1971-08-01

Comparative anatomy and histology of the mantle cavity of the chitons (polyplacophora) Mopalia muscosa and Mopalia lignosa

Theodore P. Winfield
Brigham Young University - Provo

COMPARATIVE ANATOMY AND HISTOLOGY OF THE MANTLE CAVITY OF THE CHITONS (POLYPLACOPHORA) MOPALIA MUSCOSA AND MOPALIA LIGNOSA

A Thesis

Presented to the

Department of Zoology

Brigham Young University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Theodore P. Winfield Jr.

August, 1971

This thesis, by Theodore P. Winfield Jr., is accepted in its present form by the Department of Zoology of Brigham Young University as satisfying the thesis requirement for the degree of Master of Science.

Typed by Marilyn Hall

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to the members of my committee for their continued help and support in my graduate research endeavors. Dr. Braithwaite's help was invaluable in formulating ideas, techniques and creating effective research procedures. I would also like to express my appreciation to the graduate division in the Department of Zoology for allowing me the opportunity to pursue my graduate education at Brigham Young University.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

Figure Page

Figure Page

INTRODUCTION

In 1939 Yonge published a paper describing the anatomy and currents of the mantle cavity and gills of the chitons Lepidochitona cinereus, Tonicella marmorea, Acanthochitona crinitus and Lepidopleurus asellus, and the histology of the same systems of all except Tonicella marmorea. This was the first paper that correlated anatomical and histological features together with the currents within the mantle cavity.

The anatomy, general histology and structural relationships within the mantle cavity of chitons are well described by several authors, (Plate 1897, 1899, 1901; Hoffmann 1930). Previous to Yonge's work, Arey and Crozier (1919) had observed and described currents in the mantle cavity, but they did not mention how these currents originated or functioned. Hyman (1967) has given a review of what is known about the anatomy, histology and currents of the mantle cavity and gills.

There has been limited work published concerning the relationship of the mantle cavity with the distribution of chitons. Ricketts and Calvin (1968) described the intertidal zones where chitons of the West Coast of the United States dwell. Barnawell (1954) worked on the distribution of the chiton family Mopaliidae

in San Francisco Bay, California, but did not consider the impact of the mantle cavity on this distribution. Arey and Crozier (1919) showed that the structures within the mantle cavity constitute the most sensitive area of the chiton body in response to stimuli such as touch, local osmotic disturbances, chemical and temperature. This fact would suggest that the mantle cavity and its contained structures are quite adept at sensing the environment and possibly aid in controlling the range of the chiton within its environment.

Mopalia lignosa (Gould, 1846) and Mopalia muscosa (Gould, 1846) are two related chitons from the West Coast of North America which have adapted to different habitats within the intertidal area. Mopalia lignosa is limited to the outer rocky coast where the concentration of suspended particles is relatively low when compared to the bay environment. Mopalia muscosa ranges from the outer coast to the bay environment. The bay environment is subject to high concentrations of suspended particles. Mopalia hindsii (Sowerby, 1847) has a range similar to Mopalia muscosa but according to Barnawell (1954) is more successful at inhabiting the bay environment. In this study I will explore the structure and function of the mantle cavity in an attempt to elucidate the factors enabling Mopalia muscosa (and Mopalia hindsii) to successfully adapt to the problem of increased suspended sediments encountered in the bay environment.

MATERIALS AND METHODS

Specimens of the chiton Mopalia lignosa were collected from Boiler Bay, Oregon and Mopalia muscosa from Tomales Bay and San Diego, California. Mopalia hindsii specimens, used for comparison were collected at Tomales Bay, California. Chitons used for histological preparations were relaxed in a 7.5% solution of Magnesium chloride ($MgCl_2$) and placed in Bouins fixative for at least 24 hours, then transferred to 70% ethyl alcohol. The foot was cut lengthwise to insure complete fixation of all tissues. Some of the specimens of Mopalia lignosa were fixed in 10% formalin and several individuals of Mopalia muscosa were fixed in Maximov's variation of Zenker's solution. Chitons fixed in Maximov's fluid were not iodized until the staining process.

Dehydration and infiltration with paraffin of specimens for histological observation were accomplished using a Fisher Dual Unit Tissuematon. Specimens were then embedded in "Tissuemat" (Fisher Scientific Company). The tissuemat blocks were then sectioned on a microtome at 5, 8, 10, 15 or 20 microns and affixed on to the slides using an albumin-glycerine solution. The procedure involved placing the slides on a slide warmer at 40°C after the affixative was applied. Distilled water was added to the slide and the sections were then

floated on this water to remove paraffin section wrinkles. Slides were left on the slide warmer until the water evaporated, allowing sections to affix to the albumin solution. During the sectioning every other ribbon of 35-50 sections was kept and 3 to 5 slides of 7 to 10 sections each were made from these ribbons.

The majority of the slides were stained using Ehrlich's haematoxylin and 1% alcoholic eosin. Other stains used included toluidine blue, Mallory's triple stain, thionin, and alcian blue. "Permount" (Fisher Scientific Company) was used as the mounting medium.

Some specimens were kept alive in a well aerated aquarium for observation of ciliary currents in and about the mantle cavity. Half of these specimens were kept in fresh sea water at 12 to 16°C while the others were kept in artificial sea water maintained at 18 to 21°C. During the observation, several were placed on thin glass lantern slides within their aquaria and allowed to attach. They were then observed, ventral side up, in a large photographic print tray. The glass slide supporting the chiton was held in place by four anchored corks. Water level was such that it just covered the glass slide with the chiton's ventral surface showing through the glass slide. Water used during observations was the same that the chiton was maintained in. A suspension of carmine red and starch was then introduced to the area around the chiton and, using a dissecting microscope, the currents throughout

the mantle cavity were traced by observing the particle pathways. Solutions of methylene blue, toluidine blue and aniline blue were injected around the specimen in an attempt to determine where the mucus originated. Illumination was kept to a minimum during the observation period so as to minimize any abnormal behavior which the heat from the illuminator may have produced.

Individual gills from Mopalia lignosa and Mopalia muscosa were placed in the following stains to observe distribution of mucous cells: methylene blue, alcian blue, thionin-aqueous, aniline blue and toluidine blue. The stains were diluted to a 10% solution. Gills were allowed to remain for several days in the stain before being moved to 70% ethyl alcohol for 15 minutes prior to observation.

Photomicrographs of the histological sections were taken on a Zeiss Photomicroscope II using bright field illumination. Kodak Panatomic-X was used for the photographs.

Sediment samples were collected from Boiler Bay, Oregon and the north exposure of Mission Beach Jetty in San Diego, California. These were analyzed to determine sizes and relative percentages of particles. Sediment samples were dryed, weighed and then seived through a seive series. The particles remaining on each seive were then weighed to calculate their mass percent composition.

RESULTS

Anatomy of the Mantle Cavity and Gills

The mantle cavity is a distinct groove found on the chiton's ventral side between the girdle, foot and head regions. It houses the gills, nephridiopores, gonopores, various sensory and mucous tracts and the anus. The anatomy of the visible structures will be described first. Descriptions of histology of the mantle cavity and gills will follow. The mantle cavity of Mopalia lignosa and Mopalia muscosa is not unlike that described by Yonge (1939) for Lepidochitona cinereus. The multiple gills form a linear sequence running anterior to posterior, effectively dividing the mantle cavity into two chambers (Fig. 1, 2, 5). The inhalant chamber extends from the area anterior to the first gill down the length of the gill series to the last gill on the distal side (Fig. 1, 2, 5). The exhalant chamber is located between the gills and foot and includes the area around the anus (Fig. 1, 2, 5).

The gill series is of the holobranch type, extending through most of the mantle cavity length (Fig. 1, 2, 5). The series starts near the edge of and just behind the foot's anterior margin and extends back to the posterior margin of the foot (Fig. 1, 2, 5).

Fig. 1. Lepidochitona cinereus, ventral view, showing mantle cavity currents (after Yonge, 1939). Inhalant currents (solid lines) and exhalant currents (dotted lines).

