). Site A is an exposed shore (Scale No. 5), site B is semi-exposed (Scale No. 4) while sites C and E are mainly sheltered (Scale No. 3) and site D is sheltered (Scale No. 2). The shore profile decreases in steepness from A to E and at site D, the surface of the rock platform is thinly covered with sand.

The distribution of Siphonaria at these sites clearly showed that the type of substrate is not a critical factor so long as the surface is intact and solid. S. pectinata has been reported on hard substrates ranging from limestone cliffs and rocks, granite blocks, cement seawalls and even on wood pilings and mangrove roots (Voss, 1959) while S. lessoni was also found on rocks of varying hardness from hard quartzite to most brittle sedimentary rocks (Bastida et al., 1971). In New Zealand, a brief survey has shown that S. zelandica can exist upon hard andesitic Manukau breccia as Lion Rock, Piha, west coast of North Island, as well as the concrete seawall at the port of Nelson in the South Island (Fig. 1). Its absence at site D at Goat Island Bay is probably due to its inability to withstand sand abrasion. Voss (1959) also noted the absence of S. pectinata from a sandy beach strip. Siphonaria was found at sites A, B, C and E with highest population numbers at the latter two sites, indicating a preference for semi-exposed to moderately sheltered conditions. In the exposed shore at Site A, S. zelandica is found only at relatively sheltered spots such as on the edge of a rock pool or
along rock crevices. Bedford (1967) related this distribution to the weak adhesive strength of the foot, rendering *Siphonaria* vulnerable to wave dislodgement under high exposure.

*Siphonaria zelandica* in general occupies the middle eulittoral zone, reaching into the higher eulittoral in places with strong wave splash, as at site B. Its lower limit also varies with the degree of exposure of the shore. On very wave-exposed shores, *S. zelandica* usually occupies a very narrow band in the mid-eulittoral, localised in rock pools or crevices. On sheltered shores, the distribution generally stops short of the lower eulittoral owing probably to the effect of sand and gravel abrasion as at site E. In moderately sheltered or semi-exposed shores, *S. zelandica* extends from the lower eulittoral zone to the upper eulittoral zone.

*Siphonaria zelandica* has, for the first time, been found to settle subtidally on experimental asbestos plates at a depth of 30 m (J.H.C. Choat & R.C. Willan, pers. comm.). These specimens were of small size, with thin shells covered with a thin layer of lithothamnion. *S. lessoni*, an intertidal species in South America, has also been found to extend down to a depth of a few metres (Bastida et al., 1971). Intertidal specimens of *S. zelandica* have been found, in the laboratory, to be unable to withstand more than six hours submergence, after which they crawl out of the water. It would be interesting to compare the physiology of these subtidal populations with that of the intertidal ones.

The mid-littoral distribution of *Siphonaria zelandica* appears
very irregular and patchy. Voss (1959) related the patchiness of S. pectinata in the balanoid zone to space competition with other organisms. In an exposed shore such as sites A and B, the upper limit of S. zelandica coincides with the lower limit of the large barnacle Elminius plicatus. The extremely rough shell surface of this barnacle probably prevents the soft foot of S. zelandica from moving across it.

Also at this level, the substratum dries off rapidly and the animal may face desiccation problems. Lower down, S. zelandica is found in association with the barnacle Chamaesipho columna, usually in areas of no more than moderate barnacle density. Also associated with Siphonaria are some algal grazing molluscs such as Sypharochiton pelliserpentis, Cellana radians, C. ornata and Patelloida oortiota. The first three species, though usually distributed with S. zelandica in the eulittoral zone, may also be found on bare rock surfaces or on pink coralline "paint". S. zelandica, on the other hand, is generally abundant on surfaces covered with a visible film of algae or macroscopic algal mat.

Beckett (1969) compared the radular characteristics of Cellana ornata, C. radians and Sypharochiton pelliserpentis. These were all classified as "heavy rasping" forms and from the study of the gut contents it was concluded that S. pelliserpentis had a preference for macroscopic Rhodophyceae while C. ornata and C. radians ingested everything on the substrate. The radula of Siphonaria is much more adapted for browsing (Section 3.3.1.4) and the stomach contents (see section 2.2 for details) are mainly composed of macroscopic algal fragments and diatoms. Thus it appears that S. zelandica is occupying
a distinctive niche from the grazing molluscs on the same shore. Although there may not be direct food competition, the distribution of *S. zelandica* may still be regulated by the role of these grazers in preventing dense growth of macroscopic algae by rasping off the sporelings and young plants.

The distribution of *Siphonaria zelandica* closely corresponds to that of a number of small macroscopic algae. On the greywacke conglomerates *S. zelandica* is most abundant on slopes clad with *Gelidium pusillum* or platforms and shallow pans with thick tufts of *Enteromorpha, Cladophora, Chaetomorpha* and *Ulva* (Fig. 4). The soft sandstone flats in Echinoderm Reef at site E support a wholly different algal flora and *Siphonaria* is often found browsing on the brown algae *Hafnia verrucosa* (Fig. 4F). A similar association was observed by Bedford (1967) on the sandstone reef at Long Bay, near Auckland.

In general, it can be concluded that *Siphonaria zelandica* inhabits the mid-eulittoral zone in semi-exposed to moderately sheltered shore. Its weak foot attachment, in contrast with the true limpets, probably excludes it from very exposed shores, while its poor resistance to sand abrasion limits it at low levels of sheltered shores. By its browsing habit, it is restricted to areas with abundant growth of softer or succulent algae.

2.2 Algal flora and stomach contents

A detailed study of the stomach contents in relation to the composition of the algal mat was made. Samples were collected from
Fig. 4  Association of *Siphonaria zelandica* with various small macroscopic algae.

A.  *Gelidium* in the upper-eulittoral zone of the shore at site B.  
Note the abundance of barnacles in this region.

B.  *Gelidium* mat in the mid-eulittoral zone of the shore at site B.  
Note the mucous trail left by two *Siphonaria* which are actively feeding on the *Gelidium*.

C.  *Chaetomorpha* in the shallow rock pans in the mid-eulittoral zone of the shore at site C.

D.  Fine *Cladophora* filaments in shallow rock pans in the mid-eulittoral zone of the shore at site C.  Note the yellowish crescent-shaped spawn mass of *Siphonaria* on the bottom left.

E.  *Ulva* on a sheltered rock cliff near site D.

F.  *Ralfsia* on the smooth 'papa' mudstone in the mid-eulittoral zone at site E.
algal patches in areas dominated by *Siphonaria zelandica* at sites B and C (Section 2.1, Fig. 2). The different algal species were identified by Dr C. Johnston, Department of Botany, University of Auckland. Individual *Siphonaria* feeding at low tide at these sites were collected and the stomach contents extracted. The different algal components were identified and their amount estimated. The results are presented in Table 1. The dominant alga at site B is *Gelidium pusillum* along with some species of *Enteromorpha*, *Chaetomorpha*, various small Rhodophyta and some filamentous blue green algae (e.g. *Oscillatoria* spp.). The stomach contents of *S. zelandica* from this region were predominantly *Gelidium* along with some *Enteromorpha* and *Chaetomorpha*. Site C, on the other hand, is visually dominated by *Enteromorpha* and *Chaetomorpha* but actually has masses of finer filaments of *Cladophora* associated. Also present among these is the phaeophyte *Bachelotia fulvescens*, several species of *Oscillatoria* and numerous diatoms. The stomach contents from this area contain much *Cladophora*, some *Enteromorpha* and also a high proportion of diatoms. *S. zelandica* thus appears to be an indiscriminate browser ingesting the full composition of the algal mat.

The relationship of the stomach contents to the whole complex of the substrate algae was also studied by reference to their absorption spectra. The acetone-extraction method (Strickland & Parsons, 1968) was used. Pigments were extracted with 90% acetone with magnesium carbonate (0.1 g/5ml) added to prevent development of acidity which may alter pigment composition. After centrifugation the samples were
analysed with a scanning spectrophotometer over the range 350 to 700 nm. Since chlorophyll, when dissolved in acetone, rapidly decomposes in sunlight the samples were extracted in the dark and scanned within an hour after centrifugation. The absorption spectra of the substrates dominated by different algal species were closely similar with high peaks at 665 and 435 nm and small peaks at 580 and 627 nm (Fig. 5). They also resemble samples reported by Beckett (1969). Identification of other major algal species by the absorption spectra is thus impracticable. The absorption spectra of the stomach contents of *Siphonaria* from various localities are all very similar with high peaks at 665 and 435 nm and small ones at 540 and 615 nm, only slightly different from those of the substrate algae (Fig. 6).

The spectral patterns closely coincide, too, with the absorption spectra of the digestive gland pigments, the faeces and the digestive gland excreta (Fig. 6). Contamination of the stomach content by the digestive gland pigments was reduced to a minimum by sampling just after feeding when the stomach was full of ingested food with little contribution from the digestive gland. On starvation, the digestive gland turned lighter in colour; the pigment absorption spectra, however, remained unchanged, except that the peaks were much reduced. It is thus evident that the digestive gland pigments originate from the food though the food pigments may become slightly altered soon after ingestion by the enzymes or acidity in the crop. Food pigments are absorbed by the digestive gland cells and ultimately excreted (Section 3.6).

The general resemblances between the absorption spectra of the
TABLE 1. A comparison of the algal component in the habitat and the stomach content of *Siphonaria terebriformis* from shores at site B & C.

<table>
<thead>
<tr>
<th>SITE</th>
<th>ALGAL SPECIES IN THE HABITAT</th>
<th>STOMACH CONTENT (N = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DOMINANT SPECIES : Gelidium pusillum (Rhodophyta)</td>
<td>Gelidium sp. 65 ± 5%</td>
</tr>
<tr>
<td></td>
<td>ASSOCIATED SPECIES : Enteromorpha compressa var australiensis</td>
<td>Enteromorpha sp. 22 ± 4%</td>
</tr>
<tr>
<td></td>
<td>E. nana (Chlorophyta)</td>
<td>Chaetomorpha sp. 7 ± 2%</td>
</tr>
<tr>
<td></td>
<td>Chaetomorpha aerea</td>
<td>Miscellaneous : algal spores</td>
</tr>
<tr>
<td></td>
<td>Rhizosolenia riparium</td>
<td>diatoms</td>
</tr>
<tr>
<td></td>
<td>Bostrychia sp.</td>
<td>detritus</td>
</tr>
<tr>
<td></td>
<td>Dasya sp. (Rhodophyta)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caloglossa leprievi</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oscillatoria sp. (Cyanophyta)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diatoms (Bacillariophyceae)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>DOMINANT SPECIES : Cladophora sp.</td>
<td>Cladophora sp. 80 ± 4%</td>
</tr>
<tr>
<td></td>
<td>Enteromorpha intestinalis (Chlorophyta)</td>
<td>Enteromorpha sp. 10 ± 2%</td>
</tr>
<tr>
<td></td>
<td>Chaetomorpha aerea</td>
<td>Chaetomorpha sp. 6 ± 2%</td>
</tr>
<tr>
<td></td>
<td>ASSOCIATED SPECIES : Bacheletia fulvescens (Phaeophyta)</td>
<td>Miscellaneous : algal spores</td>
</tr>
<tr>
<td></td>
<td>Oscillatoria mixed sp.</td>
<td>diatom (numerous)</td>
</tr>
<tr>
<td></td>
<td>Diatoms (Bacillariophyceae)</td>
<td>detritus</td>
</tr>
</tbody>
</table>
Absorption spectra of substrate, gut content, digestive gland pigment and digestive gland excreta of *Siphonaria zelandica* from mid-eulittoral zone at Castor Bay, Auckland.

--- substrate with predominantly *Enteromorpha*

---- gut content of *Siphonaria*

.... digestive gland homogenate

--- excretory granules from the digestive gland
- substrate with predominantly Enteromorpha
- gut content
- digestive gland
- excretory granules from digestive gland
Fig. 5

Absorption spectra of substrates dominated by different algal species.

Enteromorpha
Gelidium
Hildenbrandia
gut contents and the substrate algae suggest the animal ingests indiscriminately the whole algal material from the substrate. Slight differences may be due to the effects of digestion on the algal pigments. Thus the classification of the gastropods based on the absorption spectra into selective and unselective grazers as suggested by Beckett (1969) would appear very questionable.

2.3 Movement pattern and homing behaviour

Previous studies on siphonariid behaviour suggest that this limpet moves either on ebb tides only (Borland, 1950; Thomas, 1973) or on both ebb and flood phases (Yonge, 1952; Ohgushi, 1954, 1955; Marcus & Marcus, 1960; Cook, 1969). Recently Cook & Cook (1978) studying the effect of tide amplitude upon activity of S. normalis and S. alternata found two distinct behavioural patterns. In the first, movement begins as the animals are wetted by splash during both ebbing and rising of the tides; in the second, activity begins only on or slightly before the ebb and continues for one to two hours during aerial exposure. Wetness of the site during exposure may have important effects on behaviour.

For Siphonaria aelandica, Bedford (1967) reported movement just after aerial exposure, feeding for three hours and homing prior to low water. Beckett (1969) found the same species to move actively during splash periods of both flowing and ebbing tides. Approximately 20% of the sample moved upon submergence and none moved while dry-exposed. The present study has confirmed the effects of wetness of the substrate on behaviour of this animal. In sites B and C at Goat Island Bay, Leigh (Fig. 2), movement was observed only when the tide was out and is
prolonged (approximately 2-3 hrs), with return to the home before the rock dries out or the tide begins to flow. At Castor Bay, however, most individuals moved when splashed during ebb or flow and with the tide out, a few continued to move within sheltered locations such as rock crevices to the leeward of a rock. This pattern of movement, observed also by M. Williams (pers. comm.) at Leigh Cove, is probably related to the degree of exposure to wave and wind which in turn affects the surface dampness of the rock. Sites B and C at Goat Island Bay, Leigh are exposed to strong surge and wave-break, keeping the emersed substrate moist except at the middle of very hot days. On such shores *Siphonaria* can move around during tidal ebb for a long period without desiccation risk. With the strong incoming tide, movement may be reduced by the risk of dislodging. At Castor Bay the shore is sheltered and the rock surfaces are dried off rapidly by wind after the receding tide. *Siphonaria* adapts to this desiccation risk by moving in short periods of splash at both high and low tides.

The movement of *Siphonaria* can thus be affected by wave action, the topography of the shore and also by the weather on a particular day. On calm days, with little or no splash, fewer limpets move. During afternoon, low tides on a hot, dry day *Siphonaria* rarely moves at all. Moreover, some limpets were found to move on each tide, others on alternate tides. The movement pattern is thus a complex response to various interacting physical factors of the microhabitat.

All *Siphonaria zelandica* have been observed to home by Bedford (1967) while Beckett (1969) reported that 100% of his population homed. Among the fifty *Siphonaria* observed in this study during different times
of the year, all except two on the Gelidium mat at site B returned to the "home site". The significance of the exceptions will be discussed later.

The features of the "home site" vary with the substrate. On surfaces covered with thick Gelidium or Enteromorpha, the site is a cleared patch with little algal growth. On several occasions, a limpet was observed manoeuvring within its scar, feeding on the peripheral algae. The shell of Siphonaria is usually covered with the same algae and may be totally concealed when clamped down. With less algal growth in the habitat, Siphonaria usually homes against an irregularity of the rock. The shell is then usually very irregular in shape fitting closely with the corresponding features of the rock (Fig. 4A). The home sites are not depressed or concave as it reported for S. pectinata (Thomas, 1973). The scar is usually closely demarcated by thin algal turf up to the perimeter of the shell. On dry clean surfaces, this rim is more diffuse, and the scar may carry pink patches of lithothamnion, since it is kept moist by the limpet at low tide, being exposed briefly to sunlight when the limpet moves.

Homing to a scar at high tide may reduce dislodgement by waves (Wolcott, 1973) or by predators (Branch, 1975; Cook, 1976), and at low tide reduces desiccation stress (Davies, 1969; Wolcott, 1973; Cook, 1976) or osmotic stress from freshwater run off (Cook, 1976). Homing behaviour may maintain a pattern of dispersion which maximises the availability of food (Underwood, 1979). All Siphonaria species have a relatively weak attachment, dislodging more readily when moving than at rest. Under wave surge, dislodgement is thus avoided by
homing. Unlike true limpets *Siphonaria* is unable to clamp the shell tightly to the rock. The desiccation stress during low tide is avoided by exact homing with accurate shell fit especially by those limpets in dry places. Those on substrates with thick algal growth on the other hand face less desiccation stress which may account for their less strict homing behaviour.

The distances covered by *Siphonaria* during each feeding journey from the home site vary but are usually less than 15 cm for a limpet approximately 10 mm long. The greatest distance recorded was 45 cm for a limpet 22 mm long. The tracks of *S. aelandica* revealed no standard pattern either in direction or extent of movements (see also Beckett, 1969). As in *S. peclinata* (Thomas, 1973) there is no constant speed. The outward rate is usually slow (0.1 - 0.2 cm/min.) interrupted by frequent pauses during which some feeding takes place. At the end of the outward journey the limpet usually stays for a long period in approximately the same spot, swinging its head from side to side as well as periodically shifting the direction of its body. On the outward journey, it scans the habitat, sampling the algal growth periodically and at a suitable patch, it feeds vigorously until the stomach is full or until dryness stimulates the return journey. The return trip is usually much faster than the outward one (0.4 cm/min.). A common movement pattern involves going out in a more or less straight line and returning along or close to this line (Fig. 7). Less frequently *S. aelandica* returns home by a different path, describing a loop or circle (Fig. 7f-i) as in *S. peclinata* (Thomas, 1973). Contact with home sites does not in itself terminate movement nor does crossing the
Typical paths followed by *Siphonaria zelandica* during feeding excursions on the steep *Gelidium* bank at site B.

- □ - home of limpet
- • - positions during movement at intervals of five minutes.
previous path necessarily initiate a return trip (Fig. 71). Other activities such as copulation and egg-laying may also occur during the journey. On several occasions when large limpets encountered each other, copulation took place. From November to February, yellowish jelly-like spawn crescents are deposited (Fig. 4D). Copulation or oviposition may interrupt a journey for as long as 30 minutes.

The mechanism of homing of intertidal gastropods has been studied intensively and reviewed recently by Underwood (1979). Several possible methods such as kinaesthetic memory, topographic memory or mucus trailing were suggested (Thorpe, 1963; Wells, 1965). Chemical means however have been found to be the most likely (Cook et al., 1969). Cook (1969, 1971) found S. normalis and S. alternata closely followed the mucus trails already laid down and may accept another limpet's scar. S. zelandica usually produces a thick mucus trail and more than 80% of the animals observed homed either along or very close to the mucus trail of the outward journey. This need not eliminate the possibility of either kinaesthetic or topographic memory in homing. Physical and chemical clues may both be used.
CHAPTER 3

THE MORPHOLOGY AND HISTOLOGY OF THE DIGESTIVE SYSTEM

3.1 INTRODUCTION

The digestive system of pulmonate gastropods has been widely studied since the end of the last century and the vast literature has been reviewed by Owen (1966), Hyman (1967), Franc (1968), Purchon (1968) and more recently by Runham (1975). Most of these studies, however were concerned with freshwater or terrestrial pulmonates, important respectively as disease vectors and as agricultural and horticultural pests (Runham, 1975). The most detailed works are those on *Lymnaea stagnalis* by Carriker (1946a, b) and Carriker and Bilstad (1946) and on *Agriolimax reticulatus* by Walker (1969, 1970a,b, 1972).

Work on the digestive system of marine pulmonates has been very limited, the most extensive studies being those of J.E. Morton (1955a,b,c) on the primitive family Ellobiidae. With Siphonariidae, the literature is very scanty indeed. Very brief accounts of the morphology of the alimentary system of various siphonariids were included in the works of Cottrell (1911) on *Siphonaria obliquata* (= *Benhamina obliquata*), and of Marcus & Marcus (1960) on *S. hispida*. A more extended description of the structure and function of the digestive system of *S. zelandica* was given by Bedford (1967) but her histological work concerned only the digestive gland and was very brief.

This study therefore investigates in detail the structure and function of the whole digestive system of *Siphonaria zelandica*. For
convenience of presentation, the whole digestive system is arbitrarily divided into four parts: 1) the feeding apparatus which includes the mouth and the buccal mass (Section 3.3); 2) the digestive tract which runs from the buccal cavity, through the oesophagus, stomach, intestine to the rectum (Section 3.4); 3) the salivary gland which opens into the buccal cavity, producing mucus for lubrication and some enzymes to initiate digestion (Section 3.5); 4) the digestive gland which opens into the stomach, acting as the main site of absorption and intracellular digestion (Section 3.6).

Morphological techniques included detailed microdissection, histology with both light and electron microscopy and various histochemical tests. The function of different parts of the digestive system was studied by various procedures. The rate of production of radular teeth was investigated by two methods. The feeding movements of the buccal mass were observed in Siphonaria and compared with those reported for other gastropods (Hubendick, 1957; Mürkel, 1957; Fretter & Graham, 1962). The functions of various parts of the digestive tract as well as the salivary and digestive glands were ascertained by feeding experiments and by histochemical tests. The cycle of histological and functional changes in the cells of the digestive glands was studied in relation to feeding activity.
3.2 MATERIALS AND METHODS

Specimens of *Siphonaria zelandica* were collected from three localities (Castor Bay in Auckland, Goat Island Bay, Leigh, and Piha Beach, West Coast) (Fig. 1B) during low tide.

A. Morphological Study

The animals were dissected fresh or after narcotisation with menthol crystals sprinkled on the surface of the seawater for 2-3 hrs. The animals were carefully removed from the shell and dissected in seawater under a dissecting microscope. Observations were recorded by drawings with the aid of a camera lucida. Any movements or contractions of the digestive system were noted and timed when necessary. Ciliation on different parts was studied by dropping a suspension of carmine particles or Aquadag colloidal graphite on the surface in question and the direction of any ciliary current was noted.

For study of the buccal musculature, both fresh and fixed materials (either in Bouin's fixative or 10% buffered neutral formalin) were used, but fresh material was found more suitable, as fixation renders the muscle too brittle for manipulation. The muscle bundles were traced one by one to determine their origin and insertion. The relative position of the various musculature was derived by drawing the different buccal muscles in a simple way in their natural spatial relations and comparing similar drawings of different views and sections of the buccal mass.

For study of morphology of the radula, the radula sac was dissected out and the radula isolated according to the method suggested by Meeuse
(1950). Tissue attached to the radula was removed by soaking in 5% KOH for 12-14 hrs. The radula was cleaned in a drop of glacial acetic acid and washed thoroughly with distilled water. It was then flattened and dried between two glass slides, and when dry, mounted with DPX directly or after staining with chryosodin or Van Gieson stain.

B. **Histological Study**

1. **Light Microscopy**

   Animals were fixed whole after removal from the shell either immediately or a few hours after collection. For routine histological examination, Bouin's, Zenker's, Susa fixatives or 10% buffered neutral formalin were used; of these Susa gave the best overall histological results. Material was dehydrated in an ethanol series and embedded in paraplast wax (melting point 56 - 57°C). Sections at 6-10μ were cut and stained with Heidenhain's azan, Haematoxylin with Van Gieson, Pollock's rapid trichrome or Mallory-Heidenhain's trichrome stain. In addition, thick sections (1-2μ) of materials, fixed and embedded in Epon for electron microscopy as described later, were also examined by light microscopy using Loeffler's methylene blue stain, 1% toluidine blue in borax solution or a polychrome stain according to Jha (1976).

2. **Histochemical Tests**

   Both wax sections and Epon thick sections were used for this work. When the latter were used, the procedures for removal of the resins recommended by Snodgress *et al.* (1972) or Maxwell (1977) were followed prior to application of any histochemical tests. Special techniques employed for the detection of tissue components and cellular contents
follow Humason (1967) and Pearse (1968, 1972) except where otherwise stated. They were:

- Acrolein–Schiff method for protein.
- Mercury–Bromophenol Blue method for proteins.
- Periodic Acid Schiff (PAS) technique for carbohydrates.
- Alcian–Blue method for acid mucopolysaccharides.
- Colloidal Iron method for acid mucopolysaccharides.
- Aldehyde Fuchsin for sulphate and carboxyl groups.
- Toluidine Blue for metachromasia.
- Sudan Black B method for bound lipid (Eurenius & Jarskar, 1970).
- Schmorl's method for lipofuscins (and other reducing substances).
- Chrome Alum Haematoxylin method for lipofuscins.
- Nile Blue method and Alternative Nile Blue for distinguishing melanins from lipofuscins.
- Von Kossa method for "calcium" deposits.
- Alizarin Red S method for calcium.

3. **Electron Microscopy**

Different parts of the digestive system were dissected out from the animal and fixed in three types of fixatives at 4°C: 1% glutaraldehyde in 0.2 M cacodylate buffer pH 7.2 with calcium acetate added; 3% glutaraldehyde in 0.4 M cacodylate buffer pH 7.2; or 6% glutaraldehyde in 4/3 marine phosphate buffer pH 7.5. All these fixatives gave satisfactory results but the 3% glutaraldehyde in cacodylate buffer was the most successful. The tissues were cut into small pieces (< 1 mm thick) and fixed in the chilled fixatives for 2 hrs and then washed in several
changes of the corresponding buffer. Some tissues were stored in buffer before post fixation. Post fixation was with 1% osmium tetroxide in the appropriate buffer for 1 hr. Dehydration was carried out using an ethanol or rapid acetone series. The tissue was embedded in Epon 812 and sectioned on glass knives on a manual Porter Blum or a Reichert ultramicrotome. Sections were picked up on formvar coated single-hole grids or mesh copper grids, then stained in a 1:1 solution of 4% aqueous uranyl acetate solution for 3-5 mins and counterstained with lead citrate for 30 sec. Ultrathin sections (6000-7000 nm) were viewed and photographed on a Philips EM200 or EM301 electron microscope.

4. **Scanning Electron Microscope**

Osmium post-fixed material for electron microscopy as described above was dehydrated in a graded acetone series and dried in a Polaron critical point dryer. For luminal surfaces of the alimentary system and the mantle cavity which contain thick mucus obscuring the surface structures, the material prior to the critical point drying procedure was bathed in 16% glycerol in the appropriate buffer for 24 hrs and transferred to 20% ethyl alcohol for another 2 hrs to remove the mucus (Dirksen, 1974). The material was mounted on double sided sellotape or PVC glue on brass stubs, coated with gold using a sputter coater, then viewed on a JOEL U3 scanning electron microscope. For scanning of the radula, the radula was dissected and prepared as for light microscopy, then dehydrated and mounted as above.

C. **Radula Growth**

The radular teeth of gastropods are known to be periodically shed
and passed out in the faeces (Geyer, 1927; Carriker, 1943; Quick, 1960; Kühnelt, 1961). Ten animals were kept under a simulated tidal cycle (by immersion in seawater for 3-4 hours twice a day in the laboratory) and fed with Enteromorpha collected from the shore. The faeces produced by each animal each day was collected. The number of radular teeth and the number of rows of teeth in the faeces were noted.

The rate of radula growth was also measured directly using the cold shock treatment of Isarankura and Runham (1968). The animals were collected from their natural habitat and allowed to settle in a jar with moist Enteromorpha. The jar with the animals was then kept in the refrigerator at 4-6°C for 24 hours. Afterwards the animals were maintained in a tidal tank. After 10 days they were killed and the radula extracted for observation and measurement.

D. Feeding Tracks

Glass plates were left on the upper area of an outdoor tidal-simulated tank for three to four weeks until they became opaque with a layer of algae. Active specimens of Siphonaria selandica were collected from the shore, starved for 24 hrs. and placed on the plates to observe their feeding activity. Feeding traces left were studied and recorded.

E. Cycle of Activity of the Digestive Gland

Ten animals were collected at two-hourly intervals for 24 hrs. from the reef at Goat Island Bay, Leigh in October 1976, fixed in 10% buffered formalin, dissected and sectioned. The position of food material in the gut was recorded and the sections of the digestive glands were examined for the features of the tubules. The experiment was repeated
in February 1977 with five animals for each one and a half hour intervals for 24 hrs and the results were compared.

A laboratory experiment was also conducted to confirm the relation of the cycle of the digestive gland tubule with the feeding cycle. Animals were collected from the shore and kept in the laboratory under a simulated tidal cycle. They were starved for 48 hrs and then fed on Enteromorpha filaments coated with Aquadag colloidal graphite. After they started feeding two of them were fixed hourly for 12 hrs and the fixed specimens were sectioned. The feature of the digestive tubules and the location of the graphite particles were recorded and the results compared with those from the field experiments.
3.3 THE FEEDING APPARATUS

3.3.1 OBSERVATIONS AND RESULTS

3.3.1.1 General Morphology of the Mouth and Buccal Cavity

The mouth is situated mid-ventrally towards the anterior extremity of the body. When the animal is not feeding, it appears as an inconspicuous, crescent shape aperture lying in a slight depression. The mouth is bordered with folds, the labial or oral lappets, a ventral one with two distinct lobes and the dorsal one plicated into at least ten small lobes (Fig. 8a). Internally it is delineated by the buccal sphincter muscle which fuses with the skin and the wall of the snout. With the contraction of this muscle and also the muscles in the lappets, the mouth can be reduced to an indistinct transverse slit. The mouth encloses a small vestibule anterior to the cavity of the buccal mass proper.

The buccal mass is a pyriform muscular organ, bright red in colour from the presence of muscle myoglobin (Section 6.3). It is large in comparison with the size of the body (Length of buccal mass : Length of body = 0.26 ± 0.03), and contains two supporting structures: the odontophore cartilage and the radular collostyle, with numerous muscle bundles functioning together for the feeding movements (Fig. 9,10,11).

Dorsally from near the mouth and leading backwards along the roof of the buccal cavity, the buccal epithelium carries a cuticle which is soft and velvety in consistency. This cuticle thickens on the dorsal edge of the mouth to form a dark-brown crescent, the jaw. The buccal sphincter muscle is attached to the concave posterior surface of the jaw. The dorsal food channel extends as a median groove along the roof
of the buccal cavity into the oesophagus. Its epithelium differs from that of the buccal cavity by its heavy ciliation.

The buccal epithelium is securely affixed to the subepithelial tissues everywhere but upon the surface of the cartilage which is free to move beneath the epithelial lining. Behind the crest of the odontophore, the epithelium evaginates to form the radular sac. The sac consists of a single-layered envelope of epithelial cells which folds upon itself partially leaving a median longitudinal slit on the dorsal side so that it appears horse-shoe shape in cross-section (Fig. 8c). The radula lies within this fold between the two epithelial layers, the subradular epithelium below and the suprabradular epithelium above. Supporting the fold beneath the suprabradular epithelium is the collostyle. At the proximal end of the radular sac the epithelium folds to form the collostyled hood. Proximally the subradular epithelium with the radula curves downwards to invest the anterior tip of the odontophore cartilage. Distally the whole radula sac curves upwards to protrude slightly behind the buccal mass. The whole organ is thus in form of a horizontal S (Fig. 11).

3.3.1.2. The Buccal Musculature

I. Arrangement of the Muscles

There are altogether twenty-eight muscles separately identifiable in the buccal mass. The terminology and classification proposed by Carriker (1946a) and later used by Demian (1960, 1962) and Hembrow (1973), are adopted here for convenience of comparison. Each muscle in the
Fig. 8  Buccal Mass

a. Scanning electron micrograph of the mouth region of *Siphonaria zelandica* showing the dorsal labial lappets (La), the small ventral labial lobes (Lo), the deeply sunken mouth (x) and the foot (F).  (x 100).

b. Scanning electron micrograph of the mouth region with the jaw (J) protruded and the ventral labial lobes (Lo) distended. The mouth (M) becomes T-shaped.  (x 175).

c. Transverse section of the radular sac showing the arrangement of the radular teeth (T), the subradular epithelium (E₁), the supraradular epithelium (E₂) and the collostyle (C).
(Paraffin section, Mallory Trichrome staining;  x 975).  
following description is given a number which corresponds to the illustrations (Fig. 9,10,11).

A. **Extrinsic Muscles from the Body Wall to the Buccal Mass**

1. **Dorsal Lateral Protractors** (Fig. 9,10c)

   This pair of thin but wide bands of muscle originate from the anterior body wall, on either side of the mouth and are inserted externally on the posterior dorsolateral surface of the buccal mass. On contraction they pull the buccal mass forwards and at the same time tend to elevate it behind.

2. **Preventral Protractors** (Fig. 9b,10a)

   These are two short and relatively thin muscles, dividing into a sheet of slender bands that flare forwards below the buccal mass. They originate on either side of the anterioventral part of the body wall immediately behind the mouth and insert on to the anteroventral and ventrolateral external walls of the buccal mass.

3. **Postventral Protractors** (Fig. 9b,10a)

   This pair of thin muscle bands originates from the ventral part of the body wall below the middle part of the buccal mass and inserts onto the posteroventral side of the buccal mass, on the lower posteromedial angles of the cartilage. From their shortness and their oblique position with respect to the whole buccal mass, it appears that instead of affecting a powerful protraction, their main role is probably to pull the buccal mass down and at the same time expand the posterior portion of the cartilage.
4. **Dorsolateral Retractors** (Fig. 9,10c)

These are two thin, narrow muscles sheets originating from the anterior lateral body walls above the mouth and inserted externally on the anterior dorsolateral surface of the buccal mass. With their anterior origin, they seem unable to effect the actual backward retraction, but rather pull the anterior position of the buccal mass upwards.

5. **Buccal Retractors** (Fig. 9b,10b,c)

A pair of very short, thick and strong muscle bundles which originate from the sides of the foot below the middle part of the buccal mass and run obliquely forwards to insert on the ventral surface of the anterior-most portion of the buccal mass and lips. As distinct from other pulmonates (Demian, 1962; Hembrow, 1973) they do not pass through the nerve ring.

6. **Preventral Levators** (Fig. 9,10c)

This pair of thin, narrow muscles runs forward on each side of the anterior portion of the buccal mass. They originate on the anterior lateral body wall above the origin of the dorsolateral protractors (1) and below the dorsolateral retractors (4), being inserted externally on the side of the buccal mass on the mandibular approximator (16).

7. **Postventral Levators** (Fig. 9,10a,c)

These appear as two flattened muscular bands, greatly divided and running parallel on each side of the buccal mass. They originate from the lower lateral body wall beside the points of origin of the preventral
Fig. 9  Buccal Musculature

a. Dorsal view  and  b. Lateral view of the buccal mass showing the position and arrangement of the extrinsic buccal muscles on the buccal mass. Part of the oesophagus and salivary gland have been removed to reveal the muscles beneath. The muscles are numbered according to the descriptions in the text.

BG  -  buccal ganglion
OE  -  oesophagus
RS  -  radular sac
SG  -  salivary gland.
levators (6) and are inserted on the posteroventral external surfaces of the buccal mass, in front of the preventral protractors (3). Their low position of origin indicates that they are less powerful than the homologous levator muscles in other pulmonate snails (Demian, 1962).

8. **Suboral Dilators** (Fig. 9b,10a,b)

These consist of a number of short, slender muscle bands, radially arranged in a semicircle around the ventral anterior end of the buccal mass.

9. **Dorsal Mandibular Dilators** (Fig. 9,10c)

These consist of a number of short, slender muscle bands that work in conjunction with the suboral dilators (8) in opening the mouth, as well as in drawing the jaw upwards and downwards. They radiate dorsally and dorsolaterally from the anterior edge of the buccal mass along the site of attachment of the jaw and originate from the dorsal body wall above the mouth.

10. **Suspensor of the Radular Sac** (Fig. 9)

This is a short and diffuse median muscle originating on the buccal commissure at the base of the oesophagus. It passes over the posterior buccal nerve to be inserted at the distal end of the radular sac and serves to suspend the posterior part of the radular sac protruding behind the buccal mass.

11. **Labial Retractors**

These are formed of a large number of fine fibres located within the anterior wall of the snout, radiating all round the lips. They draw the lips away from the mouth in a "snarling" movement during the protrusion
of the odontophore.

12. **Labial Sphincter**

This is a circular diffuse muscle band located within the anterior wall of the snout. Surrounding the mouth, it serves to open and close its aperture.

B. **Intrinsic Muscles from the Cartilage to the Jaw**

13. **Anterior Jugalis** *(Fig. 9b, 10a, 11)*

This is a conspicuous, thin external muscular sheet that originates from the median dorsal surface of the anterior portion of the buccal mass and the lateral corners of the jaw. Passing through to the buccal sphincter (15) it continues superficially on either side of the posterior half of the buccal mass to insert on the ventrolateral edges of the cartilage. It functions in suspending, elevating and protracting the odontophore (c.f. similar muscle in *Lymnaea* (Carriker, 1946a)).

14. **Posterior Jugalis** *(Fig. 9b, 10c, 11)*

This is a broad, but very thin superficial muscular coat that originates within the dorsal wall of the anterior portion of the buccal mass. It spreads over the dorsal wall of the buccal cavity under the buccal sphincter (15) and then extends superficially over the posterior portion of the buccal mass, covering most of its dorsal, lateral as well as posterior surfaces, to insert on the ventrolateral external edges of the cartilage and the distal end of the radular sac. It serves to pull the ventral end of the odontophore upwards and forwards and, together with the other intrinsic and extrinsic muscles, helps to tilt the
Fig. 10  Buccal Musculature

a. Ventral view of the buccal mass after removal from the body wall with the pre-ventral protractors (2) and the post-ventral protractors (3) trimmed down.

b. Ventral view of the buccal mass after removal of the extrinsic muscles (except the buccal retractors (5) and the suboral dilators (8)), the anterior jugalis (13), the posterior jugalis (14) and the ventral odontophoral protractors (19). The left halves of the buccal sphincter (15) and the infralateral cartilage tensor (23) are also removed to expose some of the deeper intrinsic buccal muscles.

c. Posterior dorsal view of the buccal mass with the posterior jugalis (14) partly removed and the right supralateral radular tensor (21) cut and pulled sidewardly to expose the odontophore.

d. Ventral view of the buccal mass with the cartilage cut in the mid-line and the left half pulled laterally to show the insertion of the intrinsic muscles from the cartilage to the radula and radular sac.

J  -  jaw
M  -  mouth opening
OD -  odontophore
OE -  oesophagus
R  -  radula
RS -  radular sac
SD -  salivary duct

The muscles are numbered according to the descriptions in the text.
odontophore axis from its resting position (sloping backward) to a forward sloping position during its protrusion from the mouth. By its attachment to the radular sac it appears also to help in suspending that organ.

15. **Buccal Sphincter** (Fig. 10a,b,11)

This is a fairly broad and thick muscle encircling the anterior part of the buccal mass between the cartilage and the mouth. It is covered dorsally by the anterior jugalis (13) and anteroventrally by the mandibular approximator (16). This obviously encloses the buccal cavity, helping on contraction to push the food bolus into the dorsal channel and also to thrust the odontophore further inwards in the swallowing stage.

16. **Mandibular Approximator** (Fig. 9b,10a,b,d,11)

This is another thick and broad muscular band that is inserted on the side of the jaw at the dorsolateral angle. It passes around the ventral wall of the buccal mass lying in front and partly adjacent to the buccal sphincter (15). On contraction, it depresses and bends the jaw.

17. **Dorsal Odontophoral Flexors** (Fig. 10b,c)

These are two thin and inconspicuous muscles that originate from the dorsal wall of the buccal cavity and the lateral angles of the jaw. They spread close over the dorsal epithelium of the buccal cavity, below the posterior jugalis (14) and the buccal sphincter (15) to insert anteroventrally on the lateral aspect of the cartilage about one-third of the way from the anterior tip. They help in orientating and directing the odontophoral cartilage and also in drawing the whole odontophore
forwards towards the mouth. The pull applied to the sides of the
cartilage tends to bulge the cartilage slightly, and thus stretch out
tightly the radula on its surface.

18. **Ventral Odontophoral Protractors** (Fig. 10b,11)

These are two broad, thin sheets which originate from the lips
and the ventral wall of the buccal cavity at the anterior edge, close
to the jaw. Running longitudinally below the epithelial lining of the
buccal cavity, they then pass below the inframedian radular muscle (22)
to insert on the anterior part of the ventrolateral face of the cartilage.
The muscle bundles thicken laterally where they meet the dorsal odontophore
flexor (17).

19. **Infraventral Odontophoral Protractors** (Fig. 9b,10a,b)

These thick, broad muscles form the external ventral wall of the
buccal mass. They originate externally from the anteroventral wall of
the buccal mass, on the anterior mandibular approximator (16) and the
buccal sphincter (15) and are inserted upon the ventral posterior edge
of the cartilage adjacent to the insertion of the anterior jugalis (13).
The ventral portions of the mandibular approximator (16), the buccal
sphincter (15) and the infralateral cartilage tensor (23) lie sandwiched
between the infraventral and the ventral odontophoral protractors (19
& 18) both of which serve together to protract the odontophore and
posterior buccal mass.

C. **Intrinsic Muscles from the Cartilage to the Radula and Radular Sac**

20. **Supramedian Radular Tensors** (Fig. 9,10,11)

These are two thick, muscle sheets originating from the ventrolateral
Fig. 11

Drawing of the right half of the buccal mass seen from the left side after the buccal mass has been cut in the median sagittal plane.

C - collostyle
CH - collostylar hood
J - jaw
OD - odontophore
OE - oesophagus
R - radula

The buccal muscles are numbered according to the descriptions in the text.
edges of the cartilage. They pass ventrally inwards to run forward in the posterior median concavity of the cartilage to insert on either side of the radular sac.

21. **Supralateral Radular Tensors** (Fig. 9b,10,11)

This pair form the broadest and bulkiest muscle bands of the buccal mass. They are conspicuous on either side of the posterior half of the buccal mass through the transparent posterior jugalis (14). They originate from the ventrolateral edge of the cartilage and are inserted partly on the anterior dorsolateral aspect of the radular sac, partly on the under-surface of the subradular epithelium in front of the radular sac, and partly under the buccal epithelium on either side, in front of the odontophoral cartilage.

22. **Infframedian Radular Tensors** (Fig. 10b,11)

These two broad, thin sheets originate ventrally from the posterolateral edge of the cartilage, and run forward, close beneath the cartilage over its curved anterior end to insert near the mid-ventral line beneath the subradular epithelium. Muscles 20, 21, 22 evidently bind the radula firmly to the cartilage preventing it from slipping, yet allowing forward and backward movement over the cartilage during rasping.

23. **Infrralateral Cartilage Tensor** (Fig. 10b,d,11)

This broad, thick, bow-shaped muscle passes transversely between the two lateral edges of the cartilage. It is covered by the buccal sphincter (15) anteriorly, partly by the infraventral odontophoral protractor (19) and partly also by the anterior jugalis (13); it lies between the ventral
and infraventral odontophoral protractors (18 & 19). The tensor maintains the radula in the stretched condition as muscles 20, 21 and 22 contract to fit the proximal end of the radula tightly over the anterior tip of the cartilage.

24. **Intracartilage Tensors**

These are constituted by diffuse fibres running through the cartilage at right angle to its dorsal and ventral surfaces. In *Lymnaea*, such fibres with the turgid vesicular cells in between act as a pair in altering the shape of the cartilage during the feeding cycle (Carriker, 1946a). Contraction of the fibres in the mid-portion results in a flattened and expanded cartilage while the lateral fibres slightly bulge and draw together the lateral extremities of the cartilage.

24a. **Intercartilage Contractor** (Fig. 10b)

This is a thin, narrow band running transversely between the two posterior processes of the cartilage, holding them together.

D. **Intrinsic Muscles Passing Outwards from the Radular Sac**

25. **Lateral Suspensor of the Radular Sac** (Fig. 10b,c,d)

These are two short, horizontal muscle bands which originate from the medial posterior surface of the supralateral radula tensor (21) and are inserted at either side on the distal end of the radular sac.

26. **Inferior Suspensor of Radular Sac** (Fig. 10a,b,d)

This is a short, thin muscle band that flares forward from the posteromedian edge of the cartilage to insert distally on the ventral surface of the radular sac.
27. **Superior Suspensor of the Radular Sac** (Fig. 9a, 10c)

This relatively long and thick muscle band arises from the posteroventral edge of the buccal epithelium near the opening of the radular sac and runs medially over the radular sac to insert on its dorsal surface near the distal end.

28. **Tensor of the Hood** (Fig. 10c)

This is composed of short diffuse muscle bands originating from the inner surface of the radular sac to insert on the under-surface of the collostylar hood. It serves to hold the hood during rasping and prevents it from obstructing the passage of food to the dorsal food channel.

3.3.1.3 **Buccal Epithelium and Jaw**

**Buccal Epithelium**

The buccal epithelium is made up of tall columnar cells (25-35μ) carrying a thick cuticle (Fig. 13a). Scattered between these columnar cells are mucous goblet cells similar to the muciparous cells of the epidermis (Section 5.3). Some of the mucous cells are displaced subepithelially to open through the epithelial layer and cuticle by narrow extensions.

The cuticle is a clear layer staining lightly with PAS and Bromophenol Blue. Its secreting cells (buccal chitinogenic cells) (Fig. 12) are slightly vacuolated with an ovoid nucleus and are structurally very different from the radular chitinogenic cells (Section 3.3.1.5).

Ultrastructurally, the cuticle is a dense homogeneous layer with
its bottom third penetrated by microvilli and large cell processes 
(Fig. 13b). Some of the latter extend to the middle of the cuticle 
forming a sort of canal system. These processes contain dense filaments 
in the centre and round the periphery. The microvilli contain dense 
bundles of filaments and small clear vesicles, both of which are very 
abundant in the apical cytoplasm. Some filaments and vesicles are also 
present in the newly-produced basal layer of the cuticle, indicating 
that they are being secreted by the cell to form the cuticle.