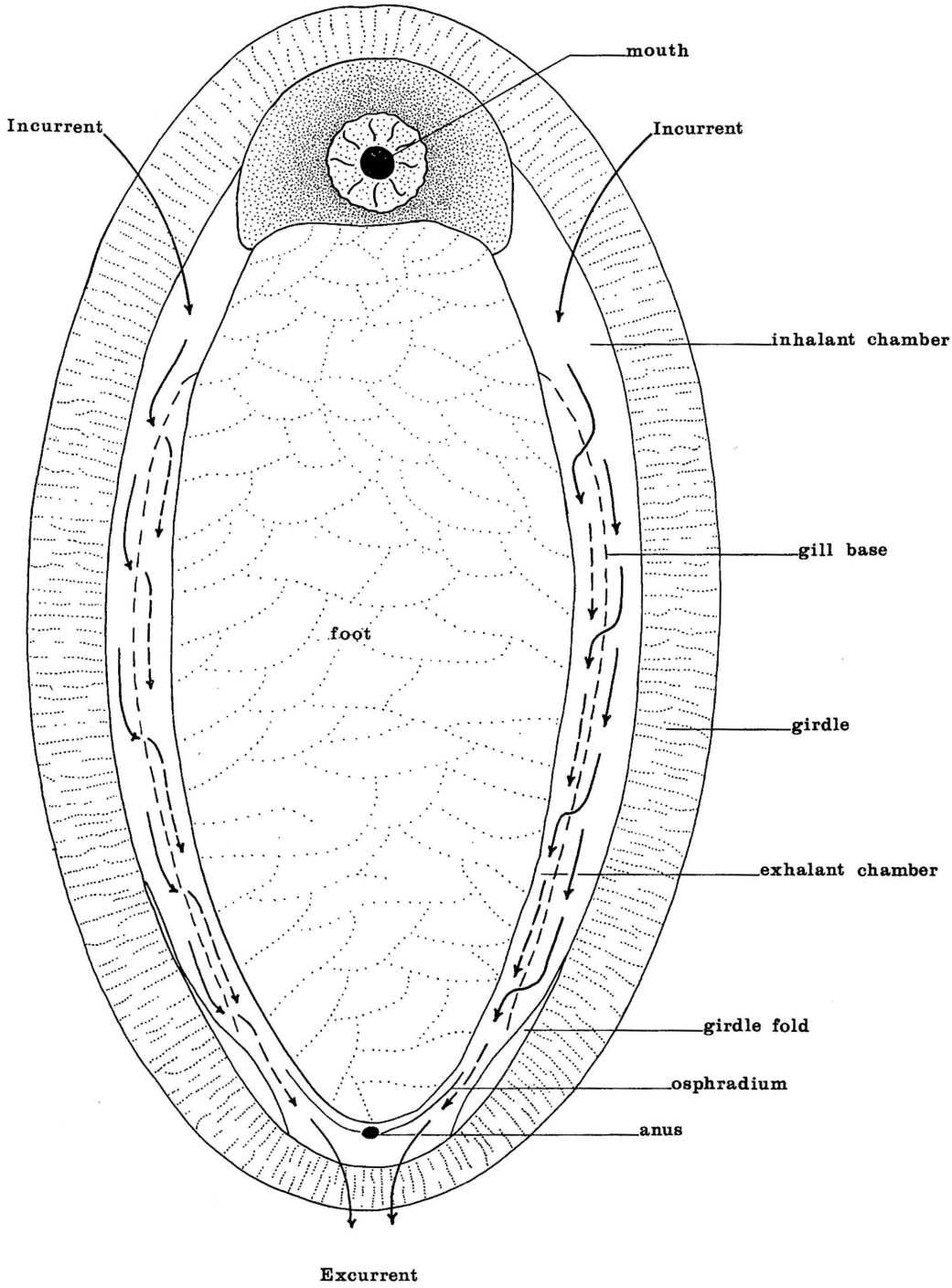

mouth
Incurrent
Incurrent
inhalant chamber
gill base
foot
girdle
exhalant chamber
girdle fold
osphradium
anus
Excurrent

Fig. 1

The posterior portion of the gill series angles toward the girdle (Fig. 1, 2, 5). The anterior-most gill is the smallest and youngest, and gills gradually increase in size up to the last several gills which are the largest in the series. Gills are oblong-shaped in cross-section (Fig. 9) and are arranged with their long axis either perpendicular to or with a slight anterior acute angle to the main body axis. In the transverse plane gills are triangular-shaped (Fig. 6) and are oriented with the broad lateral surfaces facing anterior-posteriorly. The edge of the gill near the girdle possesses a conspicuous vessel, the efferent branchial vessel (Fig. 6). The afferent branchial vessel (Fig. 6) is found facing the foot. Gills are quite flexible in life but are usually oriented with the tips facing posteriorly, and the entire gill curves slightly over the exhalant chamber.

Gills are attached to the mantle cavity roof below the afferent and efferent branchial sinuses. Blood-swollen sinuses form a prominent ridge; which in most of the specimens examined, extends into the mantle cavity and effectively increases the separation of the inhalant and exhalant chambers. This ridge structure is transitory, and when present increases the area of the exhalant chamber at the expense of the inhalant chamber.

The portion of the mantle cavity that is found around the head is shallow. Near the anterior edge of the foot the cavity deepens noticeably and quite abruptly. The cavity widens to

Fig. 2. Mopalia lignosa, ventral view, showing general mantle cavity currents. Inhalant currents (solid lines) and exhalant currents (dotted lines).

Fig. 3. Mopalia lignosa, enlarged view of incurrent area showing dispersal of currents.

Fig. 4. Mopalia lignosa, enlarged view of posterior area showing exhalant current (solid lines) and other observed current pathways (dotted lines).