The apical region of the chitinogenic cell has, as well as filaments 
and vesicles, numerous mitochondria and some multivesicular bodies 
(Fig. 13c). The middle of the cell is filled with numerous irregular-
shaped, dense granules and some electron-lucent vacuoles (Fig. 14). 
Free ribosomes, short lengths of granular endoplasmic reticulum (GER) 
and several Golgi complexes are also present, the latter consisting of 
unusually compressed stacks of dense lamellae surrounded by small dense 
vesicles (Fig. 13d). The basal nucleus has little chromatin, but 
ocasionally a nucleolus is present.

The lateral plasma membrane is much folded, interdigitating with 
that of the neighbouring cells (Fig. 13e). Towards the apex are 
junctional complexes with a distinct zonula adherens followed by a region 
of septate junctions (Fig. 13e). The folded basement membrane lies on 
a thin layer of connective tissue backed by muscles. A separate basal 
lamella is absent. Desmosomes with distinct macula adherens and filaments 
extending on both sides are present on the basement membrane (Fig. 14).

Jaw

Across the dorsal anterior end of the buccal cavity just inside the
Fig. 12  The Buccal Chitogenic Cell.

bc  -  buccal cuticle

d  -  dense body

f  -  microfilaments

g  -  Golgi complex

jc  -  junctional complex

m  -  mitochondrion

ma  -  macula adherens

mv  -  microvilli

n  -  nucleus

pc  -  pore canal

pm  -  plasma membrane
Fig. 13  Buccal Epithelium

a. Light micrograph of a section of the buccal epithelium showing the buccal cuticle (↑) and the buccal chitinogenic cells (B). (Epon thick section, Toluidine Blue staining; x 2200)

b. Electron micrograph of apical region of the buccal chitinogenic cell showing the dense homogeneous cuticle (c) penetrated by microvilli (mv) and large processes (p) from the cell. In the middle region of the cuticle there is a canal system (↑) with dense central and peripheral filaments. The apical cytoplasm of the cell contains bundles of microfilaments (↑) and small clear vesicles. (x 10800)

c. Apical junctional complexes of buccal chitinogenic cells with zonula adherens (za) followed by septate desmosomes (sd). (x 42650)

d. Electron micrograph of the mid-region of the buccal chitinogenic cell. Note the active Golgi complex (g) surrounded by strands of GER (↑), some dense bodies (d) and a few electron-lucent vacuoles (v). (x 43950)

e. Interdigitation of the lateral plasma membrane of the buccal chitinogenic cell. (x 24400)
Fig. 14 The Buccal Chitinogenic Cell

The microvilli (mv) of the cell contain dense microfilaments (f) and extend into the lower half of the buccal cuticle (c). The middle region of the cuticle is penetrated by a few larger processes resembling pore canals from the cell. The cell has numerous dense bodies (d), a few mitochondria (m) a Golgi complex (g) and a large basal nucleus (n). The basal plasma membrane is slightly folded and a few macula adherens (↑) are present. (x 11500)
Fig. 15  The Jaw-secreting Cell

ap  -  apical process
bc  -  buccal cuticle
ger -  granular endoplasmic reticulum
J   -  jaw material
jc  -  junctional complex
m   -  mitochondrion
n   -  nucleolus
pm  -  plasma membrane
sg  -  secretory granule
Fig. 16 The Jaw

a. Scanning electron micrograph of jaw region showing the rows of rod-like structures of the jaw. (x 2500)

b. Light micrograph of a section of the jaw showing the jaw secreting cells (J), each secreting a rod-like structure (R). Note the adjacent buccal chitinogenic cells (B) have secreted extra thick buccal cuticle (C). (Paraffin section, Alcian Blue staining; x 1600)

c. Electron micrograph of the apical region of the jaw secreting cells (j) showing the dome-shaped layers (↑) of dense alveolar material secreted by the cell. Note the less dense buccal cuticle material (C) in between the rods. (x 8300)

d. Electron micrograph of the jaw-secreting cell showing the dense secretory granules (↑), the apical concentration of mitochondria (m) and the secreted jaw material (s). (x 21000).
Fig. 17  Jaw-secreting Cells

a. Electron micrograph of the jaw-secreting cell showing the extensively developed GER in the cell body (ger). Note also the dense connective tissue lining the cell (])-> (x 5000).

b. Electron micrograph of the basal region of the jaw-secreting cell. Note the irregular-shaped, densely chromatic nucleus (n) and the extensive GER producing the dense secretory granules ( )->). The mitochondria (m) have distinct parallel cristae. Note also the much interdigitated lateral plasma membrane (Δ). (x 18000).
mouth, the buccal epithelium forms a slight fold, producing a brown, crescentic piece, the jaw. Though apparently continuous with the buccal cavity, the jaw is built of rows of rod-like structures (Fig. 16a) which were inert to all the histochemical tests employed. The cells secreting the rods are located at the end of the epithelial fold, and are large and pyramidal (40 - 45μ in height) (Fig. 15, 16b). The buccal chitinogenic cells on either side of the fold secrete a thicker cuticle than elsewhere, forming a matrix from which these rods appear to project.

Ultrastructurally each rod consists of dome-shaped layers of dense alveolar material lying on top of its secretory cell (Fig. 15c). The space between the base of the rods is filled with less dense, homogeneous cuticle. The lowermost layer of each rod, immediately above each cell is penetrated by cell processes from the cell. These processes and the apical region of the cell are filled with numerous dense granules (Fig. 16d). Beneath these granules, the cell has a dense layer of mitochondria with parallel cristae. The cell body is filled with distended GER cisternae, from which the dense granules appear to arise (Fig. 17a). No Golgi complex has been seen. The irregular basal nucleus is densely chromatic. The lateral plasma membrane is much folded interdigitating with the adjacent cell (Fig. 17b). The basal membrane with a thin basal lamella is only slightly folded.

3.3.1.4 The Odontophore

The odontophore in *Siphonaria selardica* forms a horse-shoe-shaped piece, gently convex ventrally and deeply excavated dorsomedially, to form a furrow between two thick side limbs (Fig. 18). The medial
posterior notch is deep allowing the penetration of the ventral buccal artery into the radular sac which rests within the dorsal cavity. The side limbs of the odontophore are connected posteriorly by a narrow muscle band - the intercartilage contractor (24a). The anterior part of the radula spreads over the front tip of the odontophore like a hood. The posture of the odontophore is adjusted by the buccal muscle which produces the rasping action of feeding. (The detailed movement of the odontophore during ingestion is described in Section 3.3.1.6).

The odontophore tissue, for which the term "cartilage" is widely used, contains numerous muscle fibres, mostly running dorsal-ventrally (24). Between these are large vacuolated connective tissue cells which did not react to any of the histochemical tests employed (Fig. 19a).

The long muscle cells have myofilaments arranged lengthwise but irregularly distributed leaving areas of the cytoplasm, especially near the nucleus, free of filaments (Fig. 19b). Both thick paramyosin and thin actin filaments are present. In longitudinal section, the paramyosin filaments have a variable diameter with slight periodic banding (Fig. 19c) while in transverse section they are roughly circular, 20-100 nm across (Fig. 19d). Vesicles and small tubules are frequently present beneath the sarcolemmal membrane. Tubules also occur within the cell body (Fig. 19c). Characteristic dense bodies, occasionally with filaments attached are sometimes fastened to the plasma membrane or may appear free within the cell. Other organelles include mitochondria, ribosomes, short GER and occasional Golgi complexes. Numerous glycogen granules are usually present. The oblong nucleus contain very little chromatin material but a large distinct nucleolus.
Fig. 18  The Odontophore

a. Posterior view, and b. Dorsal view of the odontophore

Muscle 24a  -  intercartilage contractor.
Fig. 19  The Odontophore

a. Longitudinal section of a limb of the odontophore. The odontophore contains numerous muscle fibres (↑) mostly running dorsal-ventrally with large connective tissue cells (V) in between. (Paraffin section, Mallory-Heidenhain's Trichrome stainings; x 600).

b. Electron micrograph of the middle region of an odontophore muscle cell. The nucleus (n) has a prominent nucleolus (no). The myofilaments (f) are arranged lengthwise but irregularly distributed. Note also the tubules (t) near the nucleus. (x 8100).

c. Electron micrograph of a longitudinal section of the odontophore muscle cell showing the paramyosin fibres (↑) of variable diameter and the fine actin filaments (↓). Note also the dense bodies (d) which are attached to the myofilaments and the tubules (t) in between the myofilaments. (x 35000).

d. Electron micrograph of a transverse section of the odontophore muscle cell showing the fine actin filaments (↓) surrounding the thick paramyosin fibres (↑) and the occasional dense body (d). (x 64000).

e. Electron micrograph of part of the odontophore muscle cell showing the extensive tubular system (t). Dense bodies (d) with filaments attached are randomly distributed. Mitochondria (m) with densely packed parallel cristae are present in between the myofilaments. (x 10000).
Fig. 20 Odontophore Connective Tissue Cell

a. Electron micrograph of a connective tissue cell (V) surrounded by muscle cells (M). The connective tissue cell has a large oblong nucleus (n). (x 5500).

b. Electron micrograph of part of a connective tissue cell of the odontophore showing the reticulate material which is loose in some places (rl) and dense in others (rd). Note also the tubular structures (t) which appear to project from the adjacent muscle cell. (x 27500).
The connective tissue cell of the odontophore is large and elongate. Apart from a few vesicles or cisternae and a large, oblong, central nucleus, the cells are devoid of other organelles (Fig. 20a). The cytoplasm is filled with a moderately electron-dense reticulate material which is very loose in some places and very dense in others (Fig. 20b). The nucleus is sparsely chromatic. Adjacent to the muscle cells, the connective tissue cells have attached to their plasma membrane numerous dense vesicles and tubules which appear to project from the muscle cells.

3.3.1.5 The Radula

The Structure of Radula and Radular Sac

The radula is a thin membranous cuticular structure bearing rows of teeth secreted by the epithelial cells at the posterior end of the radular sac. For *Siphonaria*, the variation of the radula length (r) and of the ratio of radula length to body length (r/l) with the body length (l) is shown in Fig. 21. A comparison of the average r/l ratio for *Siphonaria* and various neighbouring species of limpets and chitons with their usual habitat characteristic is given in Table 2. In *Siphonaria*, each row of teeth consists of a central tooth, flanked by a series of laterals with a further series of marginals towards the edge (Fig. 22a). The height of teeth decreases from the tall laterals in the middle of the radula across to small triangular marginals at the edge (Fig. 23b). The central tooth is narrow and wedge-shaped with a Y-shaped base, bearing a single, pointed cusp near the posterior half (Fig. 23b). The lateral has a rhomboidal base and a large, lanceolate central cusp (mesocone) and a small, pointed cusp on the outer side (ectocone) (Fig. 22b, 23b).
The first lateral may have a slightly bidented mesocone (Fig. 22b & c). The marginals carry three cusps; a large, blunt mesocone with a small, pointed endocone and ectocone.

The number of teeth in each row varies with the age or size of the animal and also within a population. The general radula teeth formula from a population ranging from 11-20 mm long was found to be $(18\pm3) + (18\pm3) + (18\pm4)$. The number of rows of teeth also varies and for the above population, it was found to be $144 \pm 31$. The teeth appear to be fully formed after the tenth to the twelfth row. At the anterior end, the cusps, especially those of the laterals, are worn down to a rounded stub by the browsing activity (cf. Fig. 22c and d).

The radular sac has three types of cells that are involved in the production and formation of the radula. The subradular epithelium is made up of radular chitinogenic cells which secrete a basal layer forming the subradular membrane (Fig. 25a). At the posterior tip of the radular sac, this epithelium is replaced by a special wedge-shaped strip, the odontooblasic cushion, with a number of cuneiform odontoblasts which secrete the teeth. Overlying the radula is the supraradular epithelium, made up of sclerotoblasts which are involved in hardening of the teeth. Both the subradular and supraradular epithelium abut onto the buccal epithelium. The detailed cellular constituents of the radular sac are as follows:

1. **Radular Chitinogenic Cells** (Fig. 24)

These cells are columnar (10-30u in height) with layered cuticle which stains strongly with PAS and Bromophenol Blue.
TABLE 2. The ratio of the radula length to body length \((r/l)\) of various herbivorous limpets from the Pumphouse Reef at Leigh (site B, Fig. 2) and their apparent feeding substrate.

<table>
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<tr>
<th>SPECIES</th>
<th>(r/l)</th>
<th>FEEDING SUBSTRATE</th>
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</thead>
<tbody>
<tr>
<td>Cellana ornata</td>
<td>2.71</td>
<td>visually bare rock</td>
</tr>
<tr>
<td>Cellana radians</td>
<td>2.22</td>
<td>visually bare rock</td>
</tr>
<tr>
<td>Patelloidea corticata</td>
<td>1.07</td>
<td>corallina paint</td>
</tr>
<tr>
<td>Sypharochiton pelliserpentis</td>
<td>0.41</td>
<td>corallina paint</td>
</tr>
<tr>
<td>Siphonaria zelandica</td>
<td>0.31</td>
<td>lush algal growth</td>
</tr>
</tbody>
</table>
Fig. 21

Relationship of the radula length (r) and the body length (l) of *Siphonaria zelandica*.

A. a plot of r to l.

B. a plot of r/l to l.
Fig. 22  The Radula and Radular Teeth

a. The radula and radular sac viewed from the right dorsal side.

b. Radular teeth from a row on the middle region of the radula.

(i) The central tooth (dorsal view)
(ii) The central tooth (dorsal lateral view)
(iii) The first lateral
(iv) The eighteenth lateral
(v) A marginal (the 24th tooth from the central)
(vi) The marginal at the end of the row.

E  -  ectocone
M  -  mesocone
Fig. 23 Scanning Electron Micrograph of the Radula

a. Mature region of the radula showing the narrow central (c) flanked by a series of laterals (L) and a further series of marginals (m). † indicates demarcation between laterals and marginals. (x 490).

b. The first lateral tooth with a bidented mesocone. (x 3500).

c. A lateral tooth with a pointed mesocone. (x 4200).

d. A worn lateral tooth with a blunt mesocone. (x 4200).

e. The first four marginals flanking the laterals. (x 2000).

f. Marginals further near the edge. (x 2000).
<table>
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<tr>
<td>g</td>
<td>Golgi complex</td>
</tr>
<tr>
<td>ger</td>
<td>granular endoplasmic reticulum</td>
</tr>
<tr>
<td>jc</td>
<td>junctional complex</td>
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<td>lysosome-like body</td>
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<td>mitochondrion</td>
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<td>n</td>
<td>nucleus</td>
</tr>
<tr>
<td>RM</td>
<td>subradular membrane</td>
</tr>
<tr>
<td>va</td>
<td>electron-lucent vacuole</td>
</tr>
<tr>
<td>vi</td>
<td>dense vesicle</td>
</tr>
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</table>
Fig. 25  Radular Tooth Production

a. Light micrograph of a section of the posterior end of the radular sac showing the odontoblastic cushion (Od) which produces the new radular teeth (T), the radular chitinogenic cells (Rc) which secrete the subradular membrane (Rm), the sclerotoblasts (Sc) which lie above the radular teeth and the basophilic cell (*) between the radular chitinogenic cells and the odontoblasts. (Epon section, Toluidine Blue staining; x 2000).

b. Electron micrograph of the subradular membrane (Rm).
   It consists of three layers:
   1 - a homogeneous layer of moderate electron-dense material,
   2 - a moderately dense layer pervaded by a reticulum of denser material,
   3 - an electron-lucent layer penetrated by slender processes from the radular chitinogenic cells (Rc). (x 3600).
Ultrastructurally the cuticle appears to contain three distinct layers when it is being actively secreted (Fig. 25b). The topmost layer is homogeneous and moderately electron-dense; the middle layer is less dense and pervaded by a denser reticulum; while the lowermost layer is highly electron-lucent, and penetrated by slender, branching processes drawn out from the apical end of the cell. These are probably responsible for the secretion and consolidation of the cuticle substances. The cytophasm of these processes and also of the apical region of the cell is dense with free ribosomes, small dense vesicles, and a few electron-lucent vacuoles of various sizes and shapes (Fig. 26b). Some of the vacuoles and the dense vesicles were found to fuse with the plasma membrane in the apical processes indicating a process of secretion. The dense vesicles appear to originate from Golgi complexes and, probably contain enzymes for hardening the cuticle and rendering it insoluble. Within the apical region there are also a few mitochondria with parallel cristae, and bundles of microfilaments which extend into the apical processes.

The mid-region of the cell is occupied by five to six Golgi complexes and stacks of GER (Fig. 26a). Each Golgi complex consists of a closely-packed stack of three to five lamellae with large distended vacuoles on the outer side and numerous dense vesicles on the inner side. The vacuoles appear to fuse to form the larger electron-lucent vacuoles in the apical region. The lamellae of the extensive GER are distended at parts so that the stacks appear to be irregular. Among the GER and the Golgi complexes lie some mitochondria and a few electron-dense granules.
The nucleus usually lies just below the Golgi and GER. It has dense clumps of heterochromatin with particulate euchromatin and occasionally a distinctly large nucleolus.

Mitochondria are found throughout the cytoplasm but they appear to concentrate in the basal region just below the nucleus (Fig. 26b). In the basal region, they are very elongate, with dense stroma and long, parallel, distended cristae (Fig. 27c). Some of the mitochondria are enlarged and filled with a crystalloid material of varying density (Fig. 27d). Its similarity to the dark granules nearby suggests that the granules may originate from the enlarged mitochondria.

The lateral cell junctions are typical of other epithelial cells described, with zonula adherens and septate desmosomes (Fig. 27c). The base of the cell is supported by a thin basal lamella with fine filaments (Fig. 27f). The basal plasma membrane is thrown into a few folds and occasional desmosomes are present with dense filaments extending on both sides.

2. Odontoblasts (Fig. 28)

Each odontoblast is a pyramidal cell taller than the neighbouring chitinogenic cell (30-35μ) with a large spherical nucleus (Fig. 25a).

Ultrastructurally the cell bears long and dense microvilli embedded in the tooth matrix they secrete (Fig. 29a). The apical cytoplasm is packed with numerous ribosomes, vesicles and mitochondria with dense stroma and distended cristae (Fig. 29b). In the mid region, there is usually extensive GER and several Golgi complexes (Fig. 29c).
Fig. 26 Radular Chitinogenic Cell

a. Apical processes from the radular chitinogenic cell extending into the bottom layer of the subradular membrane (3). These processes contain numerous dense vesicles (v), free ribosomes (r) and a few mitochondria (m). (x 27300).

b. Electron micrograph of the whole radular chitinogenic cell. The cell has large central nucleus (n). Above the nucleus are several extensively developed Golgi complexes (g) producing some electron-lucent vacuoles (v) and dense stacks of GER (ger). A few dense bodies (d) are scattered throughout the cytoplasm and mitochondria (m) are concentrated near the base. (x 8250).
Fig. 27  Radular Chitinogenic Cells

a. The extensively developed Golgi complexes. Each consists of 5 – 7 lamellae (l), the outer lamellae are distended forming the electron-lucent vacuoles (va) while the inner ones produce the electron-dense vesicles (vi). The Golgi complexes are surrounded by GER cisternae (ger). (x 22300).

b. GER of the radular chitinogenic cell. Note the GER cisternae are distended at parts (▲). (x 37800).

c. Mitochondria (m) and dense bodies (d). The mitochondria have numerous parallel cristae, dense stroma and a few intramitochondrial granules. (x 21350).

d. The development of the dense bodies (d) from the mitochondria (m). The degenerating mitochondrion increases greatly in density (me) and is filled with some dense crystallin material (▲). (x 37300).

f. Lateral junctional complexes with terminal zonula adherens (za) followed by septate desmosomes (sd). (x 47970).

g. Basal region of the radular chitinogenic cell showing the infolding of the basal plasma membrane (p) and the concentration of mitochondria (m) in that region. Note that there are a few macula adherens (▲) at the base. (x 34700).
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>av</td>
<td>apical vesicle</td>
</tr>
<tr>
<td>g</td>
<td>Golgi complex</td>
</tr>
<tr>
<td>ger</td>
<td>granular endoplasmic reticulum</td>
</tr>
<tr>
<td>jc</td>
<td>junctional complex</td>
</tr>
<tr>
<td>ly</td>
<td>lysosome-like body</td>
</tr>
<tr>
<td>m</td>
<td>mitochondrion</td>
</tr>
<tr>
<td>mv</td>
<td>microvilli</td>
</tr>
<tr>
<td>n</td>
<td>nucleus</td>
</tr>
<tr>
<td>no</td>
<td>nucleolus</td>
</tr>
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</table>
Fig. 29 Odontoblasts

a. Electron micrograph of the odontoblastic cushion showing the secretion of a new tooth. The long microvilli of the odontoblast secrete dense material in between forming the tooth matrix. The odontoblasts on the first row (O₁) have forwardly directed microvilli secreting material on top of the subradula membrane (Rm) forming the tooth base (Tb). The microvilli from the second row of odontoblasts (O₂) run upward and forward above those of the first row forming the base of the tooth body (To). The microvilli from the third row (O₃) run mainly upwards and slightly backwards secreting the tooth body while those from the fourth and fifth row of odontoblasts (O₄ & O₅) are directed backwards secreting the tip end of the tooth cusp. Hardening of the tooth cusp has already begun by the sclerotoblasts (Sc) above the newly formed tooth. (x 3900).

b. Apical region of odontoblasts containing numerous vesicles (v) and a few mitochondria (m). (x 17300).

c. GER (ger) and dense lysosomal bodies (l) in an odontoblast. (x 27450).

d. Golgi complexes of an odontoblast with lamella (l) budding off vesicles (v) which contain some dense material. (x 27450).

e. The poorly chromatic nucleus (n) of an odontoblast. Note the prominent nucleolus (no) in the nucleus and the extensive GER (ger), a few dense bodies (l) and mitochondria around the nucleus. (x 22300).
Each Golgi complex consists of a stack of five to six lamellae with numerous associated vesicles. The lamellae are filled with electron dense material and the vesicles appear to arise from the distended end of the lamellae (Fig. 29d). There are also a few vacuoles, occasionally very electron-dense or containing dense granules and membrane structures, resembling lysosomal bodies (Fig. 29e). The oval nucleus is poorly chromatic. There is no infolding of the basal plasma membrane, nor were any desmosomes identified.

3. **Sclerotoblasts** (Fig. 30)

These cells lie in close opposition to the newly formed teeth. As the teeth mature, the sclerotoblasts become increasingly compressed until, where the supraradular epithelium abuts on the cellostytalar hood, only cell debris remains. The tooth cusp shows distinct histochemical changes as it matures. When first secreted both the cusp and its underlying radular membrane stained blue with Mallory's Trichome. The staining reaction of the cusp then changes to red and finally colourless while the tooth base changes only from blue to red. The radular membrane which is not in contact with the sclerotoblast remains blue. This indicates the sclerotoblasts must, in some way, be altering the composition of the tooth. The results of various histochemical tests are listed in Table 3. No obvious histochemical change was detected for the radular membrane, but the body and base of the young teeth gradually become negative to Alcian Blue and Aldehyde Fuschin, and more positive to PAS and Bromophenol Blue. The histochemistry of the tooth base remains constant along the whole radula but the body of the mature tooth becomes negative to all tests.
Electron microscopy showed that the matrix of the tooth, where the sclerotoblast is in contact with, becomes more electron-dense (Fig. 31a). The sclerotoblast has closely-compacted apical processes extending onto the tooth surface. The apical region of the sclerotoblast is filled with dense vesicles, some membrane-bounded secretory granules, multivesicular bodies and mitochondria. An oval, nucleolated nucleus occupies the lower third of the cell (Fig. 31b). Lying above the nucleus is a large Golgi apparatus with two or three complexes. All the Golgi complexes are arranged with their lamellae aligned with the longitudinal axis of the cell (Fig. 31c). Each consists of a stack of three to four lamellae, long and flat towards the inner side and more dilated and sacculated on the outer. Around the Golgi apparatus are small, membrane-bounded secretory granules, vesicles and small cisternae of both smooth and granular endoplasmic reticulum. Clusters of free ribosomes are common throughout the cytoplasm (Fig. 31d). Microtubules and bundles of fine filaments run parallel to the long axis of the cell.

The basal cytoplasm contains clusters of mitochondria and numerous glycogen granules. Zonula adherens and septate junctions are found in lateral cell junctions near the apical end (Fig. 31c). The basal plasma membrane adjacent to the collostylar connective tissue has only slight infoldings.

**Radular Tooth Production**

Tooth production in *Siphonaria* involves three cell types, the chitinogenic cells, the odontoblasts and the sclerotoblasts. The subradular membrane carrying the tooth is secreted by the chitinogenic cells of the subradular epithelium. Every successive row of teeth
TABLE 3. Staining reactions of different parts of the radular teeth at different stages of development. The row of teeth immediately after the odontoblastic cushion was counted as the first row. (C = Cusp and body of the tooth, B = Base of the tooth, M = the subradular membrane on which the tooth is attached. + = positive reaction, ++ = strong reaction, - = negative reaction, ± = variable reaction).

<table>
<thead>
<tr>
<th>Staining method</th>
<th>New Teeth (1st &amp; 2nd row)</th>
<th>Young Teeth (4th to 10th row)</th>
<th>Hardening Teeth (12th to 18th row)</th>
<th>Mature Teeth (&gt; 20th row)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>C  B  M</td>
<td>C  B  M</td>
<td>C  B  M</td>
<td>C  B  M</td>
</tr>
<tr>
<td>Mallory-Heidenhain</td>
<td>purple purple purple</td>
<td>red purple purple</td>
<td>yellow red purple</td>
<td>yellow red purple</td>
</tr>
<tr>
<td>Azan</td>
<td>blue blue blue</td>
<td>blue blue blue</td>
<td>red purple blue</td>
<td>yellow red blue</td>
</tr>
<tr>
<td>Toluidine Blue</td>
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<td>blue blue blue</td>
<td>colourless blue blue</td>
<td>colourless colourless blue</td>
</tr>
<tr>
<td>PAS</td>
<td>- - -</td>
<td>± - ±</td>
<td>+ - +</td>
<td>+ - +</td>
</tr>
<tr>
<td>Alcian Blue pH2.5</td>
<td>- - -</td>
<td>+ + -</td>
<td>- - +</td>
<td>- - -</td>
</tr>
<tr>
<td>Colloidal iron</td>
<td>- - -</td>
<td>+ - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>Aldehyde Fuchsin/Alcian Blue</td>
<td>blue blue blue</td>
<td>purple purple blue</td>
<td>pink purple blue</td>
<td>pink purple blue</td>
</tr>
<tr>
<td>Bromophenol Blue</td>
<td>- - +</td>
<td>+ + +</td>
<td>++ ++ +</td>
<td>- ++ +</td>
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</table>
Fig. 30  The Sclerotoblast

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ap</td>
<td>apical process</td>
</tr>
<tr>
<td>bm</td>
<td>basal plasma membrane</td>
</tr>
<tr>
<td>ct</td>
<td>collostylar connective tissue</td>
</tr>
<tr>
<td>g</td>
<td>Golgi complex</td>
</tr>
<tr>
<td>ger</td>
<td>granular endoplasmic reticulum</td>
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<tr>
<td>jc</td>
<td>junctional complex</td>
</tr>
<tr>
<td>m</td>
<td>mitochondrion</td>
</tr>
<tr>
<td>mb</td>
<td>multivesicular body</td>
</tr>
<tr>
<td>n</td>
<td>nucleus</td>
</tr>
<tr>
<td>no</td>
<td>nucleolus</td>
</tr>
<tr>
<td>sm</td>
<td>material secreted from the cell</td>
</tr>
<tr>
<td>T</td>
<td>radular tooth</td>
</tr>
<tr>
<td>v</td>
<td>vacuole</td>
</tr>
<tr>
<td>vi</td>
<td>vesicle</td>
</tr>
</tbody>
</table>
Fig. 31 Sclerotoblasts

a. Apical processes and microvilli (mv) of the sclerotoblast apposed on the surface of the radular tooth (T). These secrete a moderately dense material (s) between them and the tooth material near the processes becomes denser (↑). The apical region of the sclerotoblast contains dense vesicles, vacuoles, multivesicular bodies (mb) and mitochondria (m). (x 13000)

b. Electron micrograph of a sclerotoblast. Note the central nucleus (n) with a prominent nucleolus (no) and the extensively developed Golgi complexes (G) above the nucleus. Lining the cells are the connective tissue of the collostyle (t). (x 6850)

c. Apical junctional complexes of the sclerotoblasts with zonula adherens (za) and septate junctions (sd). (x 42000)

d. Golgi area of the sclerotoblast. The Golgi complexes are arranged with the axes of the lamellae (l) along the longitudinal axis of the cell. The outer lamellae are vacuolated (va) while the inner ones are surrounded by dense vesicles (vi). All round the Golgi complexes are aggregates of ribosomes (r). (x 34700)
originates by secretion at the posterior end of the radular sac. Each tooth is oriented horizontally when secreted by the odontoblasts, with its basal plate attached to the end of the subradular membrane material (Fig. 29a). Five rows of odontoblasts are raised into a slight ridge and the direction and extension of their microvilli determine the shape of the teeth. The long microvilli from the first row spread forward along the subradular membrane and secrete the basal plate. The next row of odontoblasts have slightly shorter microvilli lying on top of the basal plate. The microvilli from the third, fourth and fifth rows continue to shorten. Those from the third spread out with anteriorly-directed in front and posteriorly-directed behind, while fourth and fifth rows are mainly posteriorly-directed, secreting the tip of the tooth. All the microvilli appear to take part in active secretion of the tooth material and retract slowly so that the young tooth section showed a streaky appearance as from a brush. Between the odontoblastic cushion and the subradular epithelium lies a strip of strongly basophilic cells (Fig. 25a). This probably produces new chitinogenic cells by cell division, thereby moving forward that part of the subradular membrane secreted by the chitinogenic cells immediately in front. In its forward passage, the subradular membrane lifts the new row of teeth from the odontoblasts, while the new chitinogenic cell continues to secrete more subradular membrane and new secretion from the odontoblasts forms a further row of teeth.

As the row of teeth is secreted, the sclerotoblasts that lie just above it, secrete material to harden the newly formed teeth. Behind the odontoblastic cushion is a zone of cell proliferation which
continuously gives rise to the sclerotoblasts forming the supraradular epithelium. The sclerotoblasts appear to move forward with the new teeth as they grow and decrease in size as the teeth mature. Near the collostylar hood where it abuts on the buccal epithelium, the supraradular epithelium becomes detached from its basal connective tissue to lie on top of the buccal cuticle as an extra cell layer (Fig. 32a). After being lifted off from the basal lamella, the sclerotoblasts disintegrate and pass out as cell debris attached to the teeth.

At the distal end of the radula, the teeth are worn down and the chitinogenic cells decrease in height and disintegrate where they abut on to the buccal epithelium (Fig. 32b). In this way, the subradular membrane with its teeth is detached, to be gradually broken up by mechanical abrasion during feeding.

The rate of radular teeth production measured by the faecal teeth count was 3.7 ± 0.6 rows per day while that by the cold shock experiment was 4.9 ± 0.3 rows per day.

3.3.1.6 The Feeding Processes

The buccal complex consists of four integrated systems, the radula, the odontophore, the musculature around the odontophore and the external body wall, all of which act efficiently in the function of ingestion. At resting stage, the mouth is closed with the buccal mass retracted (Fig. 33A,a). The mouth may sometimes be retracted deeply into the groove between the head and the foot. The odontophore slopes back at 45°, its longitudinal axis being bent forming an anteroventral convexity and a posterior concavity. The scoop-shaped anterior tip of the odontophore covered by the radula is directly below the collostylar
Fig. 32

a. Light micrograph of part of the buccal mass showing the lifting off of the supraradular epithelium (SU) as it abuts on to the buccal epithelium (B). ↑ indicates the point where the supraradular epithelium is lifted off and forms a disintegrating layer (D) attached to the radular teeth. Note that the tooth body (To) is inert to the staining reaction while the subradular membrane (Rm) is strongly stained. (Paraffin section, Alcian Blue staining; x 1600)

b. Light micrograph of part of the buccal mass showing the disintegration of the subradular epithelium (SB) on meeting the buccal epithelium (B). ↑ indicates the position of the disintegrating nuclei. The radula (R) is then lifted off from the buccal cuticle (C). (Paraffin section, Mallory's Trichrome staining; x 1600)

c. Scanning electron micrograph of the anterior end of the radula (R) supported by the odontophore in its retracted position. The collostylar hood (Ch) is lifted back to reveal the scoop-like tip leading into the collostyle. (x 60)

d. Scanning electron micrograph of the anterior end of the radula supported by the protracted odontophore. The tip is widen into a U-shape with the radular teeth erected. (x 270)

e. Scanning electron micrograph of the part of the radula just on the crest of the odontophore tip showing the swinging of the radular teeth from an upward to a downward position. (x 1600)

f. Scanning electron micrograph of the radula showing the flaring of the radular teeth on the protruded odontophore at the beginning of the feeding stroke (stage 3). (x 530)
Fig. 33 The Feeding Processes

A, B, C, D & E Schematic drawings of the median sagittal sections of the buccal mass at different phases of the feeding cycle.

a. to g. Drawings of the mouth at various stages of the feeding cycle.

a. - at resting phase
b. - beginning of phase 1
c. - transitional stage between phase 1 and 2.
d. - at phase 2, note the radula is protruded out of the mouth.
e. - at phase 3, note the U-shaped end of the radula is being folded as it is retracted.
f. - transitional stage between phase 3 and 4, the mouth is nearly closed with the jaw still protruded.
g. - end of phase 4.
hood (Fig. 32c).

The feeding cycle involves movements of the mouth, the buccal mass, the odontophore and the radula which are all sequential and overlapping in their action. Four phases may be described as follows:-

Phase 1 (Fig. 33b)

The mouth opens, with the buccal mass and radula being simultaneously protracted; this continues until the tip of the odontophore and the radula are brought to the opening of the mouth just above the substratum.

The opening of the mouth commences with the contraction of the suboral dilators (18) pulling the two lobes of the ventral oral lappets sideways and transforming the mouth from a transverse slit to a triangular space (Fig. 33b). Contraction of the dorsal mandibular dilators (9) lifts the oral lappet and pulls the jaw downwards, so that the jaw protrudes above the mouth. The labial retractors (11) contract to pull the palps away from the mouth widening the mouth ultimately to an oblong-ovate shape.

Meanwhile the buccal mass is tilted through 90° until it slopes forward with its tip right over the mouth opening. These movements are brought about by the dorsolateral protractors (1) and the preventral and postventral protractors (2 & 3) which pull the buccal mass towards the mouth, and also the preventral and postventral levators (6 & 7) which raise the posterior part of the buccal mass anterodorsally. The odontophore is correspondingly turned forwards by the dorsal odontophoral flexor (17). During the forward movement, the folds in the floor of the buccal cavity in front of the dorsal food channel are straightened out, and the tensor of the collostylar hood (28) contracts to pull the hood backwards. The radula thus becomes uncovered at the tip of the odontophore. The pull
on the radular sac by the tensor of the hood is counteracted by the suspensors of the radular sac (25, 26, 27) keeping the radula in its position. The radula at this stage is not stretched over the odontophore but hangs free below its tip. At the end of phase 1, it bulges out slightly from the oval mouth (Fig. 33c).

Phase 2 (Fig. 33c)

This involves further tilting of the odontophore and stretching of the radula until it is ready to rasp the substratum.

The buccal mass is maintained as at the end of phase 1, but the odontophore is further tilted to a near vertical position, through a total angle of about 125°, by the contraction of the anterior and posterior jugales (13 & 14) and the dorsal odontophoral flexor (17). The anterior jugalis (13), the ventral odontophoral protractors (18) and the infraventral odontophoral protractors (19) then pull the odontophore still further forward, to engage the radula on the substratum. The odontophore is now straightened and its lateral regions bent upwards by the contraction of the median intracartilage tensor (24) widening the tip into a U-shape (Fig. 32d). This protraction and straightening of the odontophore tenses the radula round its tip. The dorsal odontophoral flexor (17) and the infra-lateral cartilage tensor (23) stretch the anterodorsal part of the odontophore sideways. The radular tensors (20, 21, 22) also actively stretch the radula over the odontophore tip. The infra-median radular tensor (22) and the supralateral radular tensor (21) pull the radula backwards while the supramedian radular tensor (20) retracts the radular sac. As a result, the radula is stretched tightly over the tip of the odontophore, pressing against the substratum (Fig. 33d).
With the stretching of the radula over the odontophore, the rows of teeth on its crest swing from an upward to a downward position (Fig. 32e), the crescent shape of the odontophore tip causing the rows of teeth to flare slightly sideways (Fig. 32f). At the end of phase 2, the whole buccal complex is thus in the position ready for the rasping action.

Phase 3 (Fig. 33D)

This involves the forward movement of the odontophore tip causing the radula teeth to scrape the substratum followed by an upward movement to pass the food material into the buccal cavity.

The tip of the odontophore is thrust forwards and upwards by the infraventral odontophoral protractors (19) and the ventral odontophoral protractors (18); this is the beginning of its return to the resting position. During this movement, successive rows of downwardly-directed teeth are engaged on the substratum. Food is lifted up and dragged forwards. The jaw is then depressed by the contraction of mandibular approximator (16) and simultaneously relaxation of the dorsal mandibular dilators (9), so that it is held against the radula at the end of the rasping stroke, tearing the algal filaments off from their attachment on the substratum. Gradual relaxation of the infralateral cartilage tensor (23) allows the folding up of the sides of the radula as it retracts into the mouth with the food. Contraction of the lateral intracartilage tensor (24) relaxes the odontophore tip, thus rotating the rows of teeth there back to their original position, dragging the food towards the groove.
At the end of this phase, the mouth commences to close with the relaxation of the labial retractors (11) and suboral dilators (9) and contraction of the labial sphincter (12) reducing it to a triangular opening (Fig. 33e).

**Phase 4 (Fig. 33E)**

This is a direct continuation of phase 3, with the return of the odontophore to the resting position and the closure of the mouth. The buccal mass is also being returned to resting position by the separate, though synchronous, action of the dorsolateral and buccal retractors (4 & 5). Contraction of the preventral and postventral protractors (2 & 3) also help to lower the posterior part of the buccal mass to its resting position. The mouth continues to close from behind, the contraction of the labial sphincter (12) reducing it to a slit (Fig. 33f). Finally the buccal sphincter (5) closes the buccal cavity (Fig. 33g). When the food is brought to the dorsal food channel, both the buccal mass and the odontophore resume their resting position.

Feeding tracks of *Siphonaria* on algal-coated glass plate revealed clearly the feeding movements of the animal. As the animal moves over the surface, it randomly executes feeding strokes approximately following the line of movement with the head swinging slightly (Fig. 34a), so sampling the food. When it reaches a suitable patch the head is moved from side to side as they feed with very slow forward movement so that the feeding tracks are close together and may become partially superimposed (Fig. 34b). Each feeding stroke produces an elongate triangular trace. The posterior part of the trace is usually completely clean of algae, while the anterior half, where the teeth traces appear to converge slightly,
may still have some patches of algae (Fig. 34c).

Examination of the crop and intestine contents following a meal reveals a mixture of large and small pieces ranging from minute diatoms to pieces of torn Gelidium, and whole lengths of Cladophora or Enteromorpha filaments (Fig. 34d & e) with very little inorganic material.
Fig. 34. Feeding Trace and Stomach Content of *Siphonaria zelandica*.

a. Feeding trace left on glass plate by an animal which has just started to feed, sampling the algae as it moved along.
   \[\uparrow\] indicates the direction of the movement of the animal's body.
   A to B is the swing of the head. (x 110)

b. Feeding trace left on glass plate by an animal which was actively feeding. The swing of the head (A to B) has increased greatly (compared with Fig. 34a) and the feeding strokes are close together, overlapping sometimes (e.g. at X). (x 110)

c. Trace of a single feeding stroke showing the clean posterior lateral region (P) and the small algal strands left in the central region (\[\uparrow\]). (x 330)

d. Algal fragments from the stomach. (x 120)

e. Algal fragments from the intestine. (x 120)

G - *Gelidium*

C - *Cladophora*

E. - *Enteromorpha*
3.3.2 DISCUSSION

The ingestive apparatus in *Sipho naria zelandica* which includes the buccal musculature, odontophore and radula, forms a complex and powerful mechanical system. J.E. Morton (1955b) remarked on the high development of the buccal mass in *Otina otis* as an adaptation towards a sedentary, slow grazing habit. Detailed study of the buccal mass of the limnic Basommatophora, Lymnaeacea (Carriker, 1946; Demian, 1962; Hembrow, 1973) reveals that the buccal musculature has the same general plan of organisation with great uniformity in structure and arrangement of the intrinsic muscles. *Siphonaria*, an intertidal basommatophoran is now found to possess a buccal mass of comparable basic plan, indicating the high degree of conformity of the buccal mass within the Basommatophora. Useful results could be obtained if detailed studies of the buccal mass of other bassommatophoran families (e.g. Ellobiidae and Trimusculidae) together with some of the stylommatophoran groups were available for comparison.

Demian (1962) found that the arrangement of the extrinsic muscles of the buccal mass and the shape of the odontophore present certain variations among the six genera of pulmonate snails studied by him. They fall in two distinct groups: the first includes *Lymnaea*, *Biomphalaria*, *Bulinus* and *Physa*; and the second, *Ancylus* and *Acroloxus*. The buccal mass of *Siphonaria zelandica* shows great similarity with those of the latter group, especially with *Acroloxus*. The resemblances noted are as follows:

1. Deeply notched odontophore and, as in *Acroloxus*, presence of an intercartilage contractor (24a) running transversely in the
odontophore notch and holding the two lateral portions together.

2. Extension of the radular sac behind the buccal mass.

3. The higher origin and lower insertion of the dorsolateral protractor (1) which on contraction simultaneously moves the buccal mass forward and elevates the posterior portion.

4. Thin, diffuse preventral protractors (2).

5. Short, thin postventral protractors (3), originating below the posterior portion of the buccal mass, apparently pulling the mass downwards instead of forwards when contracted.

6. Thin, dorsolateral retractor (4) originating from the anterolateral body wall.

7. Short, stout buccal retractors (5) originating from the lateral foot musculature below the area of the buccal mass and converging to insert on the ventral anterior part of the buccal mass and lips without passing through the nerve ring.

8. Forward shifting of the origin of the thin preventral levators (6).

9. The low origin of the postventral levators (7).

Demian (1962) suggested the similarities between the buccal mass of Ancylus and Acrorhynchus are secondary adaptation to the special buccal movements necessary for a patelliform snail. The similarities in Siphonaria, a limpet entirely separate in its derivation from the family Ancylidae (Lymnaeacea), would strongly confirm the above suggestion.

The buccal chitinogenic cell has been reported to be similar to that of the subradular epithelium (Carriker & Bilstad, 1946). Märkel (1958), however, showed distinct differences between the subradular membrane with its epithelium and the buccal cuticle with its secreting
cells in various pulmonates. Runham (1963a) also showed that the cuticle partly lining the buccal cavity was discontinuous with the subradular membrane. In *Siphonaria*, the subradular membrane gives a stronger reaction to Bromophenol Blue. Ultrastructural description of chitinogenic cells of molluscan origin has never been previously made. The present work with electron microscopy confirms Märkel's conclusion (cf. Fig. 12 & 24). The buccal cuticle is less dense and the microvilli or comparable processes from the chitinogenic cells below penetrate half way through the cuticle layer probably corresponding to the striations in the lower half of the cuticle described with light microscopy (J.E. Morton, 1955b; Märkel, 1958). The microvilli in the buccal cuticle contain a dense cylinder of filaments resembling the pore canals in the insect cuticle (Overton, 1966). The dense subradular membrane is seldom penetrated by microvilli except at the proximal end near the odonto plasts where it is first secreted; as the subradular membrane is formed, these microvilli become filled with dense material and are cut off from the cell to form the dense reticulum present in the lower part of the subradular membrane. The functioning microvilli from the subradular epithelium have electron-lucent material between them, which gradually becomes denser with secretions from the microvilli. Such a layer of microvilli below the subradular membrane probably corresponds to Märkel's 'Faserschicht' layer. The buccal chitinogenic cells have numerous free ribosomes, short GER, small compressed Golgi complexes and dense granules in the supra-nuclear region while the cells of the subradular epithelium are filled with distinct, long stacks of GER and large Golgi complexes with big vacuoles. All these facts indicate that the secretions of the two types of chitinogenic cells are of different nature. A study of the chemical
nature of molluscan chitins other than that of radula would be timely.

In *Otina otis*, J.E. Morton (1955a) described medium and lateral buccal glands and later (1955b) reported the presence of lateral glands in various ellobiids suggesting that the lateral buccal gland is a regular feature of lower pulmonates. In *Siphonaria*, sub-epidermal mucous cells are present sparsely in the connective tissue beneath the lateral buccal epithelium. Rigby (1963) found the buccal gland cells in the stylommatophoran, *Oxychilus cellarius*, are sparse and scattered and suggested that the well-developed pedal gland takes over their role in moistening food. In *Siphonaria* also, the pedal gland is well-developed, and the buccal glands may so become less important.