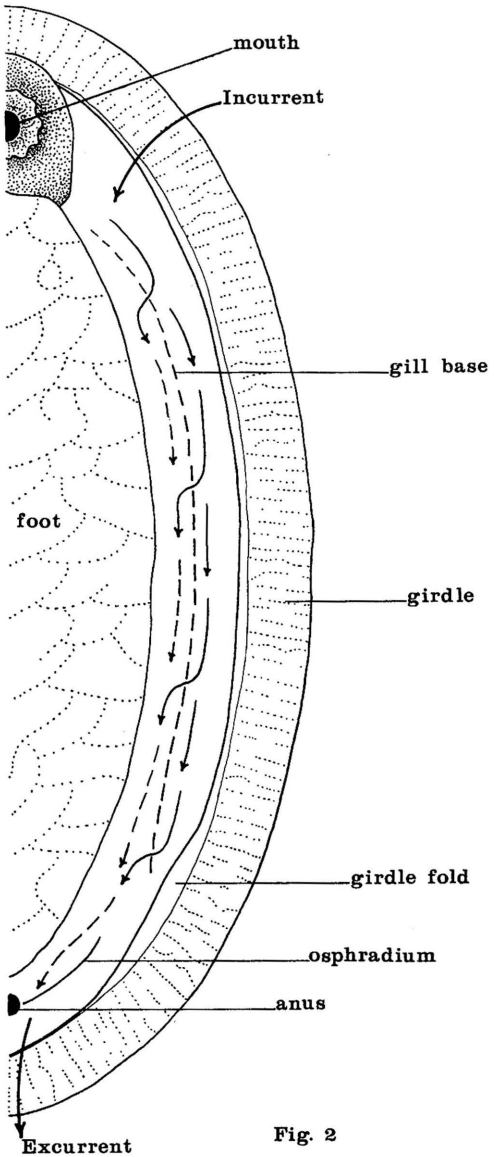


Fig. 2

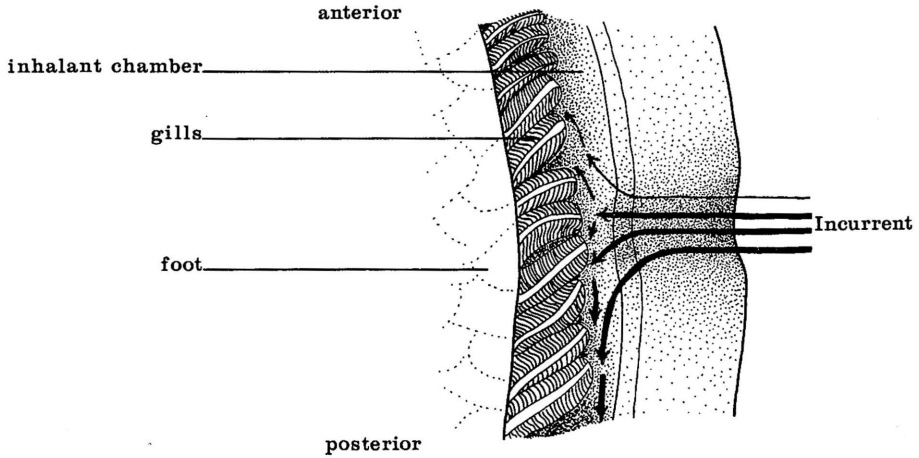


Fig. 3

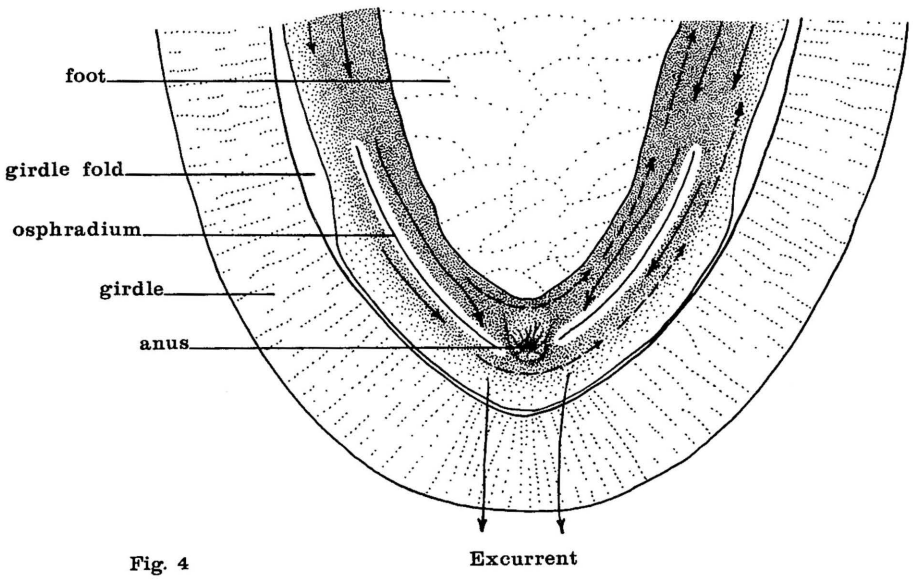


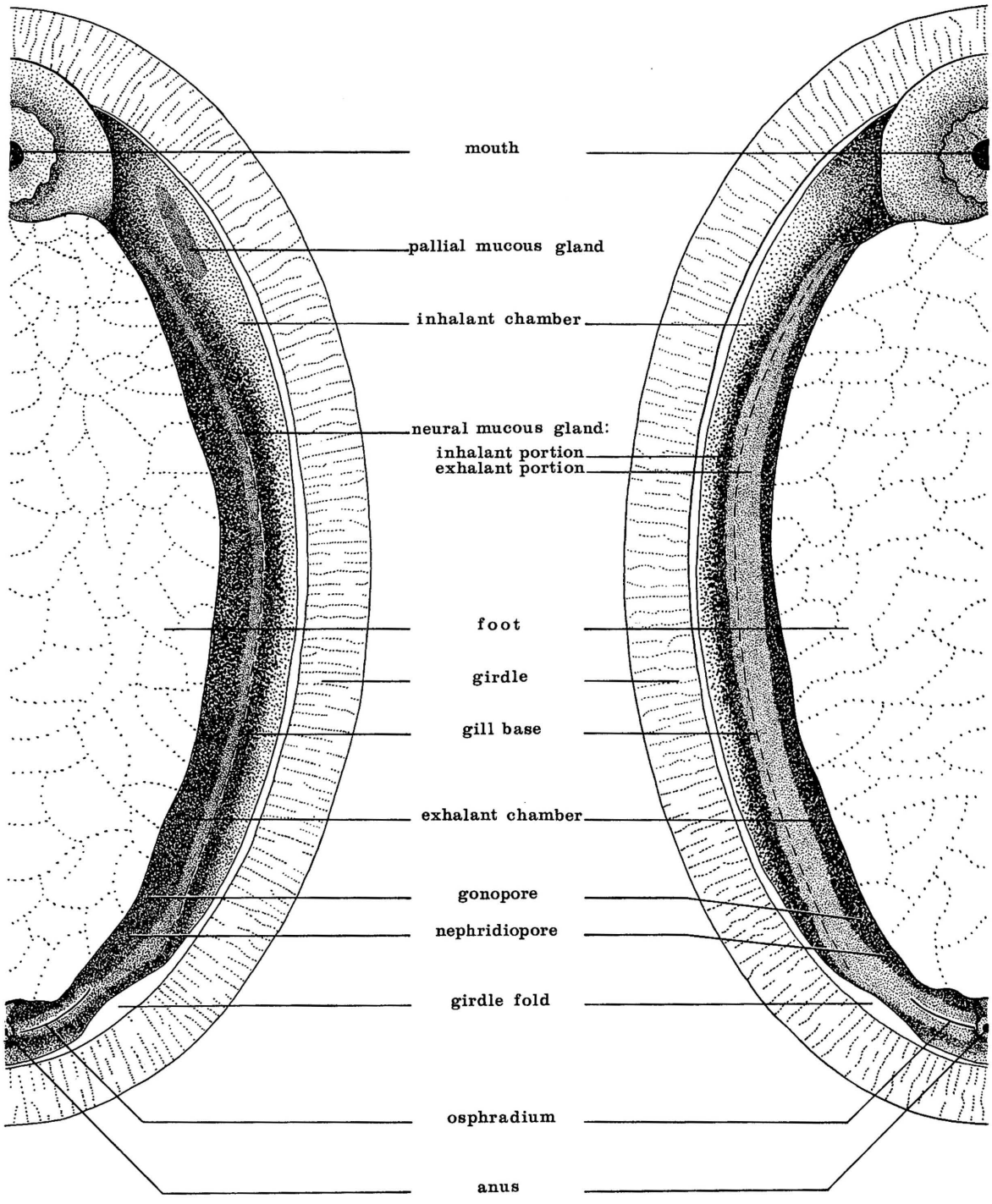
Fig. 4

accomodate the gills. It gradually widens toward the middle of the body and remains somewhat constant in width throughout the rest of the body length. There is a shallow groove that extends laterally between the foot and head. This is not considered to be a part of the mantle cavity proper.

A fold of tissue on the inner edge of the girdle completely surrounds the mantle cavity (Fig. 2, 5). This fold lacks the spines present on the rest of the girdle, and there is usually a narrow groove present which also acts to differentiate this region from the rest of the girdle. The fold is very thin anteriorly and broadens in the area lateral to the mouth. In the area of the last gill it dilates, then narrows noticeably near the anus (Fig. 2, 4, 5). This ridge of tissue is termed the girdle fold (Yonge 1939). The gonopore is located between the bases of the second and third from last gills in the exhalant chamber and the excretory pore is located between the bases of the last and next to last gills (Fig. 5).

The anus is located posterior to the foot on the tip of a conelike structure which extends into the mantle cavity from the dorsal area of the mantle cavity and foot. There is a narrow dark streak located on each side of this cone. The two streaks extend from the side of the anal cone forward, about halfway to the last gill. They have been classified as osphradia (Fig. 2, 4, 5) by various authors (Plate, 1897, 1899, 1901; Hoffmann, 1929-1930; Yonge, 1939). Yonge (1939) suggests the use of the term osphradium

Fig. 5. Mopalia lignosa and Mopalia muscosa, ventral view, comparative anatomy of mantle cavity.



Mopalia muscosa

Mopalia lignosa

Fig. 5

should not infer homology to a similarly named structure found in Gastropoda and Bivalvia.

Each gill consists of a series of flat lamellae on each side of a central axial structure. The opposed lamellae alternate in their attachment to this axial structure on either side (Fig. 7). In cross-section the gill has an oval outline with the thin axis running between two branchial vessels (Fig. 9). The two branchial vessels form the narrow edges of the lamellae (Fig. 9). Lamellae gradually decrease in size from the broad base to the free-end of the gill, giving the gill a triangular shape in transverse view (Fig. 6).

Individual lamellae are attached on their narrow sides to the branchial vessels and on their third side to the central axial structure (Fig. 6). The free edge of the lamellae is dilated and clothed with cilia. An area of dense, long cilia, termed the lateral cilia zone, is located near the efferent branchial vessel (Fig. 6, 9). This zone extends from the free-end of the lamellae to the axis. The free lamellar edge dilates slightly more in this area and clearly marks the lateral cilia zone.

Several specimens possessed gills with an abnormal gill growing from the efferent branchial vessel (Fig. 8). There were other abnormal gill growths noted, but close observation showed these gills undergoing regeneration.

Fig. 6. Mopalia lignosa, transverse view of gill.

Fig. 7. Mopalia lignosa, portion of gill, view of efferent branchial vessel, showing alternating pattern of lamellae.

Fig. 8. Mopalia lignosa, abnormal gill growing from the efferent branchial vessel of normal gill.

Fig. 9. Mopalia lignosa, cross-section of gill showing two opposing lamellae.

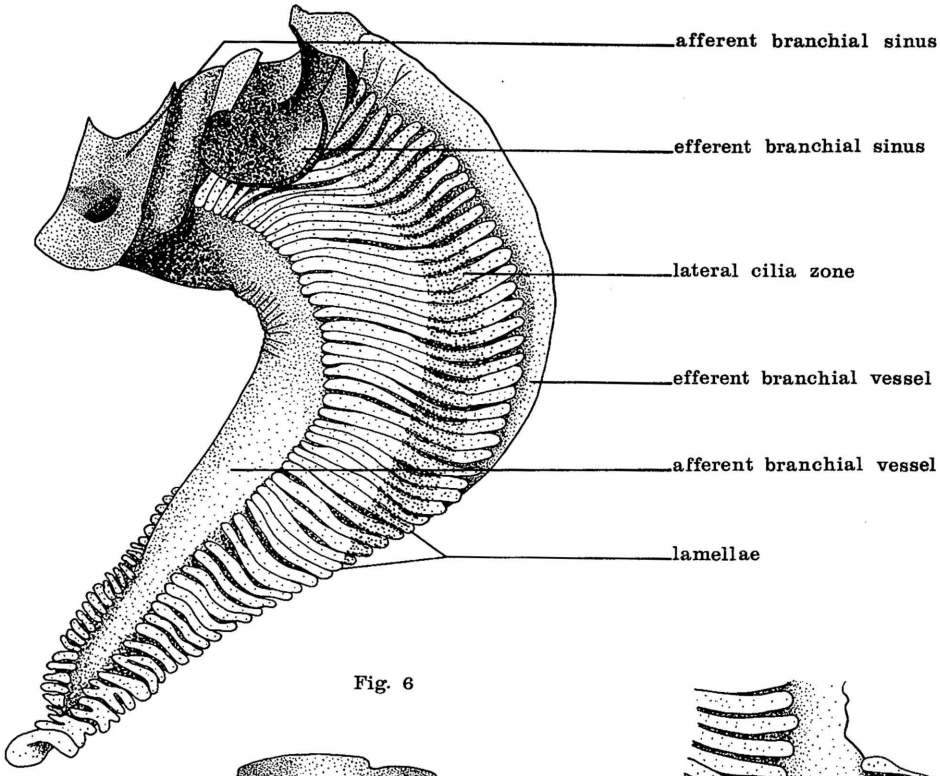


Fig. 6

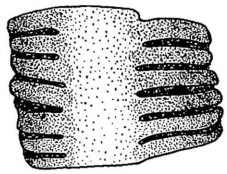


Fig. 7

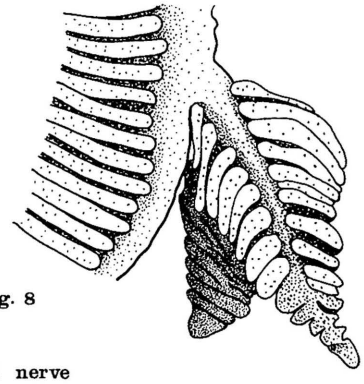


Fig. 8

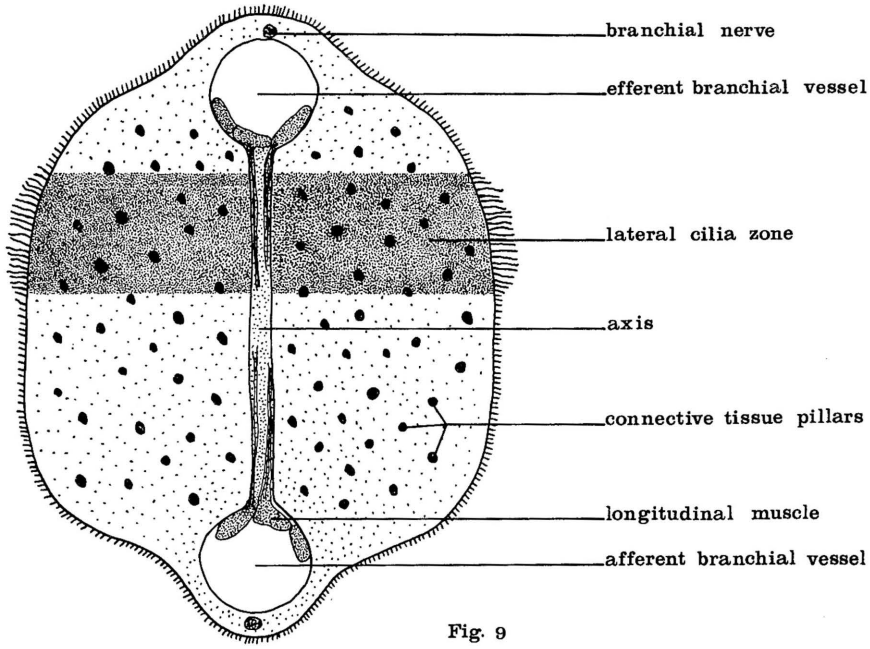


Fig. 9

Mantle Cavity and Gill Currents

The general ciliary current patterns observed in Mopalia lignosa and Mopalia muscosa are similar to those observed by Yonge (1939) for several species including Lepidochitona cinereus (Fig. 1) and those described by Arey and Crozier (1919) for Chiton tuberculatus. A current is produced within the mantle cavity whereby water is drawn into the cavity over the gills and exits behind the anus. The region of current intake is usually anterior but can be found anywhere along the body length anterior to the last several gills. Intake is accomplished simply by local liftings of the girdle. There may be more than one inhalant opening per side. The exhalant opening is created by a single lifting of the girdle behind the anal area. In general water is pulled through inhalant openings into the inhalant chamber, through the gills into the exhalant chamber and finally out the exhalant opening (Fig. 1).