Most basommatophorans have a dorsal median jaw except *Trimusculus* (Gadinia), *Amphibola* and carnivorous pulmonates (Hyman, 1967; Hubendick, 1978). Its morphology has been well described by Taylor (1894) but is considered to have little systematic value (Hubendick, 1978). The shape of the jaw of *Agriolimax* has been reported to be determined by the abrading of its surface by the radula. In *Siphonaria zelandica* the single transverse thickening consists of numerous rods. The present study has shown that the rods arise as layers of secretion from a group of cells located at the pit of a fold of the buccal epithelium. These cells with elaborate GER and numerous dense apical granules appear to be secretory. The fold of the buccal epithelium is filled with thick cuticle secreted by the buccal chitinogenic cells and the secretion from the jaw cells probably modifies and gradually hardens the buccal cuticle. The hardened material accumulates in layers on top of the cell forming the rod protruding from the buccal cuticle. This process of jaw formation
probably explains the lack of ridges in the newly regenerated jaw of
*Helix pomatia*, 12-16 weeks after removal (Bierbauer, 1957 in Runham,
1975). The jaw has been shown to consist of chitin and protein together
with some calcium, iron, carbonate, phosphate and sulphate (Spek, 1921).
The hardening of the buccal cuticle to form the jaw rods may probably
be similar to the tanning of the insect cuticle (Smith, 1968).

The odontophoral cartilage in *Siphonaria*, as in all gastropods has
no resemblance to vertebrate cartilage, consisting of a supporting cushion
of turgid connective cells and transverse muscle fibres. These are of
smooth type with paramyosin, the second type among the five described by
Hoyle (1964). The presence of so much muscle tissue in the odontophore
implies that its shape is highly plastic. Movements of isolated odonto-
phore have been observed (Grampton in Runham, 1975). The pulmonate
cartilage vesicular cells have been compared to the vesicular connective
tissue cells found elsewhere in the body which function as a glycogen
store (Smnia, 1972). In *Siphonaria*, however, neither electron microscopy
nor histochemical test results revealed any glycogen in the cartilage
vesicular cells. They appear to be filled with a reticulate material
which contains a colourless inert fluid, most likely water, in between.
The varying diversity of the reticulate material evidently related to the
presence or absence of fluid, implies that these cells are highly deformable;
they may function as a hydrostatic skeleton. The odontophore would thus
be capable of the changes in shape involved in protruding and extending
the radula (Section 3.3.1.6).

The most characteristic structure of the molluscan buccal cavity,
and indeed the hallmark of molluscs themselves, is the radula. Though
important from the taxonomic point of view in prosobranchs and opisthobranchs, the morphology and arrangement of the radular teeth have little classificatory value in pulmonates (Hyman, 1967), where tooth form and number vary with the age of the animal and also within a population. The radula of Siphonaria australis is broad and short, each row having a central tooth and variable numbers of laterals and marginals, as is typical of most herbivorous pulmonates. The number of teeth per row \((18 \pm 4) + (18 \pm 3) + (1) + (18 \pm 3) + (18 \pm 4)\) and the number of rows of teeth \((144 \pm 31)\) are very close to those of Lymnaea stagnalis \((23 + 20 + 1 + 20 + 23 \times 120)\) and Sulota fruticum \((18 + 19 + 1 + 19 + 18 \times 130)\) as described by Märkel (1958).

The lateral teeth of Siphonaria are basically similar to those of terrestrial snails which feed on succulent plant material e.g. Agriolimax (cf. Scanning electron micrograph of the radular teeth of Agriolimax reticulatus in Runham and Thornton, 1967 with Fig. 22a). The tooth shape is directly related to the kind of food the animal eats and the way which it is manipulated (Fretter & Graham, 1962). Walsby et al. (1973) have related the reduction of size of teeth of Gadinalea nivea to the mucus-trapping mode of feeding. Though Siphonaria has close resemblances in shell form and habit to the true limpets, Cellana radians (family Patellidae) and Patelloida corticata (family Acmaeidae) found in New Zealand at approximately the same shore level, the contents of its gut were found to be very different from those of the true limpets, which are scrapers and abraders of the surface algal film. Siphonaria by contrast ingests large pieces of algae such as Enteromorpha and Gelidium or smaller pieces of microscopic filamentous algae including their
attached diatoms, accompanied with very little inorganic material. It is apparently unable to rasp and scrape the surface of rock in the manner of true limpets. Runham and Thornton (1967) have compared the wear of teeth of the true limpet *Patella* and the pulmonate slug *Agriolimax* and have concluded that each tooth of the slug is used as a 'pronged cultivator' on a soft substrate. The process of wear of *Siphonaria* teeth is similar to that of *Agriolimax* with shortening and rounding of the cusps and then later complete attrition to a small stub. This further confirms that the *Siphonaria* radula is used as a 'raker' rather than as a 'scraper'.

The radula is produced by a group of odontoblasts situated at the distal end of the radular sac. From light and electron microscopic studies, the process of secretion of a radular tooth in *Siphonaria* is very similar to that described for *Lymnaea stagnalis* (Runham, 1963b), * Biomphalaria glabrata* (Wiesel & Peters, 1978), *Helix pomatia* (Märkel, 1958; Runham, 1963b) and *Limax flavus* (Kerth & Krause, 1969). Runham (1975) briefly mentioned the involvement of the long microvilli of the odontoblasts in the formation of teeth. The present study showed that the basic matrix of the tooth is mainly secreted by the odontoblasts and, as suggested for *Patella* (Peters, 1979), the tooth shape is determined by the extension and direction of the microvilli of the different odontoblasts. The detachment of the newly formed tooth involves the shedding of the microvilli as was shown in the electron micrograph (Fig. 29a), which confirms Runham's suggestion (1975) that the radula is formed as a superficial chitinization of the probably permanent odontoblasts.

Different views have been held as to the relationship of the radula with the subradular (= inferior) epithelium. Hoffman (1932) believed
that the radula is permanently attached to the inferior epithelium and that the radular only moves forward through a growth of the whole buccal mass. Märlkel (1958) suggested from histological studies that the radula and the inferior epithelium are firmly attached to each other but are both continuously replaced. He pointed out, however, that the changes in shapes of the cells imply that this attachment is not permanent. Hubendick (1945), on the other hand, maintained that the radula slides over the subradular membrane which was believed to be continuous with the buccal cuticle. Runham (1963b) established that the subradular membrane is not continuous with the buccal cuticle and on this basis questioned Hubendick's assumption. The electron microscopy studies of the radula of Siphonaria have revealed that the radular membrane detectable as a separate layer in light microscopy, is actually continuous with the base of the radular tooth. The subradular epithelium secretes an electron-lucent matrix which is gradually transformed into denser material through secretion from the microvilli extending between the matrix. The radula is, therefore, firmly attached to the epithelium but the attachment would appear to be a dynamic one which allows the alteration of the shape of the epithelial cells. The role of the microvilli in keeping the cell surface constant while it goes through several changes in shape has been suggested by Follett and Goldman (1970). This would also account for the difference in the rate of forward movement of the radula and subradular epithelial cells, as reported by Runham (1963b).

Runham (1975) suggested that the matrix material of the radula is secreted by both the superior epithelium and the odontoblasts. From
the present studies, it would appear that the basic matrix of chitin protein or glycoprotein (Hackman, 1959) is first secreted by the odontoblasts to attach to the subradular membrane produced from the subradular epithelium. The supraradular epithelium is then probably involved with the tanning and hardening of the teeth. In insects, it has been suggested that the pore canals in the endocuticle maintain a functional link between the cuticle and the epidermal cell that secretes it (Locke, 1961), probably providing a pathway for substances involved in tanning of the exocuticle (Smith, 1968). The detachment of the teeth from the odontoblasts after matrix secretion and the absence of canals or of any protoplasmic connections of the tooth with the interior epithelial cells beneath it, along with the close apposition of the microvilli and processes of the supraradular epithelium on to its surface, indicates the supraradular epithelial cells (sclerotoblasts) could be involved in the tanning process. In addition, Runham (1961, 1963a) demonstrated that the histochemistry of the subradular membrane remains constant except at the front end of the radula, while the newly formed teeth at the posterior end become negative to histochemical tests, first at the cusps then at the base, and mineral such as iron are found to be present. It is highly likely then that the microvilli from the supraradular epithelial cells are actively secreting hardening substances altering the nature of the tooth matrix.

The supraradular epithelium has been reported to move at the same rate as the radula in *Helix pomatia* and *Lymnaea stagnalis* (Runham, 1963b). Histological study of *Siphonaria* radula confirms this. As in *Lymnaea*, the supraradular epithelial cells of *Siphonaria*, on abutting onto the
buccal epithelium near the collostylar hood, do not disintegrate into an intensely staining mass as claimed by Runham for *Helix*, but pass over on top and then gradually disintegrate.

The forward movement of the supraradular and subradular epithelium with the radula poses a problem in the attachment of the musculature. Runham (1963b) suggested that either the fibres must lose their attachment at the anterior end and form new connexions at the posterior, or alternatively there must be a sliding of the epithelium over the basement membrane. The continuity of the basement membrane along its length with that of the collostylar and buccal epithelium appears to agree with the latter suggestion.

The radular tooth replacement rate measured by the faecal teeth counts and the cold shock experiments differ slightly (3.7 ± 0.6 and 4.9 ± 0.3 rows per day respectively). The result obtained by cold shock may be more reliable as the animals were returned to the natural environment for ten days while those from which faecal teeth were counted were kept in the laboratory, where their feeding activity could be reduced. The replacement rate of radular teeth of 4.9 rows per day for *Siphonaria* is very close to those obtained for *Agriolimax* (5.5 rows/day) and *Littorina* 5 - 6.5 rows/day) which are the highest among the fifteen snails studied by Isarankura and Runham (1968). Such high replacement was attributed to active herbivorous feeding irrespective of variation in the hardness of the teeth. The results obtained from *Siphonaria* support this view.

From a very detailed study of the radula of *Lymnaea limosa* Hubendick
(1945) demonstrated that the length and width of the ribbon and the number of its teeth were proportional to the size of the animal. This has been similarly demonstrated for *Siphonaria*. However the length of the radula does not increase proportionally with the body size as was indicated by the plot of radula length to body length \((r/l)\) versus body length \((l)\) (Fig. 21b).

Fretter and Graham (1962), comparing the ratio of radula length to body length of various prosobranchs, suggested that the radula length is related to the amount of work involved in feeding. No consistent trend was shown from their data, probably owing to the great disparities in body form and feeding mechanism of the animals used. A clear correlation is, however, obtained here from the comparison of radula and body length ratios of various herbivorous limpets which differ slightly in the hardness of their feeding substrate (Table 2). Increasing hardness of the substrate obviously imposes increased wear upon the teeth. The animal may adapt to this either by increasing the rate of teeth production to replace the worn off teeth or to strengthen the teeth to withstand the wear. As mentioned earlier, teeth replacement rate is equally high in both *Agriolimax* which feed on soft, fleshy plant material and *Littorina* which scrapes rocks (Isarankura & Runham, 1968). On the other hand, the hardness of the radular teeth of various snails seems to be clearly related to that of the substrate and the nature of the food (Lowenstam, 1962). Hardening, as discussed earlier, is probably regulated by the supraradular epithelial cells, which disintegrate as the teeth protrude from the radular sac onto the collostylar hood. The duration of tanning will depend on the length of the radular sac and also the rate of forward movement of the radula. With no great difference in the forward movement
rate, the time taken for the extra hardening of teeth such as by precipitation of magnetite, goethite etc. in the denticles (Lowenstam, 1962) would depend on the length of the radular sac. Therefore it is probable that the increase in radula length in snails feeding on hard substrates may be related primarily to the time necessary for the tanning and hardening process.

The feeding behaviour of *Siphonaria*, similar to many intertidal grazing gastropods, with sampling and concentrated feeding activity in favourable areas, has been suggested to lead to the most efficient removal of food material (Runham, 1975). The feeding process is basically typical of that described for other pulmonates (Hubendick, 1957; Märkel, 1958). Carriker (1946) and Märkel (1958) held that the radula slide over the cartilage during phase 3 when the radula is applied on the food while Hubendick (1957) considered this impossible. The present study revealed little movement of the radula over the odontophore. The movement of the teeth over the substratum appear to be the combined result of the active forward movement and the change in shape of the odontophore.

Using scanning electron microscopy for study of the buccal mass and radula, Runham (1969) suggested that in *Agriolimax reticulatus*, the U-shape odontophore with the teeth is used like a scoop and the cusps are used for holding and tearing off of large food pieces rather than rasping. In *Siphonaria*, as discussed before, the teeth appear to function as a 'raker'. The scoop-shaped odontophore and the composite-rod structure of the jaw also dismiss the possibility of a rasping and cutting function. The radular teeth are applied to the food by the
downward and forward movement of the odontophore and are dragged forward over it. As the odontophore is retracted back, the radula folds up against the protruded jaw, holding the food material and pulling it off the substratum, resulting in the patchy appearance in the anterior region of the feeding trace. Thus, *Siphonaria zelandica* is, in contrast, to the true limpets, a 'browser' rather than a 'grazer', and with this feeding habit, it is limited to rock surfaces with thicker algal growth.
3.4 THE ALIMENTARY TRACT

3.4.1 OBSERVATIONS AND RESULTS

3.4.1.1 General Morphology of the Alimentary Tract

The general plan of the alimentary system of *Siphonaria zelandica* is shown in Fig. 35. The alimentary canal is divisible into buccal mass, oesophagus, stomach, intestine and rectum. Opening into the canal are two glands, the salivary gland into the buccal cavity and the digestive gland into the stomach. As in most pulmonate gastropods, the oesophageal pouches or the diverticula derived from them are absent. The salivary gland and digestive gland will be described later in Section 3.5 and 3.6. The histology and histochemistry of each region of the alimentary tract, except that of the buccal mass which has been discussed in the previous section (3.3), are summarised in Table 4.

The Oesophagus

The oesophagus arises as a continuation of the ciliated dorsal food channel from the roof of the buccal mass, with a small pouch at either side where the ducts of the salivary glands enter. Behind the buccal mass, it turns downwards towards the right side, and abruptly dilates into the crop, which continues back as far as the stomach. According to its diameter, the oesophagus is divided into two regions: a slender anterior part and a dilated posterior crop.

The anterior oesophagus is lined by a ciliated epithelium which is thrown into four longitudinal folds: dorsal, ventral and two laterals, with smaller ridges in between. These folds gradually subdivide as they run down the oesophagus so that the oesophagus behind the buccal
mass has about ten to twelve folds (Fig. 37a). The epithelial cells are tallest on the crests and they all contain numerous granules which were PAS-positive and stained slightly bluish in Toluidine Blue (Fig. 37b). Mucous cells staining positively with Alcian Blue and Colloidal Iron lie subepithelially, some with long extensions through the epithelial layer. A thin band of circular and longitudinal muscles invests the oesophagus.

The crop is thinner-walled with small and numerous longitudinal ridges; it forms a spacious sac with a corrugated epithelium which is capable of expansion with food. The ridges appear to have fine cross-striations and small ciliary currents directing particles across as well as along the ridges (Fig. 36). The histology of the epithelial layer is basically similar to that of the anterior oesophagus but the cells are shorter and ciliated only on the crests of the ridges (Fig. 37c). There are also sparse oesophageal secretory cells which are filled with granules staining blue with Toluidine Blue and positively with PAS and Bromophenol Blue (Fig. 37d). Immediately beneath the epithelium is a thin layer of nerve fibres which are branches from the buccal, pleural and branchial ganglia.

The Stomach

The stomach is not clearly demarcated anteriorly from the crop, nor posteriorly from the intestine. The crop opens with only a slight constriction into the anterior end of the stomach. The stomach is an approximately triangular sac lying towards the posterior end of the visceral mass, with its tip pointing slightly towards the left. It is
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1 All staining reactions were tested with thick resin sections except those marked with *. Where necessary, the procedures for removal of resins recommended by Sneders et al. (1972) or Maxwell (1977) were followed prior to the staining procedures.

2 Staining reactions were tested with paraffin sections.
Fig. 35 The Digestive System

a. Dorsal view of the digestive system after it is removed from the animal, with the anterior digestive gland displaced towards the left.

b. Schematic drawing of the right half of the animal seen from the left side after the animal has been cut in a median sagittal plane slightly displaced towards the left showing the arrangement of the digestive system in the body.

BC  - buccal cavity
BM  - buccal mass
DA  - anterior lobe of digestive gland
DP  - posterior lobe of digestive gland
EP  - epiphallus
F   - foot
GI  - gill
GO  - gonad
\( I_1 \) - pro-intestine (A to B)
\( I_2 \) - mid-intestine (B to C)
\( I_3 \) - post-intestine (C to D)
K   - kidney
MS  - mantle skirt
OA  - anterior oesophagus
OC  - oesophageal crop
OD  - odontophore
R   - radula
RC  - rectum
RD  - dorsal raphe
RV  - ventral raphe
SG  - salivary gland
SO  - spermoviduct
ST  - stomach
The crop, stomach and pro-intestine dissected ventrally to show the internal structure and the direction of the ciliary current (indicated by arrows).

AS - smooth strip  
C - crop  
DA - aperture of anterior digestive gland  
DR - double ridge  
DP - aperture of posterior digestive gland  
I - pro-intestine  
SA - anterior chamber of stomach  
SP - posterior chamber of stomach  
SI - preintestinal chamber of stomach  
$T_1$ - major typhlosole  
$T_2$ - minor typhlosole

Inset shows the way in which the organs are cut open.
Fig. 37  The Oesophagus

a. T.S. of the anterior oesophagus (E). The oesophagus is surrounded by the salivary gland (S). Note the large left buccal ganglion (B) beneath the oesophagus and the collostylar hood (H) further below. (Paraffin section, Alcian Blue staining; x 600).

b. Light micrograph of a section of an epithelial fold of the anterior oesophagus. The epithelial layer is made up of ciliated cells (C) with interspersed mucous cells (↑). The connective tissue lining the cells contains large blood spaces (B). (Epon thick section, Toluidine Blue staining; x 3000).

c. Scanning electron micrograph of a ridge in the crop showing the ciliation (↑) on the crest of the ridge. (x 1800).

d. Light micrograph of a section of a ridge in the crop. The ridge consists of non-ciliated cells (N) on both sides and ciliated cells (C) at the crest. Interspersed randomly are some oesophageal secretory cells (↑). (Epon thick section, Toluidine Blue staining; x 1400).
closely enveloped by the two lobes of the digestive gland and on the right posterior by the gonad. The intestine arises ventrally on the left, near the crop opening. A large anterior digestive gland aperture lies between the crop and the intestine, while a smaller posterior one enters from the right side mid ventrally.

Just behind the crop, the stomach is thin-walled and transparent, with its contents visible externally. From behind the digestive openings, back to its apex, it forms a round, muscular pouch, reddish in colour, possibly from the presence of some myoglobin.

Internally the stomach can be divided into three regions: the anterior chamber, the posterior chamber and the preintestinal chamber. The anterior chamber is demarcated from the crop by the cessation of the longitudinal folds, and the appearance of a smooth annular strip, (Fig. 38a) which may be equivalent to the gastric shield area. Scanning electron microscopy showed this strip to be very sparsely ciliated (Fig. 38b). Posteriorly the anterior chamber is bordered by a double ridge running between the two digestive gland apertures and extending dorsoventrally round the stomach. The whole chamber is lined with numerous folds, directed obliquely towards the digestive diverticula. The double ridge which joins the digestive apertures continues from the anterior aperture along the preintestinal chamber forming the major and minor typhlosoles. Between these typhlosoles, the folds from the ventral side of the anterior aperture continue towards the dorsal side of the preintestinal chamber (Fig. 38h). These folds gradually diminish until there is only a single groove between the typhlosoles. The preintestinal chamber now turns to the right to run backwards as the intestine, and
the typhlosoles merge into its wall at the point of twining. The posterior chamber behind the double ridges from the digestive apertures has a thick wall with longitudinal folds. Towards the posterior apex, the wall becomes thinner with only irregular and impermanent folds.

Histologically, the 'gastric shield' strip of the anterior chamber consists of tall, unciliated cells, the gastric cuticular cells, with a dense cuticle and numerous glycogen and lipid globules (Fig. 38c). Elsewhere the anterior chamber is lined by ciliated cells similar to those of the oesophagus but with denser and shorter cilia (Fig. 38d & e). The epithelium of the double ridge is much taller than the adjacent chamber wall and has long cilia and a few goblet cells staining positively with PAS and slightly with Alcian Blue. The folds running from the anterior digestive gland aperture into the preintestinal chamber are formed by ciliated cells, tallest on the crests and shortest in the grooves (Fig. 38f). There are also a few goblet mucous cells.

The cilia in the anterior chamber appear to direct small food particles obliquely towards the digestive gland apertures while large particles are carried straight into the posterior chamber (Fig. 36). The cilia on the crest of the folds from the anterior digestive aperture direct material towards the preintestinal chamber while those in the grooves transfer particles into the digestive gland.

The epithelium of the posterior chamber resembles that of the anterior except that the cilia are shorter (Fig. 38f & g) and goblet cells are infrequent. The cilia in the anterior region direct particles towards the posterior end while those in the thin-walled terminal pocket produce a rotatory movement (Fig. 36).
Fig. 38  The Stomach

a. Scanning electron micrograph of the junction of the crop (C) and the anterior chamber of the stomach (A). The smooth strip (G) marks the beginning of the anterior chamber. (x 315).

b. Scanning electron micrograph of the smooth 'cuticular' strip showing the presence of sparse thin cilia (↑) on the cuticle. (x 1050).

c. Light micrograph of a section of the 'cuticular' strip. The gastric 'cuticular' cells carry a thin layer of 'cuticle' (↑) and contain large amounts of glycogen (dark granules in the cell). (Epon thick section, PAS/Alcian Blue staining; x 2200).

d. Scanning electron micrograph of the anterior chamber of the stomach showing the thickly ciliated surface. (x 1250).

e. Scanning electron micrograph of the anterior chamber of the stomach showing the ciliated surface and mucous secretions from the mucous cells. (x 10000).

f. Scanning electron micrograph of the posterior chamber of the stomach showing the ciliated surface and mucous secretion (S) from the mucous cells. (x 6125).

g. Scanning electron micrograph of the preintestinal chamber showing the major and minor typhlosoles (T₁ & T₂ respectively) and the small folds in between them. (x 280).

h. Scanning electron micrograph of the folds in the preintestinal chamber of the stomach showing the dense fine cilia. (x 3200).

i. T.S. of the preintestinal chamber showing the typhlosoles (T₁ & T₂) and the small folds (F) in between. (x 375).
The folds in the preintestinal chamber are lined by an epithelium similar to that of the folds from the anterior digestive aperture but with more goblet cells, while the typhlosoles are a continuation of the double ridge from the anterior chamber. The ciliary movements in the folds are as before, directing material up the preintestinal chamber on the crest, and towards the digestive gland in the groove. Cilia on the typhlosoles direct particles sideways into the ventral region where they are carried forward into the intestine (Fig. 36).

The stomach is invested by a layer of circular muscle about as thick as the epithelium in the anterior region, but increasing to about twenty times in the mid region of the posterior chamber. Outside the muscle layer is a thin sheet of connective tissue, with blood spaces and nerve fibres.

The Intestine and Rectum

The intestine continues from the preintestinal chamber, running below the oesophagus on the left and looping round the digestive gland to emerge on the right; it then runs forward below and to the left of the preintestinal chamber, loops around the digestive gland again and turns to the right to become the rectum. The anus opens at the anal lobe on the ventral side of the pneumostome (Fig. 101). The thin-walled intestine has no special external features. Histologically, however, it can be divided into three regions: the pro-, mid- and post-intestine, as in several other pulmonates (Carriker, 1946a; Runham & Hunter, 1970).

The pro-intestine leads out of the preintestinal chamber of the stomach where the typhlosoles disappear and loops below the anterior digestive gland to run forward as the middle intestine (Fig. 35, A to B).
Arising from the typhlosoles, a thick band continues forward to spread round the wall of the pro-intestine. The internal lining is smooth and ciliated throughout (Fig. 39a). The cilia beat transversely producing a rotatory movement. The epithelial cells of this region are tall and narrow with a distinct brush border and long cilia especially near the end of the typhlosoles. The cells are acidophilic with numerous apical droplets of various sizes and an oval, single-nucleolated nucleus. Mucous goblet cells, like those in the stomach are abundant everywhere except on the anterior band where there is another type of secretory cell (Fig. 39b). The secretory cells are broad-based and narrowly attenuated towards the apex. Their basal nuclei have a prominent nucleolus and the cell contents are of glycoprotein staining strongly with PAS but only slightly with Alcian Blue, Colloidal Iron, Bromophenol Blue and Acrolein (Table 4). These secretory cells may be so densely alternated with the columnar cells that the whole epithelium appears to have two layers.

The mid-intestine ends abruptly as it turns ventrally beside the stomach (Fig. 35, B to C). The internal surface on the side against the digestive gland is smooth, while the rest of the lining has longitudinal folds which are visible externally (Fig. 39c). The epithelium is also made up of ciliated columnar cells as in the anterior intestine but without the apical droplets. Cell height varies greatly (25 - 50μ) with the tallest on the ridges. As in other regions, goblet mucous cells are present. Another type of cell, oblong in shape with slightly narrow apex (the mid-intestinal secretory cell) is also common (Fig. 39d). These cells are filled with light-reflecting granules, staining positively
Fig. 39  The Intestine

a. Scanning electron micrograph of the pro-intestine showing the cilia on the surface of the ridge. The secretions (S) from the pro-intestinal secretory cells appear as elongated strands and the long cilia (†) among the secretory cells are probably for distributing the secretion. (x 440).

b. Light micrograph of a section of the pro-intestine. The pro-intestinal secretory cells (S), each with an attenuated apical half (†) appear as a separate cell layer basal to the ciliated cells (C). (Epon thick section, Polychrome staining; x 1600).

c. Scanning electron micrograph of the mid-intestine showing the cilia on the surface. (x 600).

d. Light micrograph of a section of the mid-intestine. Interspersed between the ciliated epithelial cells are a number of secretory cells (S) and mucous cells (†). (Epon thick section, Polychrome staining, x 1600).

e. Scanning electron micrograph of the post-intestine showing the ciliated surface. (x 1120).

f. Light micrograph of a section of the post-intestine showing the presence of numerous mucous cells (†) in between the ciliated cells. (Epon thick section, Polychrome staining; x 1600).
with PAS and Bromophenol Blue, and dark blue with Toluidine Blue (Table 4), indicating the presence of protein.

The post-intestine begins with a distinct transverse fold separating it from the mid-intestine. It forms the second loop round the anterior digestive gland (Fig. 35, C to D). The lining is irregularly ridged, but there is a distinct groove on the ventral side. This region is lined with similar but shorter ciliated cells (20 - 40μ) as those of the mid-intestine. Mucous goblet cells increase to the same proportions as in the pro-intestine. Cilia in the mid- and post-intestine direct material towards the anus.

The whole intestine is bounded by a thin layer of inner circular and outer longitudinal muscle fibres.

The rectum is wider than the post-intestine, with weaker infoldings of the wall. The epithelial cells have a short-ciliary coat and are much vacuolated apically and filled with fine granules. Goblet mucous cells appear slightly different from those in the intestine, staining strongly with Alcian Blue indicating a strong acid mucopolysaccharide. The muscle layer is about twice as thick as in the intestine.

3.4.1.2. Cellular Constituents of the Alimentary Tract

1. The Ciliated Epithelial Cells

The whole alimentary tract is lined by ciliated columnar cells, varying in height (Table 5) but referable with modifications to a single basic type (Fig. 40). These cells stain light purple with Ehrlich's haematoxylin and some have apical granules positive to PAS and Bromophenol Blue as well as variable amount of lipid. The nucleus is placed
between the middle and the base of the cell.

Under electron microscopy, the cilia are seen to vary greatly in length along the alimentary canal. Each cilium has a typical 9 + 2 structure (Fig. 42a); the outer fibrils continue into an ovoid basal body with its upper end protruding slightly above the general cell surface (Fig. 41). A ciliary plate is present in between the basal body and the cilium proper. The plasma membrane distends to surround the protruded part of the basal body and constrict at the level of the ciliary plate, forming a ciliary pit around the base of each cilium. A striated root-fibril reaches down from the basal body into the cytoplasm, with regular cross bands and inter-periodic sub-bands (Fig. 42b).

In addition to the cilia, these cells also bear microvilli, of varying size and density, though usually of uniform length and diameter in a given region. Each microvillus is bounded by a unit membrane, bearing a fine filamentous coat resembling the 'fuzzy cell coat', or 'glycocalyx' as described on the microvilli of the mammalian intestinal cells (Bennett & Leblond, 1977). From the centre of each microvillus, a core of fibrils extends back into the apical cytoplasm. Between the microvilli, the plasma membrane invaginates occasionally to form pinocytic vesicles (Fig. 42c).

The cytoplasm contains free ribosomes, bundles of microfilaments, occasional clear vesicles and variable amounts of glycogen and lipid. In the apical region, there are numerous small vesicles with distinct limiting membranes resembling the pinocytotic vesicles (Fig. 42b & c). Deeper in the cell lie a number of multivesicular bodies and numerous elongate or ovoid mitochondria with many irregularly-orientated cristae
TABLE 5. Comparative heights of the ciliated epithelial cells and the lengths of their cilia in the alimentary tract of *Stophoriaria zelandica* (taken from histological sections of a 11 mm. specimen).

<table>
<thead>
<tr>
<th>Region of the alimentary tract</th>
<th>Cell height (μ)</th>
<th>Length of cilia (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior oesophagus (on crest of folds)</td>
<td>20–25</td>
<td>7</td>
</tr>
<tr>
<td>Anterior oesophagus (between folds)</td>
<td>12–15</td>
<td>5</td>
</tr>
<tr>
<td>Crop (on crest of folds)</td>
<td>25–30</td>
<td>5</td>
</tr>
<tr>
<td>Crop (between folds)</td>
<td>10–12</td>
<td>5</td>
</tr>
<tr>
<td>Stomach</td>
<td>15–25</td>
<td>7</td>
</tr>
<tr>
<td>Pro-intestine (on typhlosoles)</td>
<td>80–90</td>
<td>12</td>
</tr>
<tr>
<td>Pro-intestine (on folds)</td>
<td>20–35</td>
<td>7</td>
</tr>
<tr>
<td>Mid-intestine</td>
<td>25–50</td>
<td>10</td>
</tr>
<tr>
<td>Post-intestine</td>
<td>20–40</td>
<td>10</td>
</tr>
<tr>
<td>Rectum</td>
<td>12–35</td>
<td>10</td>
</tr>
</tbody>
</table>
Fig. 40 A generalised ciliated epithelial cell of the alimentary tract.

av - apical vesicle
bl - basal lamella
bm - basal plasma membrane
c - cilium
cb - basal body of cilium
cr - rootlet of cilium
ei - endocytotic invagination
g - Golgi complex
ger - granular endoplasmic reticulum
gl - glycogen granules
jc - junctional complex
li - lipid droplet
ly - lysosome
m - mitochondrion
mv - microvilli
n - nucleus
no - nucleolus
Fig. 41  Ciliated Cells of the Alimentary Tract.

a. Apical region of the ciliated epithelial cell of the anterior oesophagus showing the cillum body (c), the ciliary plate (↑) and the basal body (b). The microvilli (mv) between the cilia have a central core of filaments which continue into the cell (↑), and are coated with a thin glycocalyx (↑) (x 27500).

b. Apical region of the crop ciliated cell showing the globular apical processes (p) between the cilia (c). (x 21100).

c. Apical region of the stomach ciliated cell. The cilia (c) have long rootlets (↑) and the microvilli between them have vesicular swellings (s). (x 22400).

d. Apical region of the mid-intestinal ciliated cells. The cilia (c) also have long rootlets and the microvilli (mv) between them are dense. (x 24800).

e. Apical region of the post-intestinal ciliated cells. c - cilia mv - microvilli. (x 22500).

f. Apical region of the rectal ciliated cell. The cilia (c) have very short rootlets (↑) and the microvilli (mv) in between are short and irregular. (x 22400).
Fig. 42  Ciliated Cells of the Alimentary Tract.

a. Electron micrograph of a transverse section of cilia from the oesophageal ciliated cells. The cilia have the typical 9 + 2 microtubular structure and are surrounded by a loose plasma membrane (↑). (x 57600).

b. Rootlet of a cillum in the stomach ciliated cell showing the cross banding (B) and the interperiodic sub-bands (I). (x 50400).

c. Apical region of the oesophageal ciliated cell showing endocytotic indentations (↑) and vesicles (v). There are also numerous mitochondria (m) and one or two multivesicular bodies (mb). (x 25000)

d. Golgi area of the stomach ciliated cell. The Golgi complex buds off electron-lucent vacuoles (va) and dense vesicles (vi). Surrounding the complex are numerous lysosomal bodies (l), free ribosomes (r) and mitochondria (m). (x 37800)

e. Apical junctional complexes of the ciliated cells of the alimentary tract. za – zonula adherens, sd – septate junctions. (x 47950)

f. Basal region of the crop ciliated cell showing the slightly folded basal plasma membrane. A few macula adherens (↑) with filaments (f) extending into the cell are also present on the basal plasma membrane. (x 19200)
in a dense, homogeneous matrix and one or two intramitochondrial granules.

Between the apical mitochondria and extending to the middle of the cell are vacuoles (0.4 - 0.8μ diameter) probably lysosomal bodies, with dense granules or membrane structures inside (Fig. 41d).

Above the nucleus, there are usually one or two Golgi complexes which appear to be associated with the formation of the lysosomal bodies. Each complex consists of a stack of six to eight, closely packed, smooth membrane lamellae, with numerous small vesicles on the inner side and vacuoles on the outer (Fig. 42d). The vacuoles appear to arise from the distended ends of the lamellae and fuse with the vesicles to form the lysosomal bodies.

The endoplasmic reticulum is poorly developed, consisting of vesicles and short membranous profiles. The elongate nucleus has a peripheral ring of heterochromatin as well numerous clumps scattered throughout. Euchromatin is sparse and the nucleolus is not always distinct.

Below the nucleus lie a few mitochondria and some small vacuoles. The lateral cell membrane interdigitates to various degrees with the neighbouring cells (Fig. 42e). Junctional complexes towards the apex consist of a zonula adherens followed by septate junctions. The basal membrane is involuted by folds resting on a smooth basal lamella (Fig. 41f). At the adhesion of the basement membrane to the basal lamella, occasional desmosomes are seen with a distinct macula adherens and filaments extending on both sides.

The ciliated cells in the anterior oesophagus have numerous lysosome-like bodies in their apical half (Fig. 43). The cilia have
long rootlets which appear to be connected to the microfilaments in the cytophasm. There is very little glycogen or lipid material. The apical lateral plasma membrane immediately below the apical junctional complexes interdigitates deeply with the neighbouring cells.

The ciliated cells in the posterior oesophagus are very similar to those in the anterior oesophagus except apically, in addition to cilia and microvilli, they bear large globular processes. These processes, filled with granular material, appear to be cut off from the cell occasionally forming globules in the oesophageal lumen.

The ciliated cells of the stomach are different from those of the oesophagus, having only a few lysosome-like bodies. The ciliary rootlets are long and the microvilli between the cilia have vesicular swellings (Fig. 44). Numerous irregular-shaped electron-lucent vacuoles appear to arise from the active Golgi complexes. Basally some glycogen and lipid may be present.

The intestinal ciliated cells are distinct from the other ciliated cells of the alimentary tract, in having very few lysosome-like bodies, numerous clear apical vesicles, a very compressed Golgi apparatus and large quantities of glycogen and lipid (Fig. 45, 46, 47). The highest lipid content is found in the pro-intestinal ciliated cells, with electron-lucent globules filling a greater part of the cell below the apical mitochondrial layer (Fig. 45a). The mid-intestinal ciliated cells have both glycogen and lipid but the glycogen content is higher than in the pro-intestinal cells, while the post-intestinal cells have mainly glycogen granules (Fig. 46). The post-intestinal cells are also distinguished by the deep infolding of the basal membrane with numerous elongated mitochondria (Fig. 47c).
Fig. 43  Oesophageal Ciliated Cells

A montage of a ciliated epithelial in the anterior oesophagus. The cell bears thin microvilli (mv) and long cilia (c) with long rootlets (r), and has apical concentration of mitochondria (m) and numerous dense lysosome-like bodies (l). Among the dense bodies are one or two Golgi complexes (g). The oblong nucleus (n) has very sparse euchromatin. The lateral plasma membrane interdigitates deeply with the adjacent cells (▲). The basal plasma membrane is lined by a thin basal lamella (la). (x 5400)
The cilia of the cell have long rootlets (r) extending down to the middle of the cell. The microvilli between the cilia have vesicular swellings (s). Apically the cell has numerous dense vesicles (▲), a few multivesicular bodies (mb) and some mitochondria (m). In the mid-region, there are a few dense lysosome-like bodies (l) and numerous electron-lucent vacuoles (v) which resembles the swellings in the microvilli. The nucleus (n) is basally located and the cell is lined basally by a distinct basal lamella (la). (x 10400)
Fig. 45  Pro-intestinal Ciliated Cell

a. Electron micrograph of the pro-intestinal epithelium showing the ciliated cells (C) with secretory cells (S) in between. The ciliated cells are filled with electron-lucent lipid globules (l). (x 2790)

b. Golgi area of the pro-intestinal ciliated cells with very compressed lamellae (g). Note also the lysosome-like (ly) bodies and the clusters of free ribosomes (r) nearby. (x 28400)

c. Part of the cytoplasm of the pro-intestinal ciliated cells. Note the lysosome-like bodies (ly), the sparse GER (†) and the bundles of microfilaments (f) among the lipid globules (l). (x 28400)
Fig. 46  Mid-intestinal Epithelial Cells

The ciliated cell (C) has several cilia with thick short rootlets (r). In between the cilia are long fine microvilli (mv). Mitochondria (m) are concentrated in the apical and basal region of the cell. The centrally-placed nucleus (n) has a prominent nucleolus. In the supranuclear region there are a few lysosome-like bodies (ly) and occasionally a Golgi complex. Throughout the cytoplasm are electron-lucent lipid globules (l) and clusters of glycogen granules (gl).

The non-ciliated cell (N) has a slightly protruded apex with long, thick microvilli. The mitochondria is evenly distributed. The cell also has abundant lipid and glycogen. Bundles of microfilaments (f) ramify throughout the cytoplasm. The basal plasma membrane are not infolded as that of the ciliated cell. (x 2700)
Fig. 47  Post-intestinal Ciliated Cells

a. Electron micrograph of the post-intestinal epithelium showing the ciliated cells with numerous lipid globules (l) and a centrally-placed nucleus (n). Note a nerve bundle (Ne) extend into the epithelium in between the basal region of the ciliated cells. (x 3900)

b. Mid-region of a post-intestinal ciliated cell showing the glycogen granules (gl), elongated mitochondria (m) and bundles of microfilaments (f). (x 22500)

c. Basal region of a post-intestinal ciliated cell showing the multi-folded basal plasma membrane (p) with elongated mitochondria (m) sandwiched in between. A nerve (Ne) is surrounded by the folded basal plasma membrane. Note also the glycogen granules (gl) in the cytoplasm and the basal lamella (la) of variable thickness. (x 57600)
Fig. 48. Rectal Ciliated Cells

a. Electron micrograph of the rectal epithelium showing the ciliated cells (C) and the mucus-secreting cells (Mu). Note the numerous nerve bundles (Ne) in between the ciliated cells. (x 7950)

b. Dense membranous structures in the lysosome bodies (l) of the rectal ciliated cells. (x 38400)

c. Dense glycogen granules (gl) in the cytoplasm of the rectal ciliated cell. (x 24800)

d. A basal process (p) of the rectal ciliated cell containing dense glycogen granules (gl) and a few mitochondria (m). The basal lamella (la) lining the plasma membrane is thickened at parts (↑) where it appears to be connected to the collagen fibres (f) of the adjacent connective tissue. (x 37800)
The rectal ciliated cells are shorter and contain lysosome-like bodies as well as glycogen and lipid. The basal membrane with the basal lamella sends out processes into the dense underlying connective tissue (Fig. 48).

2. **Mucus-secreting Cells** (Fig. 49).

This second cell type of the alimentary canal varies greatly from goblet to fusiform or cuneiform. Usually interspersed between the ciliated cells, they may also, especially in the oesophagus, be displaced sub-epithelially (Fig. 50a). Epithelial mucous cells stain lightly with PAS and some parts are positive to Alcian Blue and Colloidal Iron, while the sub-epithelial mucous cells are usually strongly positive to Alcian Blue and Colloidal iron. Lufty and Demian (1967) distinguished two types of mucous cells in the intestine of *Marisa* by their location, shape and histochemical properties. In *Siphonaria*, however, the uniform ultrastructure and the varying scale of staining intensity with PAS and Alcian Blue indicate a single cell type at different phases of secretion or secreting slightly different types of mucus. In addition, the epithelial mucous cells in the oesophagus or rectum are seldom seen with a nucleus while the subepithelial cells are usually nucleated. It could appear therefore that most of the mucous cells in the epithelial layer of the oesophagus or rectum are the apical processes of the subepithelial cell bodies.

The mucous cell exhibits the characteristic ultrastructure of an active exocrine cell. Apically the plasma membrane is invaginated to form a funnel through which the secretion is liberated (Fig. 50b). The microvilli are arranged in whorls lining the neck of this channel.
(Fig. 50c). The basal nucleus has heterochromatin in small clumps just inside the nuclear envelope. Euchromatin is dense, with a nucleolus occasionally present (Fig. 50d). Above the nucleus, the cell is packed with secretory granules, electron-lucent but reticulate throughout with a loose filamentous material. At places of contact, the granule membrane is often incomplete, indicating anastomoses into larger granules. The secretory granules are discharged apocrinally (Fig. 50f).

The cytoplasm in the apical half of the cell is reduced to a network of thin strands between the large secretory granules. Apart from a few mitochondria, there are no other organelles. Just above the nucleus between smaller secretory granules are one or two prominent Golgi complexes (Fig. 50c & e). Each complex consists of a stack of six to nine long, closely packed saccules. Electron-lucent vacuoles are formed at the inner side of the complex and small electron-dense vesicles are budded off from the outer side. The vacuoles and vesicles appear to fuse together to be incorporated into the nearby secretory granules (Fig. 50e). The neighbouring cytoplasm has a distinct endoplasmic reticulum (GER), with the cisternae greatly distended and filled with a dense crystalline material.

Mitochondria in the mucous cells are sparse and scattered, varying in shape with irregularly-orientated cristae and very dense stroma.

Cell boundaries and apical junctions resemble those of the ciliated columnar cells.

3. **The Non-ciliated Oesophageal Cells**

The ridges of the posterior oesophagus are clad with ciliated cells
Fig. 49  The Mucus-secreting Cell of the Alimentary Tract

af  -  apical funnel
cer -  cisternae of granular endoplasmic reticulum
g  -  Golgi complex
m  -  mitochondrion
mv  -  microvilli
n  -  nucleus
sg  -  secretory granule
Fig. 50 Mucus-secreting Cells

a. Electron micrograph of the crop epithelium showing a mucus-secreting cell (Mu) and a secretory cell (S) among the ciliated cells (C). (x 2000)

b. Electron micrograph of a mucus-secreting cell (Mu) showing the apical funnel (†) through which the mucous secretion (sg) is liberated. (x 6000)

c. Golgi complexes (g) of a mucus-secreting cell surrounded by mucous secretory granules (sg). Note the dense GER cisternae (¶), the occasional mitochondria (m) and the densely chromatic nucleus (n). (x 14200)

d. Electron micrograph of a crop mucus-secreting cell showing the microvilli (mv) surrounding the apical funnel (†). (x 7000)

e. Golgi area of a mucus-secreting cell showing the fusion of the vacuoles (va) and vesicles (vi) from the Golgi lamellae (l) forming the secretory granules (sg). Surrounding the Golgi complex are dense GER cisternae (ger) and a few mitochondria (m). (x 42640)

f. Apocrine liberation of the mucus from the mucus-secreting cell (Mu) in the stomach region. (x 13440)
only on the crests (Fig. 51a). The cells of the non-ciliated part (non-ciliated oesophageal cells) are similar to the ciliated cells histochemically; but structurally, apart from having no cilia, they differ in having denser microvilli and numerous electron-lucent lipid globules with dense glycogen granules.

4. The Oesophageal Secretory Cells

These cells, found sparsely in the crop epithelium, are small, spindle-shaped with attenuated apex and base (Fig. 51a). The granular contents are blue with Toluidine Blue and positive to Bromophenol Blue. The whole cell is filled with electron-dense granules (0.4 - 1μm diameter) sometimes surrounded by GER, with a few dense mitochondria in between (Fig. 51b). The cytoplasm is dense and granular and has some distended GER cisternae. Occasionally a very compressed Golgi complex is present, with a few dense lamellae (Fig. 51c). The oval nucleus is centrally located, containing diffuse heterochromatin and a small nucleolus (Fig. 51b).

Spherules of cytoplasm with dense secretory granules are extruded merocrinally from the narrow cell apex (Fig. 51d) and are probably gradually broken down in the lumen with release of the granules.