Currents in both Mopalia lignosa and Mopalia muscosa are similar. The major inhalant currents enter the inhalant chamber via distinct raised areas of the girdle (Fig. 2, 3). Weaker inhalant currents are present under broad, slightly elevated girdle areas adjacent to the main opening. A current is then drawn through the interlamellar spaces on the gills and into the exhalant chamber (Fig. 2, 3). These currents are created by the lateral cilia (Fig. 6, 9) and movements by the gills themselves. Gills of the specimens

studied are active, continually extending and contracting creating a jet-like effect upon the currents. These currents are strong as verified by the speed of the particles in the exhalant current as they exit in the anal area. The exhalant opening did not change its position directly behind the anus (Fig. 2, 4).

The last gill is situated so as to effectively form a posterior partition of the inhalant chamber. Gills are active, and in conjuction with the dilated girdle fold (Fig. 1, 2, 4) can form an effective seal facilitating the separation of the two chambers and increase the jet-like effect upon the currents.

Gills form effective filters of suspended particles within the respiratory currents. As the water is drawn through the interlamellar spaces, large suspended particles are excluded due to size restriction imposed by interlamellar spaces. Filtered particles are sent by ciliary action to the furrows between adjacent gills, either out to the tip, or in a few cases to the base, then into the exhalant current and out the exhalant opening. Smaller particles that are permitted to enter through the interlamellar spaces are released into the exhalant current and then out the exhalant opening. Cilia on the roof of the exhalant chamber move out any particles that happen to drop out of the exhalant current when the chiton is upside down. This is apparently an important cleansing mechanism. Particles that have dropped out of suspension are moved posteriorly along the mantle cavity roof, then up across the osphradium and anal cone,

and are finally released into the exhalant current. Particles falling out of suspension from the inhalant current are also moved posteriorly. Posterior to the last gill the two rejection currents meet with the particles being moved to the anal cone area before release into the exhalant current. Particles in these rejection currents are seldom observed in noticeable mucous accumulations. However, as the concentration of suspended particles increases, so does the frequency of mucous accumulation. Short spines found on the ventral girdle act as a passive filter of large suspended particles as the current is drawn in through the inhalant opening.

Particles in the exhalant current are very seldom observed consolidated into mucous accumulations when using dilute solutions of carmine red and starch. However, when a saturated solution is added to the inhalant current of Mopalia muscosa copious amounts of mucus is secreted, accumulating the suspended particles into a loose mucous accumulation. This accumulation of mucus and suspended particles is then flushed out in the exhalant current. This phenomenon occurs immediately upon contact of large concentration of suspended particles with the anterior gills and mantle cavity. If the saturated solution is continuously introduced the chiton restricts the inhalant opening to broad, slightly elevated areas of the girdle. Mopalia lignosa does not display a similar reaction. It will simply limit its inhalant opening in response to any increase in suspended particle concentration. This suggests that Mopalia lignosa is inept at

handling sudden increases in suspended particles or a high concentration of suspended particles over a period of time.

The use of mucus to cleanse the mantle cavity of particles entering with the respiratory currents is probably a primitive function, as suggested by Yonge (1939), with the ciliary currents in holobranch chitons taking over as the chief means of cleansing the mantle cavity. The function of mucus is still retained and used when the concentration of suspended particles is such that the normal ciliary currents are inadequate. This is especially true of Mopalia muscosa which has the capacity to survive in a bay environment where it would be subject to high concentrations along with sudden and rapid increases in the concentration of suspended sediment particles.

Normal respiration currents move at a constant rate. When various dyes such as methylene blue, toluidine blue and aniline blue are introduced into the inhalant current in an attempt to determine where mucus originates within the cavity, chitons react initially by reversing their currents. Normal respiration then ceases. The inhalant currents become weak, just seeping under the flatten girdle. These weak currents originate away from the direction of chemical sources in the water. When moved to fresh sea water this "testing" type of respiration continues for a short time before normal activity resumes. The mantle cavity is sensitive to chemical stimuli (Arey and Crozier, 1919) even in dilute solutions. This reaction to chemical stimuli along with the aforementioned sensitivity to

suspended particle concentrations illustrates the apparent sensitivity of the anterior mantle cavity and gills to the environmental conditions and its importance in regulating the activity of the chiton.

Close observation of the inhalant currents indicate most of the water is pulled directly in or posteriorly (Fig. 3). There is also a weak anterior current operating at the same time which pulls water through the gills anterior to the opening (Fig. 3). Mopalia lignosa and Mopalia muscosa both exhibit the ability to regulate not only the speed of the exhalant current but the direction also. They will create rapid currents in one exhalant chamber and weak currents in the other at the same time. They both demonstrate the ability to reverse current flow in one exhalant chamber and thereby draw the exhalant current from the other chamber around the posterior portion of the mantle cavity and then anteriorly instead of allowing the current to exit normally (Fig. 4). Exhalant currents also eject the products of the excretory, genital, and anal pores.

Histology of the Mantle Cavity

The greatest comparative differences are manifested in the histology of the mantle cavity and associated structures of the species studied herein. The distribution of cilia and the structure, distribution and secretory behavior of the mucous glands are the areas showing the greatest differences between Mopalia lignosa and Mopalia muscosa. This section is concerned with the lining of the

mantle cavity only. The osphradium, gills and mucous glands will be discussed separately.

The basic epithelial cell types found lining the mantle cavity do not differ between Mopalia lignosa and Mopalia muscosa. The epithelium of the mantle cavity adjacent to the head is composed of columnar cells with oval nuclei and scattered goblet cells (Fig. 10).

On the ventral side of the head the epithelium is composed of narrow, columnar cells and scattered goblet cells (Fig. 11). The concentration of goblet cells is higher than in the region adjacent to the head. Tall, narrow columnar cells and scattered goblet cells make up the epithelium of the ventral surface of the head (Fig. 12).

The ventral surface of the girdle possesses spines and a cuticle. Near its border with the distal edge of the mantle cavity there is a fold of tissue termed the girdle fold (Fig. 5). In the region of this fold the cuticle ends abruptly and an epithelial layer of tall, narrow columnar cells and goblet cells originates (Fig. 13). This layer is confined to the girdle fold and adjacent region of the mantle cavity. The epithelium of the mantle cavity adjacent to the distal edge changes gradually to columnar cells of moderate height.

Posterior to the head the foot forms the proximal region of the mantle cavity. The epithelium of the ventral side of the foot within the cavity is composed of tall columnar cells and numerous goblet cells (Fig. 14). The two ventral edges of the mantle cavity are remarkably similar in structure. The identity of both is retained

Fig. 10. Mopalia muscosa, dorsal mantle cavity epithelium in head region showing scattered goblet cells. Ehlich's haematoxylin and eosin. 205x.

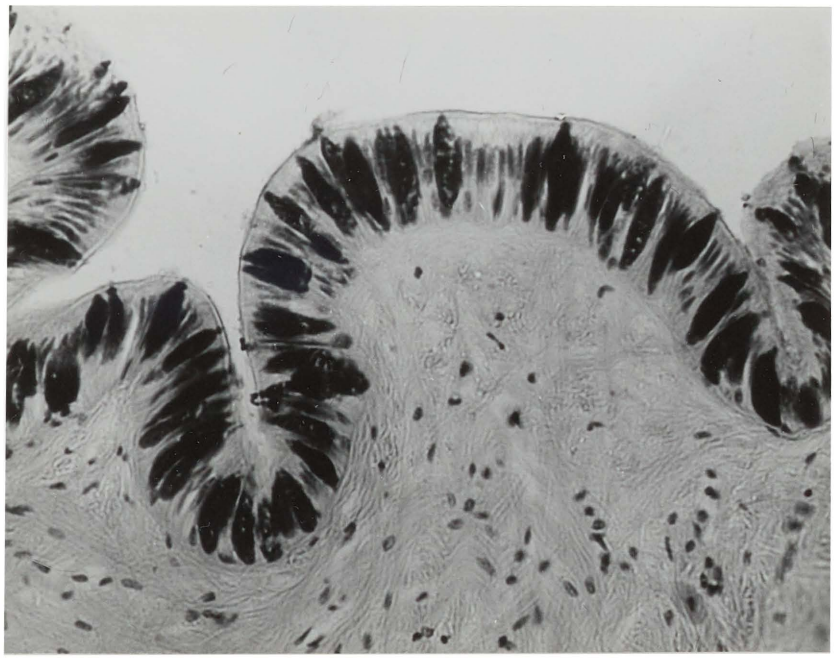


Fig. 11. Mopalia muscosa, mantle cavity epithelium on ventral side of head showing accumulations of goblet cells. Ehrlich's haematoxylin and eosin. 205x.

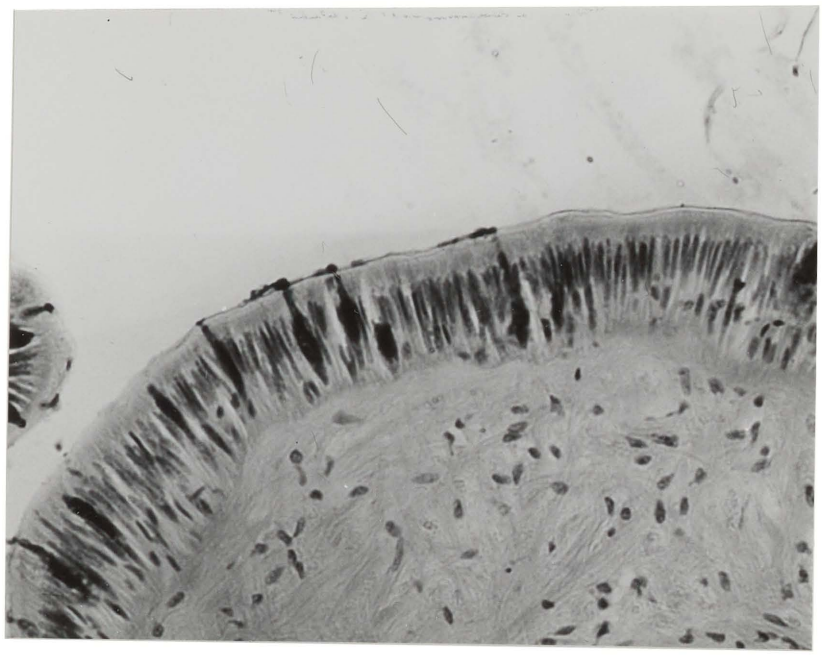


Fig. 12. Mopalia muscosa, ventral surface of head. Ehrlich's haematoxylin and eosin. 205x.