5. The Gastric 'Cuticular' Cells (Fig. 52)

These cells occur only on the narrow smooth strip in the anterior chamber of the stomach. They are columnar and dense with glycogen and lipid. The dense apical 'cuticular' layer stains slightly with Alcian Blue but densely with PAS. Ultrastructurally this layer is not a true cuticle; it is actually a layer of closely-packed microvilli with dense aggregates of filamentous material in between (Fig. 53a). The microvilli
are long (1 – 2µ) and thick (120 nm) with several short, stub-like branches on the apical half and covered with a filamentar glycocalyx coat. At the apical part where the microvilli branch, the glycocalyx may aggregate into dense clumps. The microvilli are supported by a central core of fibrils, and the plasma membrane at their base may invaginate to give rise to vesicles in the terminal web of the cell. Immediately below this, the cytoplasm is filled with dense glycogen granules and large electron-dense lipid globules. Mitochondria are numerous, mainly in the apical and basal region. Among the lipid globules are also a few spherical vacuoles with dense heterogeneous contents, many of which are similar to the lipid globules except with a vacuolar membrane (Fig. 53b).

The Golgi complex frequently consists of a stack of six to seven lamellae, the outermost being distended into vacuoles and a few vesicles (Fig. 53c). The oblong central nucleus has a prominent nucleolus, patchy heterochromatin and dense euchromatin (Fig. 53a).

The apical cell junctions are as previously described (Fig. 53c). The lateral plasma membrane interdigitates slightly while the basal plasma membrane has slight infolding.

6. The Pro-intestinal Secretory Cells

This clavate cell type is found only in the pro-intestine, having a large body located beneath the ciliated epithelial cells and reaching the lumen with an attenuated apex (Fig. 54a). The cell, especially the apical region, is filled with dense glycoprotein secretory granules.

The cell bears very sparse microvilli (Fig. 54b). The apical secretory granules (0.2 – 0.4µ diameter) is very electron-dense. The cell
body contains in addition to the secretory granules, a few moderately dense vacuoles which are probably the early stages of the secretory granules (Fig. 54c). Surrounding these granules and vacuoles are distended cisternae of GER. A number of active Golgi complexes lie peripherally, consisting of six or seven lamellae filled with dense material. The distended ends of the lamellae give rise to dense vesicles which appear to coalesce to form the secretory granules. The oblong, basal nucleus has dense chromatin and a prominent nucleolus.

The secretory granules are liberated merocrinally (Fig. 54d), with apical portions of the cell often seen free in the lumen (Fig. 54e & f).

7. **The Mid-intestinal Secretory Cells**

These cells are large and oblong, filled with granules staining positively for protein only (Fig. 55a).

The microvilli are few but thicker than in the adjacent ciliated cells (Fig. 55c). The secretory granules which fill the whole cell are dense and spherical (0.5 - 1.5μ diameter) (Fig. 55d). The cytoplasm contains numerous free ribosomes, scattered mitochondria and distended cisternae of GER which are occasionally stacked or whorled especially in the basal region of the cell (Fig. 55b). The Golgi complex if present is very small with a few compressed lamellae and some dense vesicles (Fig. 55d). The oval, basal nucleus has dense chromatin and a prominent nucleolus.

8. **The Non-ciliated Intestinal Cell**

These are found in between the ciliated cells in the mid- and
post-intestine, distinct only under electron microscopy. They bear no cilia but have dense microvilli, shorter and thicker than those in the adjacent ciliated cell (Figs. 46 & 56a). The apical cytoplasm contains numerous small vesicles which appear to aggregate into large multivesicular bodies (Fig. 56b). The lower cytoplasm is filled with glycogen granules and bundles of microfilaments and a moderate number of mitochondria (Fig. 56c). Occasionally one or two Golgi complexes may be present in the supranuclear region, with a few compressed lamellae surrounded by some dense vesicles. The basal plasma membrane has, in contrast to that of the adjacent ciliated cell, only very few infoldings (Figs. 46 & 55d).
Fig. 51 Oesophageal Secretory Cells

a. Electron micrograph of the crop (posterior oesophagus) epithelium showing the ciliated cells (C), the non-ciliated cells (E) and the secretory cells (S). (x 6050)

b. Electron micrograph of an oesophageal secretory cell. The cell contains numerous dense granules (sg), dense GER cisternae (†), a few dense mitochondria (m) and a large nucleus (n). (x 21000)

c. Golgi complex (g) between a secretory granule (sg) and a mitochondrion (m) in an oesophageal secretory cell. (x 42640)

d. Merocrine secretion (Sm) from the oesophageal secretory cell. Note the dense granules (sg) in the secreted spherule. (x 10500)
Fig. 52  Montage of the Gastric 'Cuticular' Cell

The cell has microvilli densely packed with a fibrous material (↑). The whole cell is filled with electron-dense lipid globules (li) and clusters of glycogen granules (g). The nucleus (n) is centrally-located and the mitochondria (m) are concentrated in the apical and basal region of the cell. (x 4300)
a. Electron micrograph of a section of the gastric shield strip showing the gastric 'cuticular' cells with a ciliated cell (C) in between. (x 4000)

b. Apical region of the gastric 'cuticular' cell showing the thick microvilli (mv), some of which have short branches (†). The microvilli are coated with glycocalyx (gc) which aggregates at parts into dense clumps (†), and supported by a central core of fibrils (f). The plasma membrane at the base of the microvilli invaginates to form vesicles (v) in the terminal web. The apical cytoplasm contains dense glycogen granules (gl) and a few mitochondria (m). (x 36000)

c. Mid-region of a gastric 'cuticular' cell showing the Golgi complex (g) numerous lipid globules (li) and glycogen granules (gl) above the nucleus (n). (x 25500)

d. Apical junctional complex of the gastric 'cuticular' cell. za – zonula adherens, sd – septate junction. (x 27500)
Fig. 54 Pro-intestinal Secretory Cells

a. Electron micrograph of two pro-intestinal secretory cells in between the ciliated epithelial cells (c). The cell has numerous dense secretory granules and an oblong nucleus (n). (x 6950)

b. Supranuclear region of the pro-intestinal secretory cell. The Golgi complex (g) has dense lamellae and is surrounded by GER cisternae (cer). Small secretory granules (sg) appear to arise from the dense Golgi vacuoles. (x 14000)

c. Apical region of the pro-intestinal secretory cell. The cell bears very sparse microvilli (†) and is filled with secretory granules (s). (x 25500)

d. A pro-intestinal secretory cell undergoing merocrinal secretion liberating a secretory spherule (ss) into the lumen (L) of the pro-intestine. (x 7750)

e. A secretory spherule (ss) from the pro-intestinal secretory cell in the lumen (L) of the pro-intestine. (x 12750)

f. Disintegrating secretory spherule (ss) in the lumen (L) of the pro-intestine. (x 11450)
Fig. 55 Mid-intestinal Secretory Cells

a. Electron micrograph of the mid-intestinal epithelium showing the secretory cells (S) distributed between the ciliated epithelial cells (C). (x 1400)

b. Montage of a developing mid-intestinal secretory cell. The cell has whorls of GER (ger), numerous dense secretory granules (sg) and a basal nucleus (n) with a large nucleolus (no). (x 7000)

c. Apical region of the mid-intestinal secretory cell showing the thick microvilli (mv), the dense secretory granules (sg) and a few mitochondria (m). (x 10000)

d. Mid region of a mature mid-intestinal secretory cell filled with the dense secretory granules (s). Golgi complexes (g) and some GER (†) are still quite distinct in the cytoplasm between the secretory granules. (x 9300)
Fig. 56 Post-intestinal Non-ciliated Cells

a. A post-intestinal non-ciliated cell between the ciliated epithelial cells (C). (x 6500)

b. Apical region of a non-ciliated cell in the post-intestine. The cell bears dense microvilli (mv). The endocytotic invagination (†) at the base of the microvilli form vesicles (v) which appear to aggregate into multivesicular bodies (mb). (x 27500)

c. Mid-region of the post-intestinal non-ciliated cell showing the abundance of microfilaments (f), the mitochondria (m) and glycogen granules (gl) in the cell. (x 27530)

d. Basal region of the post-intestinal non-ciliated cell showing the slightly folded basal plasma membrane (†) in contrast to the multifolded basal plasma membrane (p) of the adjacent ciliated cell. (x 17280)
3.4.2 DISCUSSION

The siphonariid digestive system, as already shown by Marcus and Marcus (1962), consists of a large buccal mass, a distensible oesophageal crop, a small and relatively simple stomach with paired digestive diverticula, and a simple intestine and rectum. All these show broad resemblances to the plan offered for the primitive Basommatophora by J.E. Morton (1955b) in his study of Otina otis. The structure of the different parts of the digestive system of Stiphonaria zealandica are discussed now with respect to its food and habitat and in comparison with related pulmonate groups.

The two regions of the oesophagus, a slender anterior part and a distensible posterior crop are common to most pulmonates (J.E. Morton, 1955a,b; Ghose, 1963; Rigby, 1963; 1965; Walker, 1972). In Lymnaea stagnalis, Carriker (1946a) however recognised three parts of the oesophagus: pro-, post- and crop, the crop being distinguished from the posterior oesophagus only by its increased diameter. The larger diameter of the crop may be due to the distention by the gizzard immediately behind it. The crop and the post-oesophagus are thus more likely to be one single part of the oesophagus.

The crop, a site of storage and initial digestion usually present in pulmonates, is according to Ghose (1963) an adaptation for uncertain food supply. This would be scarcely applicable, however, to Stiphonaria which has easy access to rich plant fodder and continues to feed very actively both night and day when the tide is out. The crop walls, though muscular, do not appear to triturate, and food breakdown appears to be entirely by enzymes, which were shown to be strongly present in the salivary
gland and also in the oesophageal wall (Section 4.3). The crop serves then as a distensible bag where food is churned and digested. Rigby (1963) suggests for Oxychilus the function of mixing and squeezing of the muscular crop wall is an adaptation to a bulky herbivorous diet and for the increasing importance of extracellular digestion.

Ciliation of the oesophagus and crop, as in Siphonaria has been reported also in the primitive Otina otis and ellobiids (J.E. Morton, 1955a,b). But for Stylommatophora such as Oxychilus and Succinea (Rigby, 1963, 1965), cilia are absent and muscles have assumed the whole function of transport and food mixing. (See also Runham, 1975).

Carriker and Bilstad (1946) reported two types of ciliated cells in the digestive tract of Lymnaea stagnalis; the normal ciliated cell in the upper oesophagus and the secretory ciliated cells in the lower oesophagus, crop, pylorus and upper intestine. The latter are distinguished by their alveolar cytoplasm, large oval nuclei and secretion of globules. The secretory ciliated cell in the crop of Siphonaria, however, is very similar to the normal ciliated cell except for its merocrine secretion. As the secretory globules do not contain any distinctive organelles or secretory granules, their function is still uncertain.

All the ciliated cells in the oesophagus of Siphonaria contain irregular, dense bodies with heterogeneous contents, which appear to originate partly from the Golgi complex and are possibly lysosomes. Bowen (1970) demonstrated acid phosphatase, a marker of lysosomes, in the Golgi saccules and vacuoles in the crop cell of Arion ater. Walker (1969) on the other hand found this enzyme absent in the crop wall of Agriolimax but the cell granules contained non-specific esterase, one of the enzymes
associated with lysosomes. The abundance of these lysosomal bodies in the oesophageal cells suggests a possible role of the oesophageal cells in absorption and further digestion. Runham (1975) suggested the presence of numerous microvilli and some pits between them may indicate the uptake of food material. In Siphonaria, pinocytotic vesicles are sometimes observed in these cells and the apical multivesicular bodies are probably aggregations of these vesicles. Simpler substances can also be absorbed by permeation or active transport through the plasma membrane with no special structures. In the crop of Agriolimax reticulatus, Walker (1972) has shown that absorbed simple monosaccharides were located diffusely in the cytoplasm while lipids were located in the granules suggesting their pinocytotic entry. In Siphonaria, the simple products of digestion by salivary enzymes could be absorbed likewise and thus would be removed from the food mass before they reach the stomach.

In Lymnaea, Carriker and Bilstad (1946) found basophilic flask cells in the post-oesophagus, crop, pylorus, caecum and pro-intestine and suggested they function in secreting a mucoid envelope for the faeces. The similarly shaped oesophageal secretory cell in the crop of Siphonaria is, however, unlikely to have such function as active digestion is in progress in the crop. The secretory granules are proteinaceous, probably containing digestive enzymes for extracellular digestion. The detection of strong enzyme activity from the oesophageal wall (Section 4.3) confirms this.

The evolutionary and functional significance of the molluscan stomach has been extensively discussed by Graham (1949) and J.E. Morton (1953). The stomach of Siphonaria carries a number of features which are
reminiscent of the primitive gastropod stomach, as postulated by Graham, as well as some characteristics of more advanced pulmonates. The surviving primitive features include the positions of the anterior and posterior digestive gland apertures and the paired typhlosoles still recognisable in the ridges bounding the posterior ciliated plicae from the digestive gland openings. Though there is no style or protostyle, the cilia on the typhlosoles produce a rotatory beat, suggesting that the pre-intestinal chamber is a vestige of the style sac.

Typical pulmonate characteristics are the thickening of the musculature and the reduction of the gastric shield to a small strip of 'cuticle' in the anterior chamber. Both indicate the beginning of a muscular gizzard. With such a structure, the stomach of *Siphonaria* resembles closely that of the primitive pulmonate *Otina otis* (J.E. Morton, 1955b). Some characteristics of higher Basommatophora and Stylommatophora are also present. These include the loss of the protostyle, the presence of an accessory fold running from the posterior to the anterior digestive gland aperture, continuing as the minor typhlosole, and the reduction in size of the posterior digestive gland, the aperture of which is surrounded by a sphincter muscle precluding the entry of large particles. All these characters are also found in the stomachs of the stylommatophorans, *Oxychilus* and *Succinea* (Rigby, 1963, 1965) and *Agriolimax* (Walker, 1972). The establishment of muscular control of the digestive gland aperture was held by Rigby (1963) to be an adaptation in stylommatophorans, higher tectibranchs and some higher basommatophorans, for dealing with a greater diversity of foods than the original microphagous gastropods. The general simplification of gastric structure in pulmonates may be attributed to the progressive development of a macrophagous feeding habit and extracellular
digestion.

The ciliated cells of the stomach of *Siphonaria* are quite different from those of the oesophagus. With light microscopy, their cilia appear very dense and thick. Electron microscopy reveals that the microvilli between them have numerous 'bead-like' vesicular swellings. Such vesicles have also been reported in the gastric shield cuticle in the protobranchiate bivalve, *Nucula* (Halton & Owen, 1968). The presence of acid phosphatase activity in these microvilli suggests a secretory-function. Although histoenzymatic tests were not conducted for *Siphonaria*, the numerous supranuclear Golgi complexes budding off electron-lucent vacuoles may be an indication of the secretory activity.

The strip of 'cuticle' in the anterior chamber is shown, under the electron microscope, to be composed of densely-packed microvilli with a dense fibrous matrix in between. A similar structure has been reported in the non-removable portion of the gastric shield of *Nucula* (Halton & Owen, 1968) and the whole gastric shield of *Lasaea rubra* (McQuiston, 1970), but with some notable distinctions. The non-removable cuticle of *Nucula* contains very little matrix material but shows strong secretory activity as was indicated by the vesicular swellings of the microvilli. In *Lasaea*, however, the matrix was distributed evenly among the microvilli, which rarely showed vesicular distention. For *Siphonaria*, the microvilli were thick, with very dense matrix in between but no vesicular distention. The reduced distention of microvilli in *Lasaea rubra* has been suggested as an indication of reduced metabolic activity. Halton and Owen (1968) suggested that the enzymes released from the 'cuticle' may play some digestive role in the stomach and predict that in Lamellibranchs where the stomach has a more extensive 'cuticle', mechanical protection may have replaced the original secretory role. This was confirmed by the work of McQuiston (1970) on
Lasaea rubra. The 'gastric cuticle' of Siphonaria would appear mainly protective with secretory activity much reduced. The small representation of GER, Golgi complexes and secretory vacuoles indicate a lack of secretory activity, which may have been assumed by the ciliated cells in the other parts of the stomach.

The high glycogen content of the gastric 'cuticular' cell in Siphonaria is paralleled in Lasaea rubra (McQuiston, 1970). This may be related to the production of chitin through transformation of glucose which along with other sugars, would be taken in from the blood via the basal lamella. The glucose taken in is probably first converted to glycogen by the enzymes contained in the basally concentrated mitochondria and then the glycogen is turned into chitin by those enzymes in the apical mitochondria. Similar processes may occur in Siphonaria as is indicated by the abundance of mitochondria in the apical and basal regions of the gastric 'cuticular' cell.

The role of the spherical vacuolar bodies in these cells is not clear. Halton and Owen (1968) found that these bodies are histochemically and histologically related to lipofuscin pigments and suggested that they are a by-product of lipid metabolism. The presence of pinocytotic vesicles at the base of the microvilli and numerous lipid globules would suggest that these cells are active in lipid uptake and metabolism. The association of these vacuolar bodies with lipid globules would appear to agree with Halton and Owen's suggestion.

In Siphonaria the preintestinal chamber of the stomach, with its typhlosoles as in Agriolimax (Walker, 1970), can clearly be regarded as the remnant of the style sac. J.E. Morton (1955a) reported the presence
of a protostyle in *Otina otis* but in *Siphonaria* this is absent. The basophilic, flask-shaped secretory cells, lying in a layer beneath the ciliated cells in the distal end of the typhlosoles and the first part of the pro-intestine, resemble the basophilic flask cells in the crop, stomach and pro-intestine of *Lymnaea* (Carriker & Bilstad, 1946), which are negative to mucicarmine. These cells in *Siphonaria* stained strongly for neutral polysaccharide and very lightly for acid mucosubstances but showed distinct metachromasia with Toluidine Blue. Electron microscopy suggests that the cell produces a sero-mucous secretion, having large cisternae of GER with dense granular material and the secretory granules arising from the Golgi cisternae, as in other serous cells (Sjostrand & Hanzon, 1954; Scott & Pease, 1959). Similar sero-mucous gland cells in the stomach of the ascidian, *Ciona* have been suggested to be possibly enzyme secreting (Thomas, 1970) but enzyme histo-chemical tests have not been made. In *Siphonaria*, enzymes in the lumen of the pro-intestine may originate from the fragmentation spherules of the digestive gland (Section 3.6). Carriker and Bilstad (1946) on the other hand suggested for the flask cells the role of secreting a mucoid coat for the faecal mass emerging from the stomach.

The circulatory action of the typhlosolar and the pro-intestinal cilia in *Siphonaria* must consolidate the faecal material as in *Lymnaea* (Carriker, 1946a) and *Agriolimax* (Walker, 1972). Graham (1949) commented on the role of the typhlosolar cilia in picking up loose particles within the stomach and passing mucus forward from the glandular typhlosolar epithelium. Carriker (1946a) named the ciliated area distal to the typhlosoles in *Lymnaea*, the 'pellet compressor' regarding it as assisting in the consolidation of faeces.
The intestine and rectum have been little studied in pulmonates (Runham, 1975). In *Siphonaria*, as in *Agriolimax* (Walker, 1972), the intestine is not distinctly separated from the region corresponding to the style sac, but is considered to begin at the distal end of the typhlosoles. Following Rigby (1963), *Siphonaria* should lie nearer the Stylommatophora with the intestine looping round the anterior digestive gland, rather than penetrating the posterior lobe as is typical in Basommatophora.

In the intestinal cells of *Siphonaria*, the dense microvilli with the pinocytotic vesicles at their base strongly indicate absorption. A similar brush border region with a profusion of mitochondria in the intestine of *Arion emปริโคrum* has been suggested to indicate the presence of an active transport system. In bivalves, with mainly intracellular digestion by the digestive gland cells, absorption by the intestine has long been held to be absent (Yonge, 1926a; George, 1952; J.E. Morton, 1953; Owen, 1955, 1956; Sumner, 1966b; Giusti, 1970). Extracellular digestion has, however, been demonstrated in gastropods (Holden & Tracey, 1950; Myers & Northcote, 1958; Evans & Jones, 1962a,b) and it seems unlikely that absorption should be restricted to the digestive gland. Uptake of calcium, strontium, phosphate and iodine from the intestinal lumen has been demonstrated in *Arion, Agriolimax* and *Helix* (Fretter, 1952, 1953). Sumner (1965b) reported the uptake of lipid by intestinal cells of *Agriolimax*, simple molecules such as glycine, glucose and galactose diffuse into the cytoplasm while palmitic acid is taken up by pinocytosis and subsequently processed by the lysosomal system. Orive *et al.* (1979) demonstrated in *vitro* that L-proline and not
L-Lysine or L-glutamic acid was actively absorbed by the intestine of *Helix pomatia* and that for *Arion empiricorum* L-glutamic acid was transported by mediated diffusion in the posterior intestine and by simple diffusion in the anterior one. In *Siphonaria*, the morphological evidence of absorption is supported indirectly by the presence of enzymatic activity in the lumen as well as the intestinal wall (Section 4.3) which indicates that digestion and absorption could continue as faeces form in the intestine.

The intestinal ciliated cells contain clear lipid globules, as distinct from the dense lipid droplets in the stomach 'cuticular' cell. The pinocytotic vesicles at the base of the microvilli indicate the origin of these lipid globules. Lipid staining by osmium tetroxide varies from electron dense to electron lucent depending on the chemical nature of the lipid (Casey-Smith, 1963). Hence, the lipid absorbed by the intestinal cells is different from that of the stomach cells. The intestinal lipid is possibly passed to the connective tissue beneath the epithelium. This, however, appears improbable in the pro-intestinal cells, which have the highest amount of lipid, for the cell base is compressed by the basophilic secretory cell. It seems more likely that these lipid globules form a temporary store, possibly to replace the lipid content of the adjacent secretory cells which are constantly lost as membrane lipoprotein in merocrine secretions.

The mid-intestine has an additional secretory cell type of its own, with granules staining lightly for neutral carbohydrate and strongly for protein. It resembles the basal cell of the intestine and rectum of *Lymnaea* (Carriker & Bilstad, 1946), and the secretory cell in the terminal
intestine of *Mytilus* (Giusti, 1970). Its whorls of distended GER and small Golgi system indicates protein production. The zymogen affinities suggest enzyme production, but the similar cell in *Lymnaea* has been held to secrete a cement for the faeces (Carricker & Bilstad, 1946).

The post-intestinal ciliated cells have strong multiple infolding of the basal membrane, with high concentration of mitochondria. Similar infolding with mitochondria is shown by cells of the intestine of *Ciona* (Thomas, 1970), the salivary duct of pulmonates including *Siphonaria* (Section 3.5), the reno-pericardial system of *Lymnaea* (Wendelaar Bonga & Boer, 1969) as well as other invertebrate and vertebrate tissues (Pease, 1955; Doyle, 1960; Copeland, 1963, 1964; Degail & Levi, 1964; Copeland & Fitzjarrel, 1968). This folding is typical of active transport sites for ions and for water (Diamond & Tormey, 1966). Guisti (1970) disputed the need of this function in marine invertebrates; but Degail and Levi (1964), describing the digestive gland of the ascidian *Molgula ovulata*, suggested that although the animal is iso-osmotic with seawater, some ionic regulation must still be required. As an intertidal pulmonate, *Siphonaria* faces great fluctuations in the water and ionic content of the environment; thus the post-intestinal cell could serve an osmoregulatory role along with control of the water content of the faeces.