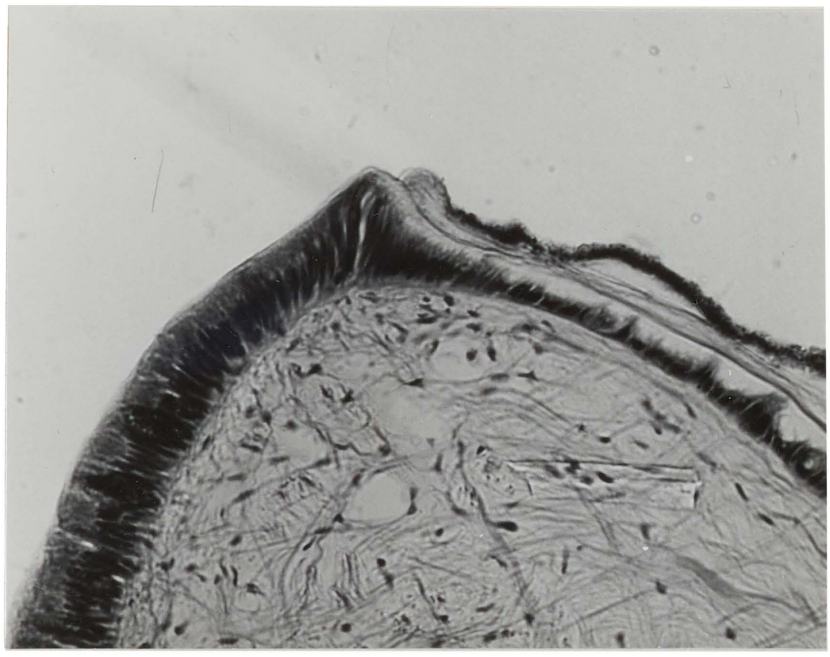


Fig. 13. Mopalia lignosa, girdle fold, shows junction between cuticle and mantle cavity epithelium. Ehrlich's haematoxylin and eosin. 205x.

Fig. 14. Mopalia muscosa, mantle cavity epithelium on ventral side of foot showing accumulations of goblet cells. Ehrlich's haematoxylin and eosin. 205x.

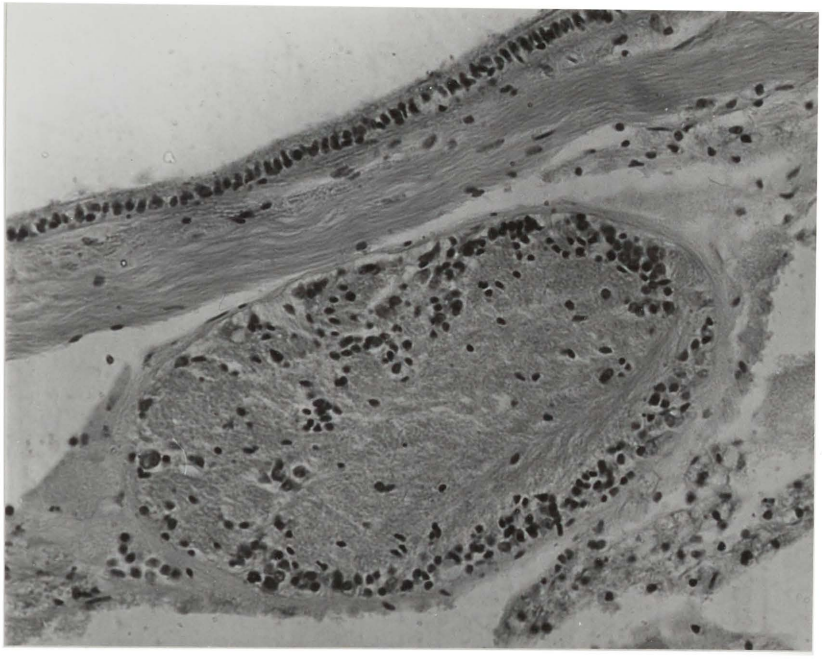


Fig. 15. Mopalia muscosa, dorsal mantle cavity epithelium in foot region overlying lateral nerve cord. Ehrlich's haematoxylin and eosin. 160x.

throughout the length of the mantle cavity. The epithelium between the two edges of the mantle cavity is composed of columnar cells with goblet cells rarely found (Fig. 15). The ventral surface of the foot (Fig. 16) is similar to the ventral surface of the head. The histology of the ventral surface of the foot is basically similar to that described by Cowden (1963) for Chiton tuberculatum.

The region of the mantle cavity along the gills changes little in basic histology except for the presence of mucous glands. Posterior to the last gill the histology outside the mucous glands and osphradium changes little. Goblet cells are rarely found on the distal wall but become more numerous on the proximal wall. The histology of the anal cone is an extension of the basic histology of the mantle cavity.

The distribution of cilia in the mantle cavity is one of the areas where Mopalia lignosa and Mopalia muscosa differ appreciably. Long cilia, 12-16 microns in length, are present throughout the mantle cavity of Mopalia lignosa originating near the anterior margin of the foot and extending the rest of the mantle cavity length. Cilia, however, do not extend onto the two ventral regions of the mantle cavity (Fig. 13, 14). Cilia in Mopalia muscosa are very short and hard to see using the light microscope. Cilia are present on the cells of the exhalant chamber throughout its full length and collectively appear as a "fuzzy" border. The exact distribution of cilia in Mopalia muscosa is conjectural at this time but is believed to

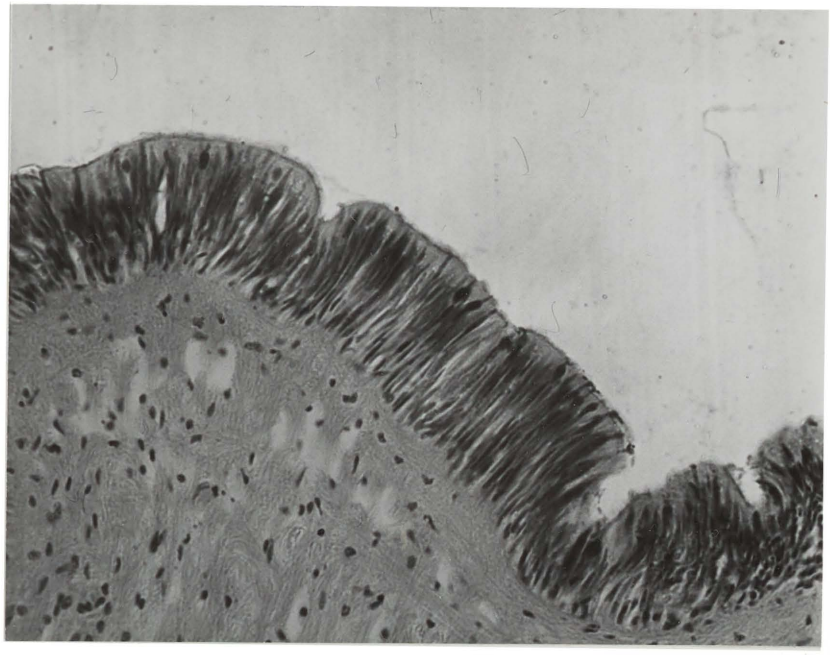


Fig. 16. Mopalia muscosa, ventral surface of foot. Ehrlich's haematoxylin and eosin. 160x.

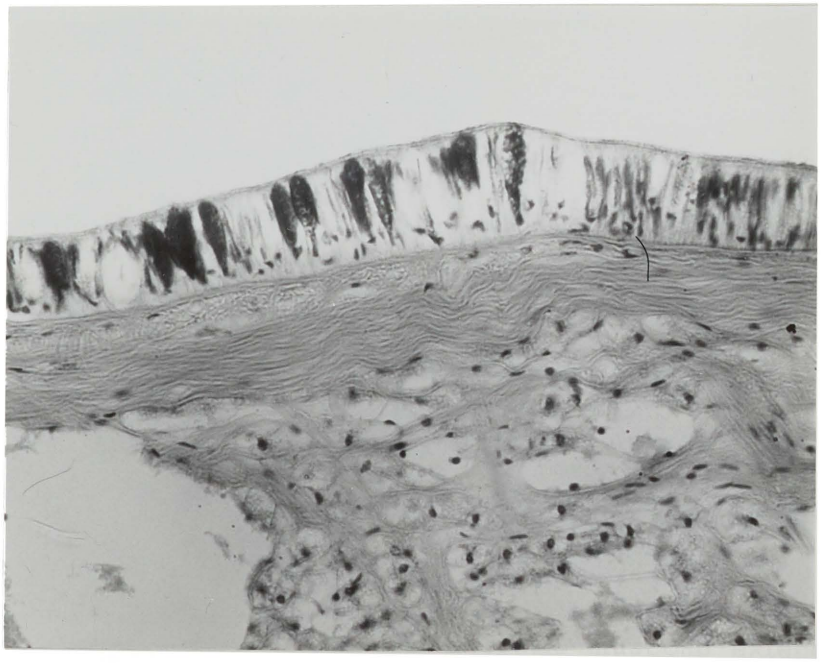


Fig. 17. Mopalia muscosa, pallial mucous gland showing goblet cells. Ehrlich's haematoxylin and eosin. 160x.

be similar to Mopalia lignosa.

Mucous Gland Histology

This section describes the histology of the mucous glands and the distribution of the various mucous tracts. Mopalia lignosa and Mopalia muscosa differ from one another distinctly in structure and disposition of mucous glands. The terms 'tract' and 'gland' are used interchangeably in this paper with both referring to the specialized aggregation of secretory cells into a unicellular layer.

Plate, as described in Yonge (1939), listed and classified four tracts of unicellular mucous glands in the mantle cavity of chitons. These tracts were named the neural, located on the roof of the mantle cavity; pedal, on the side of the foot; pallial, on the inner wall of the girdle and the branchial on the inner axis of the gill. Yonge (1939) further states that Plate did not find more than three of these tracts in any one chiton, and the number of tracts varied with the species studied.

In each lateral portion of a chiton's mantle cavity mucous tracts are found. Mopalia muscosa possesses a pallial mucous gland and a neural mucous gland whereas Mopalia lignosa possesses only a neural mucous gland.