The dense microvilli and glycogen granules in the rectal ciliated cells suggest that absorption may still be proceeding here. The function of the numerous dense lysosome-like bodies similar to those in the oesophageal cells still awaits investigation.

The microfilaments extending through the cytoplasm and into the microvilli of all the alimentary ciliated cells of *Siphonaria*, particularly
prominent in the intestinal cells, have also been reported in the
general epidermal cells of Lymnaea (Zylstra, 1972) and in the young
digestive cells of some gastropods (Meuleman, 1972; Nelson, 1976).
It has been suggested that these microfilaments serve as a cytoskeleton
or assist in contractile movements of the cell (Zylstra, 1972). In
Siphonaria, the epithelial cells of the whole alimentary tract must
receive much stress from distortions during peristalsis. The role
of the microfilaments in intracellular transport has been emphasized
(Rosenberg, 1966; Rasmussen, 1970; Rebhun, 1972; Bardele, 1973; Nelson,
1976); in Siphonaria they could provide a structural basis for dynamic
activity of the cytoplasm enabling rapid intracellular movements of
absorbed material.

The mucous cells of the gut have a very uniform ultrastructure,
with flocculent secretory granules, prominent Golgi complexes and much
distended GER. Histochemically, however, those of the rectum are
distinctive. It is now known that mucus is a mixture of at least two
components: acid-mucopolysaccharide and carbohydrate-protein complexes
(Pearse, 1968). The strong metachromasia of the rectal cells indicates
a higher acid mucopolysaccharide content as compared with other regions
which have more carbohydrate-protein. Mucus discharge is entirely
comparable with the apocrine secretion of the mammalian intestinal
goblet cells (Rhodin, 1959; Florey, 1962; Bierring, 1962).
3.5 THE STRUCTURE OF THE SALIVARY GLAND

3.5.1 OBSERVATIONS AND RESULTS

3.5.1.1 General Morphology of the Salivary Gland

The paired salivary glands in *Siphonaria zelandica* form yellow, much-branched structures at either side of the oesophagus (Fig. 35). Each leads into the dorsal food channel of the buccal mass by a narrow duct, running for a short distance through muscles and connective tissues. The glands are made up of small acinose tubules and are loosely bound to the anterior oesophagus and posteriorly to each other by connective tissue so that they may appear to be a single entity. The short salivary duct receives the interlobular ducts which collect from the intralobular ductules bearing the acini.

3.5.1.2 Cellular Structure of the Salivary Gland

The glands are inadequately preserved by most fixatives for paraffin sectioning, therefore epon thick sections were generally used for light microscopy. Seven cell types were initially identified, but with electron microscopy and light microscopy of starved animals, only six were confirmed, one type (Mucocyte III) proving to be an early stage of a second type (Mucocyte II). The six confirmed types following the terminology of Gabe and Prenant (1948) and modified by Boer *et al.* (1967) are the ciliated duct cell, the duct mucocyte, the grain cell, mucocytes I and II and the undifferentiated cell. Apart from the ciliated duct cell and the undifferentiated cell, all others appeared to be secretory in function.
The cell types are not randomly distributed but strongly localised (Fig. 57). The epithelium of the terminal acini and the intralobular tubules consists mainly of Mucocytes I and II with a few grain cells (Fig. 58b). Interspersed between these cells around the acinous lumen and the intralobular duct are small ciliated cells (Fig. 58a,c,d). The distal part of the salivary duct up to the first bifurcation is lined with duct mucocytes and ciliated duct cells (Fig. 58a).

1. **Ciliated Duct Cell**

The salivary ciliated duct cells appear in two forms: one is found in the inter- and intralobular ducts between the secretory cells and another in the proximal salivary duct. Both of these forms are quite different from the ciliated lining cells of the alimentary tract.

These ciliated cells in the inter- and intralobular ducts are small and slender. The cell apex, and sometimes the base too, expands widely over the neighbouring cells (Fig. 59a). Electron microscopy reveals fine and irregular microvilli in between the cilia. The cilia are long (7-8μ) and have the characteristic 9 + 2 microtubular structure (Fig. 59c) with a basal body and a long rootlet (Fig. 59d). Triple centre filaments were occasionally observed (Fig. 59c). The cytoplasm contains numerous mitochondria, many free ribosomes, a small amount of GER and a network of microfilaments which appear to be connected to the rootlets of the cilia. The mitochondria are mainly concentrated in the apical region below the rootlets, having numerous cristae and one or two intra-mitochondrial granules (Fig. 59e). Vacuoles are also common, some with electron-dense material on the inner surface of the limiting membrane (Fig. 59b). The basal nucleus is polymorphic, with clumps
Fig. 57

Schematic drawing of a longitudinal section of the salivary gland through the interlobular duct, intralobular duct and the tubule showing the distribution of the different cell types.

1 - proximal salivary duct
2 - posterior salivary duct
3 - interlobular duct
4 - intralobular duct
5 - salivary tubule
Cd\(_1\) - ciliated duct cell type 1
Cd\(_2\) - ciliated duct cell type 2
G - grain cell
L - lumen
M\(_1\) - mucocyte I
M\(_2\) - mucocyte II
Md - duct mucocyte
Ms - muscle layer
U - undifferentiated cell
Fig. 58 Salivary Gland

a. & b. Light micrographs of transverse sections of the salivary gland through the interlobular duct (a) and intralobular duct (b) region. Note the concentration of duct mucocytes (Md) near the posterior salivary duct (SD) and the distribution of mucocyte I (M₁), mucocyte II (M₂) and grain cell (G) in the tubules. The salivary duct is lined by ciliated cell (C) and filled with strongly stained secretion. (Epon thick section, Colloidal Iron/PAS staining; x 1800)

c. & d. Light micrographs of transverse sections of the interlobular duct of the salivary gland showing the different histochemical properties of the cell types: duct mucocyte (Md), mucocyte I (M₁), mucocyte II (M₂), grain cell (G) and the ciliated duct cell (C). Note the different luminal size of the interlobular duct (D₁) and the intralobular duct (D₂). (Epon thick sections, c. - Alcian Blue pH 2.5/PAS, d. - Aldehyde Fuchsin/Alcian Blue pH 2.5; x 1200)
Fig. 59 Ciliated Duct Cells

a. Electron micrograph of a section through the intralobular duct region showing the ciliated duct cell (C) located between a mucocyte I (M₁) and a mucocyte II (M₂). Sections of the cilia from the ciliated cell are present in the lumen of the duct (L). (x 7740)

b. Electron micrograph of a ciliated cell showing the cilia (↑), basal bodies (↑) of the cilia, the network of microfilaments (f) that are connected to the rootlet (▲) of the cilia. The cell also has a number of vacuoles (v), a few mitochondria (m) and a large nucleus (n). (x 19200)

c. Cilia of the ciliated duct cell showing one cilium with three central tubules (↑) among the normal cilia with 9 + 2 microtubular structure. (x 60760)

d. Apical region of the ciliated duct cell showing the basal bodies (b) and the long rootlet (▲) of the cilia. (x 30580)

e. Apical junctional complexes of the ciliated cells. b - basal body of cilia, m - mitochondria, sd - septate junction, za - zonula adherens. (x 34800)
of dense heterochromatin. No Golgi complex has been seen. The junctional complexes near the apex are of typical zonula adherens and septate desmosomes.

The ciliated cells in the proximal salivary duct form a complete layer backed by several layers of muscle cells (Fig. 60a). The cells appear to be less slender probably owing to the absence of secretory cells in this region of the duct. They have, in addition to the features described above, numerous glycogen granules, a denser, distinctly singly-nucleolated nucleus and a much folded basement membrane resting on a thin basal lamella (Fig. 60b).

2. **Duct Mucocytes**

These columnar to oval cells (25 - 40μm long) are located chiefly around the main salivary ducts and the interlobular ducts. They are filled with granules of strongly acidic sulphated mucopolysaccharide.

The cell apex shows no differentiation and is usually covered by apical expansions of the ciliated cells (Fig. 61a). The cell is filled with secretory granules of homogeneous granular material of varying electron density. The surrounding membrane may break down to leave a clearer area between the granules. The granules were seen to be liberated into the salivary duct, already filled with the secretion from Mucocyte I (Fig. 61a). The cytoplasm between the granules has distended cisternae of GER (Fig. 61b). Ribosomes also occur free. Oval or oblong mitochondria are sparsely present. The Golgi complex consists of numerous stacks of parallel cisternae, bulb-shaped at the ends, where small Golgi vesicles (≈ 300 nm) occur free (Fig. 61c). The central nucleus is
densely chromatic with a single distinct nucleolus.

3. **Grain Cells**

These are the smallest (10-30µ long) of the secretory cells, columnar to oval and occurring in the acini frequently near the intra-lobular ducts. They contain very dense granules (1-5µ) of neutral polysaccharide and protein (Table 6), distributed throughout the cell (Fig. 62a). The irregularly-shaped basal nucleus is dense with chromatin (Fig. 62b). The GER is very distinct, the early stages being very regular in outline, forming lamella stacks of cisternae (Fig. 62c). Clusters of ribosomes also occur free in the cytoplasm. In the actively synthesizing cell, several Golgi complexes form compacted stacks of three to four lamellae with the ends much distended. Small dense vesicles (40 – 55nm) are numerous around the complex. The later stage of this cell occasionally has a more developed Golgi complex with distinct prosecretory vesicles which appear to aggregate as an electron-lucent ring around the protein granule. Frequent mitochondria with distinct cristae and dense granules lie between the secretory granules.

4. **Mucocyte I.**

These cells are pyramidal (15 – 35µ long) and filled with small secretory granules staining positively for weakly acidic sulphated mucopolysaccharide (Table 6). They occur regularly in the interlobular duct and the acini.

The apex has irregular microvilli (Fig. 63b). The secretory granules (up to 5µ diameter) contain fine reticulate material and appear to fuse together at the cell apex by breaking down of their
TABLE 6. Staining reactions of Salivary gland cells of *Siphonaria zelandica* (+ = positive reaction, ++ = strong reaction, - = negative reaction)

<table>
<thead>
<tr>
<th>Staining Method†</th>
<th>Duct mucocytes</th>
<th>Mucocyte I</th>
<th>Mucocyte II</th>
<th>Grain cell</th>
<th>Duct ciliated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mallory-Heidenhain</td>
<td>light purple</td>
<td>light purple</td>
<td>light purple</td>
<td>red granules</td>
<td>blue</td>
</tr>
<tr>
<td>Azan</td>
<td>blue</td>
<td>blue</td>
<td>light purple</td>
<td>red granules</td>
<td>light purple</td>
</tr>
<tr>
<td>Polychrome</td>
<td>purple</td>
<td>reddish purple</td>
<td>lish blue</td>
<td>colourless dark blue granules</td>
<td>light greenish blue</td>
</tr>
<tr>
<td>Toluidine Blue</td>
<td>light purple</td>
<td>reddish purple</td>
<td>purple</td>
<td>colourless dark blue granules</td>
<td>light blue</td>
</tr>
<tr>
<td>Alcian Blue pH 2.5</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Colloidal Iron</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aldehyde Fuchsin/Alcian Blue</td>
<td>reddish purple</td>
<td>bluish purple</td>
<td>colourless globules</td>
<td>pink granules</td>
<td>pink</td>
</tr>
<tr>
<td>Aldehyde Fuchsin/Alcian Blue</td>
<td>with blue rim</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Bromophenol Blue</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Acetone Sudan Black B</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† All staining reactions were tested with thick Epon sections except those marked with *. Where necessary the procedures for removal of resins recommended by Snodgress *et al.* (1972) or Maxwell (1977) were followed prior to the staining procedures.

* Staining reactions were tested with paraffin sections.
Fig. 60  Ciliated Duct Cell in the Salivary Duct

a. Electron micrograph of a section of proximal salivary duct showing the ciliated duct cell (C) surrounded by layers of muscles (Mu). Note the nerve (Ne) that runs along the periphery of the salivary duct. (x 1950)

b. Electron micrograph of a ciliated duct cell in the proximal salivary duct showing the irregular microvilli (mv) in between the cilia (c), the concentration of the mitochondria (m) in the apical cytoplasm and the dense, oblong nucleus (n) in the basal region of the cell and the folded basal plasma membrane (↑). Note the cell is filled with glycogen granules (↓) (x 3150)

c. Apical junctional complexes of the ciliated duct cell in the proximal salivary duct. mv - microvilli, sd - septate junctions, za - zonula adherens.
Fig. 61 Duct Mucocytes

a. Duct mucocytes (Md) liberating its secretion into the lumen of the interlobular duct (L). Note the slender ciliated duct cells in between the duct mucocytes and the muscle fibres (Mu) surrounding the duct wall. (x 10000)

b. Electron micrograph of a duct mucocyte showing the mucous secretory granules (sg), the dense nucleus and the GER cisternae (cer) surrounding the secretory granules. (x 9100)

c. Golgi area of a duct mucocyte. All round the Golgi lamella (l) are dense vesicles (v). In the cytoplasm are a few mitochondria (m) and dense GER cisternae (cer). (x 25000)
a. Electron micrograph of an actively synthesizing grain cell. The cell contains numerous dense secretory granules (sg), stacks of GER (ger), extensive Golgi complex (g) and a few mitochondria (m). (x 12750)

b. Electron micrograph of a mature grain cell. Note the dense secretory granules (sg) and the large basal nucleus (n). (x 8300)

c. Golgi area of the grain cell showing the association of the GER (ger) and the Golgi complex (g) with the secretory granules (sg). Note also the dense nucleus (n) and the mitochondria (m) with distinct cristae and a few intra-mitochondrial granules. (x 22600)
limiting membranes. Their contents are liberated into the lumina of the acini apocrinally and are found in the interlobular duct (Fig. 63a). The basal nucleus has a single nucleolus with dense clumps of heterochromatin (Fig. 63c). The peripheral cytoplasm is very granular, with clusters of free ribosomes and tubular GER occasionally arranged in stacks attached to the membrane bound granules. The mitochondria have irregularly-directed cristae, a few granules and dense stroma.

The Golgi complexes of these cells are characteristic and distinct. In young cells, they consist of stacks of long dense lamellae with slightly distended ends (Fig. 63d). As the cell matures, the lamellae distend and are filled up with an electron-lucent material, until the whole Golgi is packed with such vacuoles (Fig. 64a). Dense vesicles (≈ 50 nm diameter) surround the Golgi complex and the globular secretory granules appeared to be formed by the fusion of the Golgi vacuoles with the dense vesicles. The remains of the vacuolar membrane form the reticulate material in the granules (Fig. 64b). Occasionally comb-like membranous structures are seen attached to the secretory granules (Fig. 64c & e). The resemblance of these structures to the membranes of the Golgi vacuoles (Fig. 64 c & d) suggests that they are possibly the remains of the membranes after fusion.

5. Mucocyte II.

These are the largest gland cells (25-50µ) and have a conspicuous basal nucleus (Fig. 65a). Their large secretory granules of unknown nature reacted negatively to all the histochemical tests employed (Fig. 58c & d, Table 6).
Smaller mucocytes ("Mucocyte III") (10-30µ high) can be identified from light microscopy with granules of weakly acidic sulphated mucopolysaccharide staining variably with Toluidine Blue from light purple to colourless (Fig. 65b). The granules when colourless are similar to those in Mucocyte II. Both the Mucocyte II and III are located in the interlobular duct as well as in the acini. In actively feeding animals, Mucocyte III increases in number with the reduction of Mucocyte II. In starved animals, acini were occupied by numerous large Mucocyte II with enlarged secretory granules; no Mucocyte III were found (Fig. 65c). The ultrastructure of these two cells is quite similar, and it is concluded that "Mucocyte III" is actually a developmental stage of Mucocyte II.

Mucocyte II contains coalescing granules of variable size (Fig. 68) usually electron-lucent. At the earlier (Mucocyte III) stage they usually contain fine filaments of varying density (Fig. 66b & c) which may aggregate in concentric layers (Fig. 66a), suggesting a crystalline nature. The cytoplasm is very granular with numerous free ribosomes and dense vesicles (Fig. 66a,b,c). Elongate mitochondria with irregularly oriented cristae lie between the secretory granules. Much of the cytoplasm is occupied by tubules of GER usually in parallel stacks (Fig. 67a). In some cells, the GER was concentrically packed to form whorls (Fig. 67b & c), evidently associated with the Golgi complex in the production of the secretory granules. The Golgi complex is a tightly packed stack of 9-10 lamellae with distended ends (Fig. 67d), whose electron-lucent vacuoles are incorporated into the secretory granules (Fig. 67e). The secretory granules of both young and mature Mucocyte II are liberated apocrinally (Fig. 69a & b).
6. Undifferentiated Cells

These small ovoid cells (4-5μ) are clearly identified only by electron microscopy. They are found singly between the secretory cells or in groups of two or three beneath the secretory cells at the base of the tubule epithelium (Fig. 70a). They have a large nucleus with dense chromatin and a large nucleolus. The cytoplasm is very dense with numerous free ribosomes and a few mitochondria (Fig. 70b). Small stacks of GER and Golgi complex may be present. They appear to be able to develop into any of the secretory types described above.
Fig. 63 Mucocytes I

a. Electron micrograph of a mucocyte I liberating its secretory granules (sg) into the tubule lumen (L). The cell is filled with the secretory material. Note the large nucleus (n) and the prominent Golgi complexes (g). (x 9900)

b. Apical region of mucocyte I showing the presence of apical microvilli (mv). (x 29750)

c. Electron micrograph of mature mucocytes I. The cells are filled with secretory granules with fine reticulate material. The Golgi area (g) is very compressed. The nucleus (n) becomes less densely chromatic. (x 5000)

d. A developing mucocyte I with small secretory granules (sg) and extensive Golgi complexes (g). (x 15000)
Fig. 64 Mucocytes I

a. Golgi area of a developing mucocyte I showing the formation of the secretory granules from the fusion of the Golgi vacuoles (va) and the small dense vesicles (vi). The small secretory granules (s₁) then fuse together to form larger globules (s₂). (x 18500)

b. Golgi area of a mature mucocyte I. Small secretory granules are still being produced from the fusion of the Golgi vacuoles (va) with the dense vesicles (vi). Numerous mitochondria (m) and extensive GER are present in the cytoplasm between the secretory granules (sg). (x 24400)

c. Comb-like structure in the secretory granules. (x 21300)

d. Stacks of vacuoles (v) formed from the distended Golgi lamella, appear to fuse with the secretory granules (sg). This probably results in formation of the comb-like structure in the secretory granules. (x 17200)

e. The varied appearance of comb-like structure (↑) in the secretory granules (sg). (x 39300)
Fig. 65 Mucocytes II

a. Electron micrograph of a salivary gland tubule showing the distinguished features of mucocyte II ($M_2$) in contrast with mucocyte I ($M_1$) and grain cell (G). Note the ciliated duct cells (C) extending laterally over the adjacent secretory cells appear to form a complete layer lining the tubule lumen (L). (x 3950)

b. Light micrograph of a section of the salivary gland showing the variable appearance of mucocyte II ($M_2$) with Toluidine Blue staining. The content of mucocyte I stains reddish purple and appears to be finely granular. Grain cell (G) contains dense dark blue granules. The content of mucocyte II stains variably ranging from colourless to pale purplish blue to a mixture of pale blue with fine dark blue granules (which was mistaken as a separate cell type mucocyte III). (Epon thick section, Toluidine Blue staining; x 2400).

c. Light micrograph of a section of the salivary gland from a limpet starved for 4 days. Mucocytes I & II ($M_1$ & $M_2$) are both swollen with secretory material. Mucocyte II appears only in one form with large colourless secretory globules. The grain cell (G) appears smaller probably owing to compression from the adjacent swollen mucocytes. (Epon thick section, Alcian Blue pH 2.5/PAS staining; x 2400).
Fig. 66 Mucocytes II

a. Electron micrograph of a developing mucocyte II. Note the lamellated granular material in the secretory granules (sg). (x 12750)

b. Electron micrograph of a developing mucocyte II. The secretory granules (sg) are of variable electron-densities. The cell has peripheral stacks of GER (ger), a distinct Golgi complex (▲) and a few mitochondria (m) in the cytoplasm. (x 27300)

c. Electron micrograph of a mucocyte II showing the fusion of the Golgi vacuoles (v) with the secretory granules (sg). Note also the densely chromatic nucleus (n). (x 22950)
Fig. 67 Mucocytes II

a. Electron micrograph of a part of the mucocyte II showing the lamellated GER (ger) producing the dense granular secretory granules (sg₁) which later fuse with the Golgi vacuoles (v) to form the less dense secretory granules (sg₂). (x 30500)

b. GER whorls (↑) and active Golgi complex (↑) in developing mucocyte II. (x 12000)

c. A GER whorl in mucocyte II. (x 33600)

d. A Golgi complex in mucocyte II, consisting of distended lamella (l), large vacuoles (v) and small dense vesicles (vi). The Golgi vacuoles and vesicles appear to fuse forming the secretory granules (sg). (x 27450)

e. A Golgi complex in mucocyte II showing the compact Golgi lamella (l) and the incorporation of the Golgi vacuoles (v) into the secretory granules (sg). (x 32750)
Fig. 68 Mucocytes II (Later Stage)

a. The late stage mucocyte II is filled with electron-lucent secretory granules (sg). Some GER (ger) still remain as whorls around the secretory granules. (x 10900)

b. Late stage mucocyte II with an active Golgi complex still producing vacuoles (v) which are being incorporated into the secretory granules (sg). Lengths of GER (ger) and a few mitochondria (m) are present in the cytoplasm in between the secretory granules. (x 14550)

c. Very late stage mucocyte II. The GER are no longer in stacks but become sparse and short (†). Mitochondria (m) are very dense with irregular cristae. The Golgi complex (g) is less extensive and the nucleus (n) becomes less densely chromatic. (x 16500)
Fig. 69 Liberation of the Secretory Material from Mucocyte II.

a. A young mucocyte II undergoing apocrine secretion. The lumen (L) of the tubule contains both secretion from mucocyte I (sg₁) and mucocyte (sg₂). Ciliated cells (C) are located between the mucocytes. (x 19200)

b. A mature mucocyte II undergoing apocrine secretion. The tubule lumen (L) is filled by the secretion from mucocyte II (sg₂). (x 8850)
Fig. 70 Undifferentiated Cells of the Salivary Gland

a. A cluster of four undifferentiated cells (U) between the secretory cells, mucocyte I (M₁), mucocyte II (M₂) and grain cell (G) at the base of a salivary gland tubule. (x 10900)

b. Electron micrograph of an undifferentiated cell. The cell has a densely-chromatic nucleus (n) and dense cytoplasm with numerous free ribosomes (r), a developing Golgi complex (g) and small stacks of GER (ger). (x 27550)