The pallial mucous gland of Mopalia muscosa extends along the middle of the distal wall of the mantle cavity from the anterior margin of the foot to the third or fourth gill (Fig. 5). This tract is

composed mainly of tall columnar goblet cells (Fig. 17). The nuclei of these cells are flattened in the basal portions of cells (Fig. 18). In between the goblet cells there are thin cells termed neurosensory cells by Hoffmann (1930).

The neural mucous gland of Mopalia muscosa is located proximal to the gill bases and extends from the anterior most gills posteriorly to the anal cone (Fig. 5). Posterior to the last gill this tract expands and surrounds the osphradium (Fig. 5). The histology is similar to the pallial mucous gland. Tall columnar cells are the most conspicuous with flattened basal nuclei (Fig. 19). Thin neurosensory cells, and wedge-shaped ciliated cells (Hoffmann, 1930) are also present (Fig. 20).

The neural mucous gland of Mopalia lignosa can be divided into two regions. The inhalant portion (Fig. 5) is located in the inhalant chamber near the base of the gills. This portion is narrow and extends along the entire gill series. The exhalant portion (Fig. 5) is located in the exhalant chamber near the base of the gills. This region extends from the gill base to the dorsal side of the foot and runs the full length of the gill series. Behind the last gill both portions coalesce and extend posteriorly to the anal cone (Fig. 5). The osphradium is surrounded by the neural mucous gland with the greatest portion between the osphradium and foot. The mucous tract is composed of tall columnar cells possessing medium to coarse secretory granules (Fig. 21). Wedge-shaped ciliated cells are

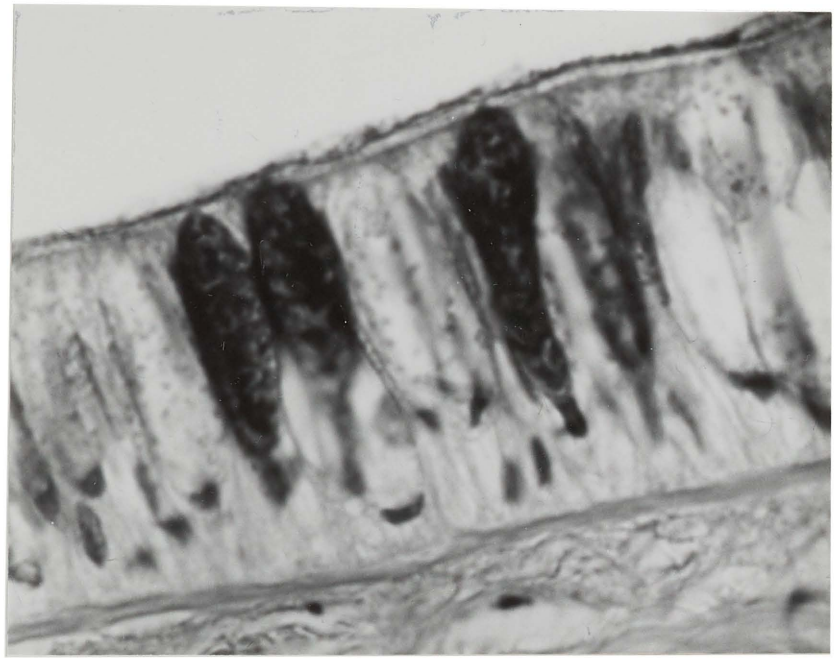


Fig. 18. Mopalia muscosa, goblet cells of pallial mucous gland showing basal, flattened nuclei. Ehrlich's haematoxylin and eosin. 640x.

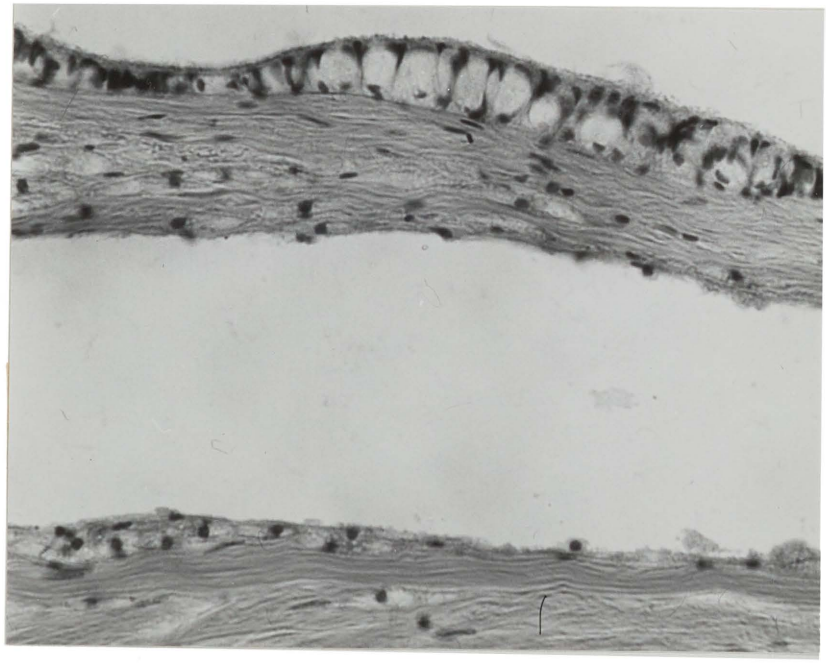


Fig. 19. Mopalia muscosa, neural mucous gland showing goblet cells and wedge-shaped ciliated cells. Ehrlich's haematoxylin and eosin. 205x.

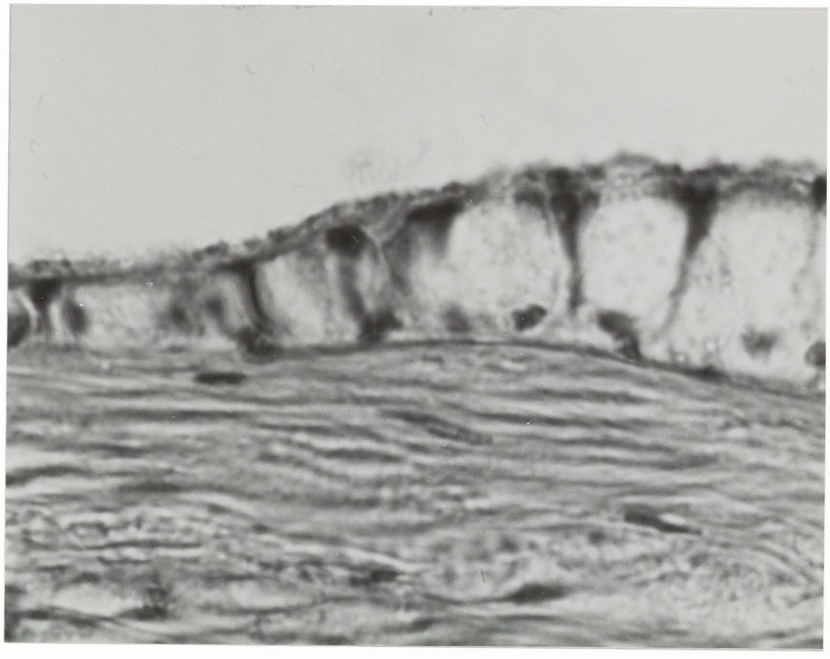


Fig. 20. Mopalia muscosa, neural mucous gland showing wedge-shaped ciliated cells and goblet cells. Ehrlich's haematoxylin and eosin. 640x.

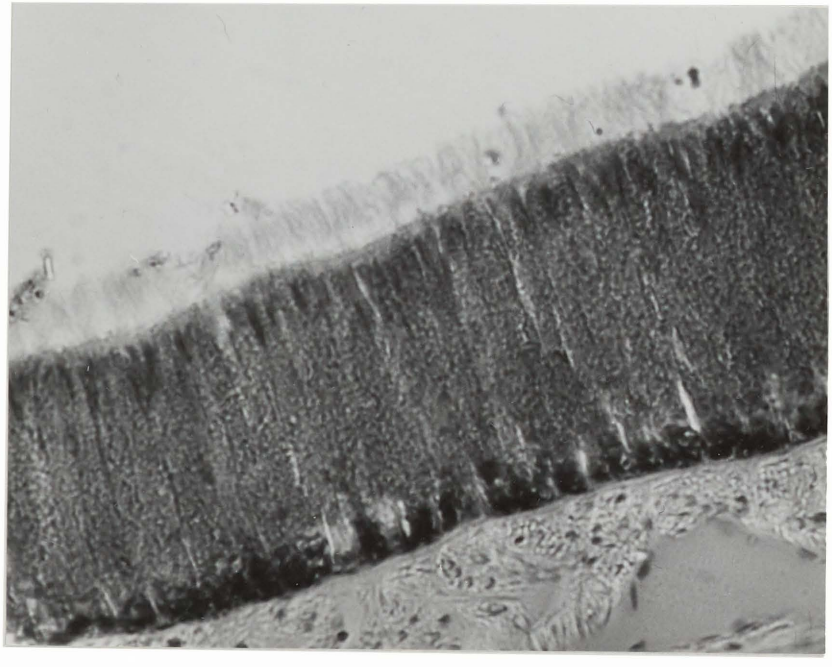


Fig. 21. Mopalia lignosa, neural mucous gland with ciliated cells and secretory granule-containing cells. Ehrlich's haematoxylin and eosin. 256x.

present near the surface of the tract with thin basal portions extending to the base of the tract (Fig. 22). Thin neurosensory cells are also present. Immediately adjacent to the osphradium there is a band of cells similar in structure to the goblet cells of the mucous glands of Mopalia muscosa (Fig. 23).

The staining reaction and inferred secretory behavior is different between the goblet cells and the granule-containing secretory cells. A majority of the goblet cells appear empty when stained with Ehrlich's haematoxylin and eosin (Fig. 17, 19). Staining with Mallory triple stain and toluidine blue, however, shows small irregular shaped secretory globules to be present (Fig. 24). The secretory product, when present, appears somewhat agranular. This product stains violet with toluidine blue and Mallory triple stain which suggests that the product is mucus. In the region of the osphradium in Mopalia muscosa the goblet cells are shown either empty, in the process of secreting their product or completely full (Fig. 25). This occurrence along with the demonstration of secretory globules leads to the conclusion that these cells exhibit rhythmic activity. Gabe and Arvy (1961) describe cells exhibiting rhythmic behavior as those where secretion and formation of products occur at different times as opposed to continuous activity where extrusion of the product, its formation and absorbtion of material from the environment all take place at the same time. Continuous secreting cells show no difference in appearance, whereas rhythmically active

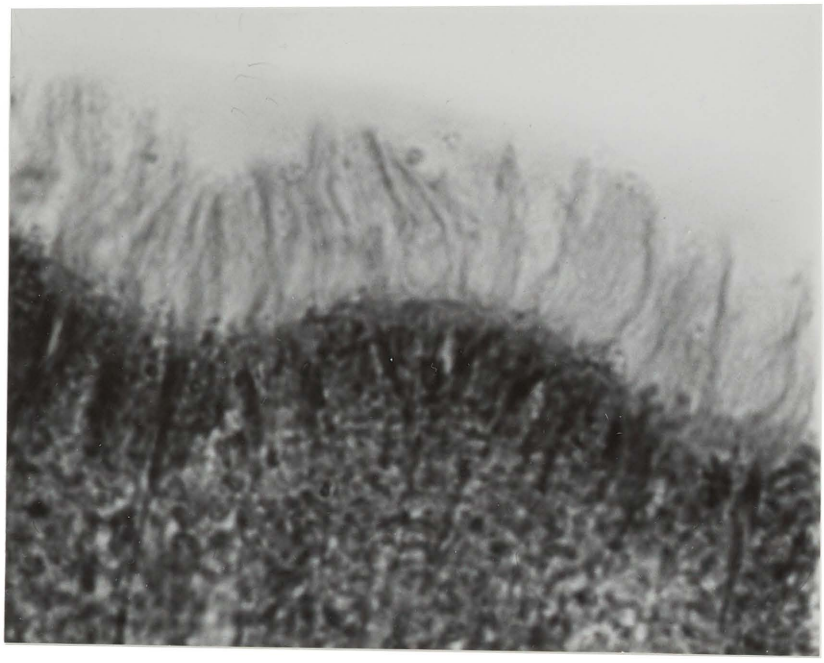


Fig. 22. Mopalia lignosa, neural mucous gland showing wedge-shaped ciliated cells. Ehrlich's haematoxylin and eosin. 640x.

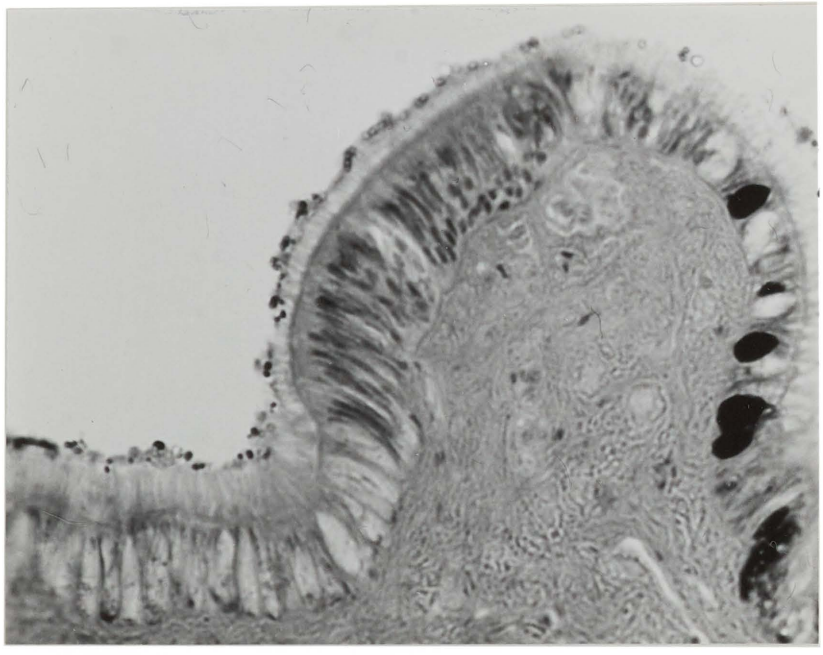


Fig. 23. Mopalia lignosa, osphradium showing narrow band of goblet cells surrounding it. Ehrlich's haematoxylin and eosin. 256x.

Fig. 24. Mopalia muscosa, secretory globules in goblet cells. Toluidine blue. 640x.

Fig. 25. Mopalia muscosa, cells of neural mucous gland near osphradium in stages of secretion. Ehrlich's haematoxylin and eosin. 512x.

cells reveal considerable differences in appearance (Gabe and Arvy, 1961). The goblet cell accumulates its product as globules which then extruded as a whole. Since the function of the mucous glands is to consolidate particles of sediment brought in with the inhalant current (Yonge, 1939) the restoration period in these rhythmically active goblet cells is, out of necessity, short, especially when the chiton inhabits environments with high concentrations of suspended sediment particles.

Secretory cells possessing granules as found in Mopalia lignosa exhibit continuous activity. These cells are taller than the goblet cells. The granules are medium to coarse and are densely packed in the cells. These cells stain violet, using toluidine blue, suggesting that their product is mucus. When stained with Ehrlich's haematoxylin and eosin the two portions react differently. The inhalant portion stains pink while the exhalant portion stains purple with isolated pink staining cells. This reaction is probably due to the pH of the cells. These secretory cells are continually secreting and restoring and all appear similar in section. The continually active secretory cells are merocrine type. Goblet cells are listed as being merocrine but may approach the apocrine type (Gabe and Arvy, 1961).

The function of these mucous glands is similar to the function of the hypobranchial glands of the prosobranch gastropods. They are thought to be analogous to the hypobranchial gland (Yonge, 1939,

1947) while the pallial mucous tract may be homologous with hypobranchial glands due to its position in the inhalant chamber (Yonge, 1939).

Histology of the Osphradium

In chitons the osphradium is located in the posterior area of the mantle cavity. In Mopalia lignosa and Mopalia muscosa the osphradium lies next to the anus and extends halfway to the last gill (Fig. 2, 4, 5). It is visible as a thin brown line on the roof of the mantle cavity. The use of the term 'osphradium' should not denote homology as compared with prosobranch gastropods (Yonge, 1939). The osphradium in the Polyplacophora is located in the exhalant chamber, while it is in the inhalant region in the prosobranch Gastropoda (Yonge, 1939). It is present in a majority of the Polyplacophora (Yonge, 1939).

The histology of the osphradium in the chitons studied herein is similar to that described for other chitons (Plate, 1897, 1898, 1901; Yonge, 1939; Purchon, 1968). The osphradium is composed of tall, thin, columnar cells with long, narrow nuclei (Fig. 26, 27). There are thin neurosensory cells interspersed between these cells and scattered cilia cells. There is a well-defined cuticle covering the entire osphradial cell layer through which sensory hairs penetrate (Yonge, 1939). The lateral nerve cord inervates the osphradium and lies dorsal to it.

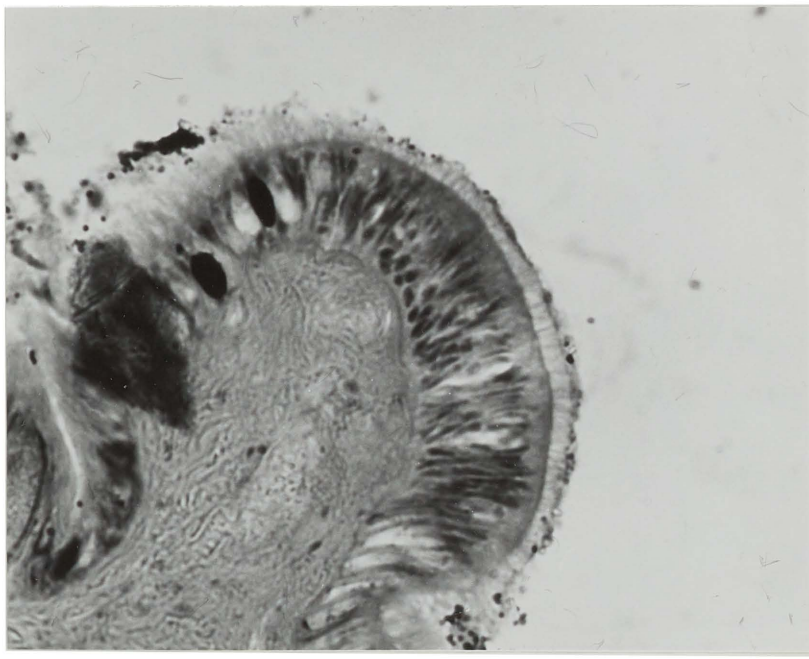


Fig. 26. Mopalia lignosa, osphradium. Ehrlich's haematoxylin and eosin. 256x.

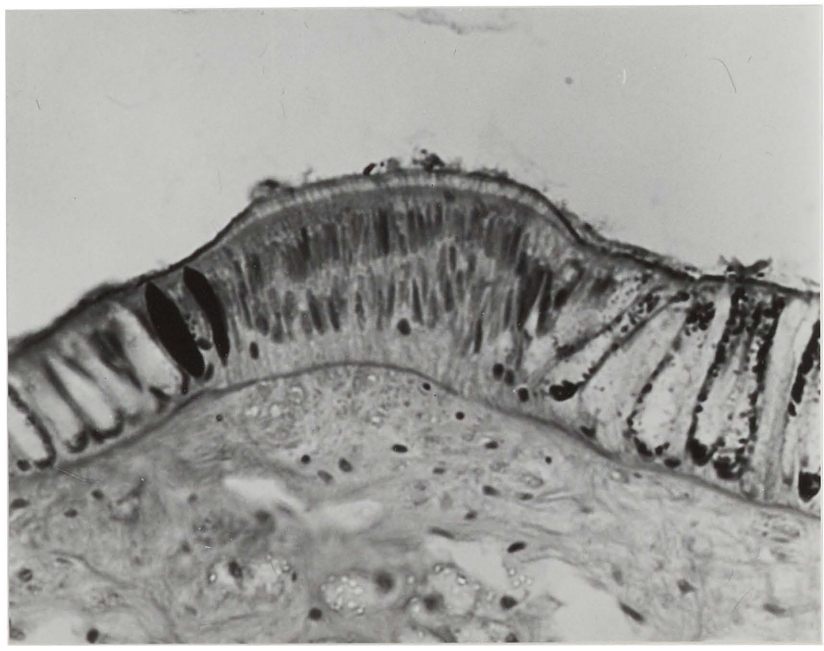


Fig. 27. Mopalia muscosa, osphradium. Ehrlich's haematoxylin and eosin. 256x.

Histology of the Gills

Gills of the studied chitons are similar to one another in histological structure. All gills in any one specimen are essentially identical in histological structure.

The base of the gill is a transitory ridge formed by the branchial sinuses. In cross-section the distal sinus is the efferent branchial sinus while the afferent branchial sinus is proximal (Fig. 6). The lateral nerve cord is located dorsal to the sinuses. It inervates the gills, neurosensory cells of the mucous tracts, osphradium and other structures. Longitudinal muscles are found running lengthwise, paralleling the branchial sinuses.

Gill lamellae are semi-hollow structures with pillars of connective tissue extending between the two outer surfaces (Fig. 28). The outer layer is one cell thick with the epithelial cells being low and columnar with short cilia and oval nuclei (Fig. 28). The epithelium is underlain with connective tissue which forms the aforementioned pillars. The remainder of the inner area is one large blood sinus.

The edge of the lamellae is dilated and contains long cilia (Fig. 29). The epithelium is composed of columnar cells and scattered goblet cells which are taller than those found in the rest of the lamellae. In the area of the lateral cilia zone the lamellar edges are slightly more dilated, clearly outlining the zone (Fig. 6).

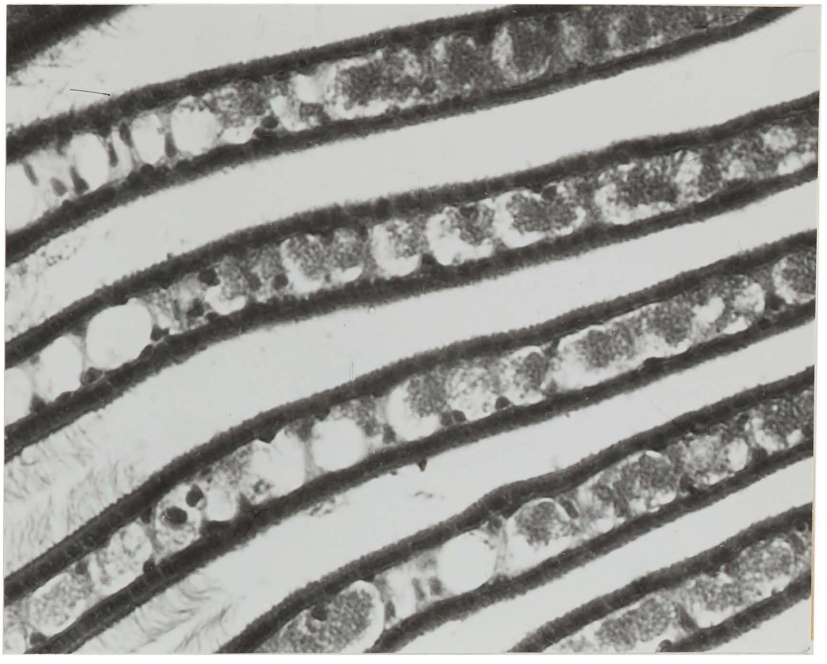


Fig. 28. Mopalia muscosa, transverse view of lamellae showing connective tissue pillars and enlarged blood sinus. Toluidine blue. 256x.

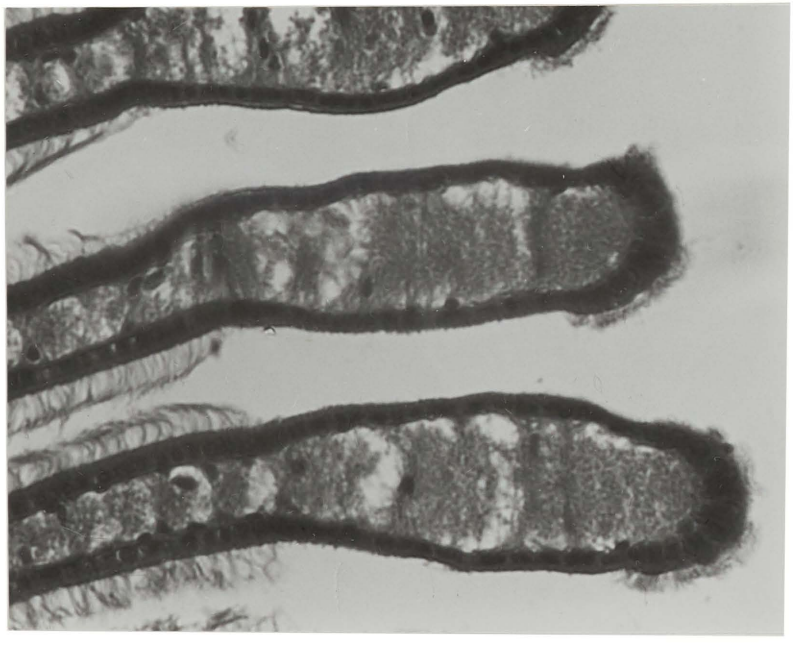


Fig. 29. Mopalia muscosa, transverse view of dilated lamellar edges with cilia. Toluidine blue. 256x.

Cilia in the zone are dense and measure up to 25 microns long (Fig. 30). The base of the gill on the transitory ridge also contains a lateral cilia zone with its histological structure essentially similar to the surface of the lamellae proper.

The epithelium extending around the branchial vessels contains columnar cells, scattered goblet cells and cells with short cilia. The branchial nerve from the lateral nerve cord runs along the outer margin of the branchial vessel while the longitudinal muscle fibers extend into the gill along the inner margin of the branchial vessels (Fig. 9). There are fibers extending between the two vessels through the central axial structure (Fig. 9).

Staining of the entire gill demonstrated that the lamellar edges and the epithelium about the branchial vessels contain mucous cells. This is confirmed by histological observation. These areas stained purple using methylene blue, toluidine blue and alcian blue. The rest of the lamellae stain very light, if at all, except for the pillars of connective tissue which appear as dark spots on the lamellar surface (Fig. 9). The lateral cilia zone appears as a fuzzy, lightly stained area.

Histology of Mopalia hindsii

The histology of the mantle cavity, gills and osphradium is the only histology investigated in this study. Mopalia hindsii is used in comparison because of its success at exploiting the bay

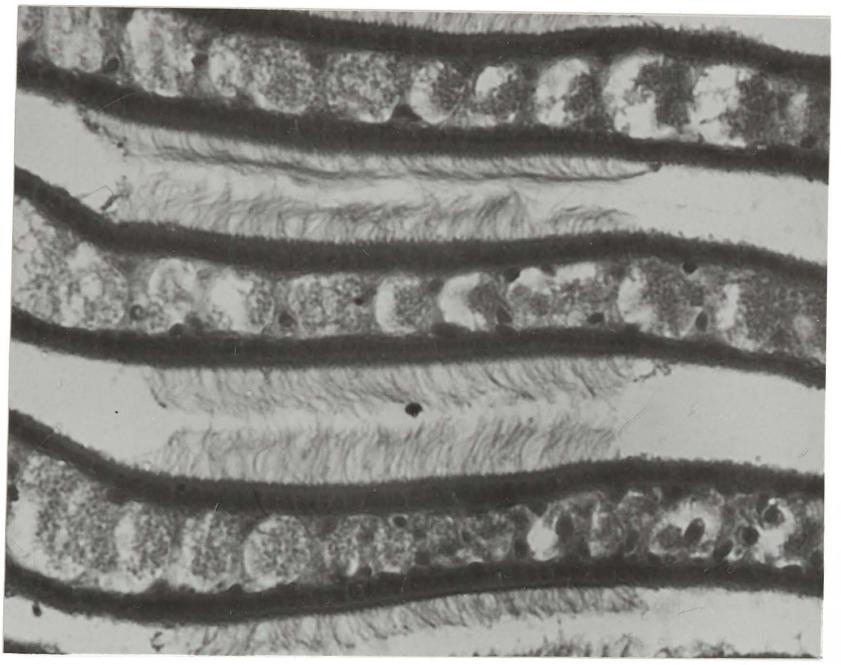


Fig. 30. Mopalia muscosa, transverse view of lamellae through lateral cilia zone. Toluidine blue. 256x.

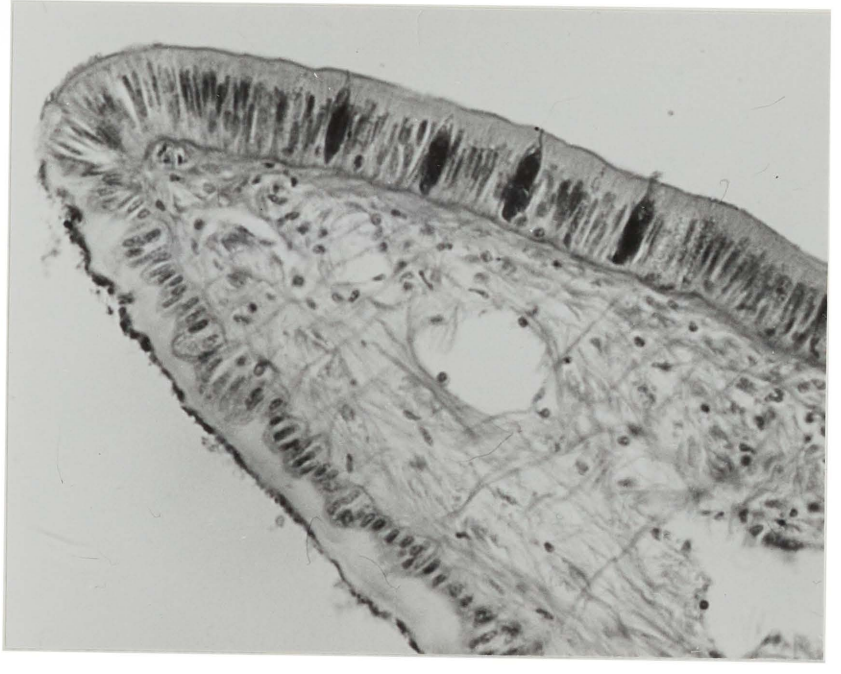


Fig. 31. Mopalia hindsii, girdle fold at junction of cuticle and mantle cavity epithelium. Ehrlich's haematoxylin and eosin. 205x.

environment (Barnawell, 1954). Although Barnawell (1954) bases his conclusion on other factors, the ability to handle increased suspended sediments in the bay environment is paramount to the success of Mopalia hindsii in its niche.

The structure of the mantle cavity in Mopalia hindsii is different from that of either Mopalia muscosa or Mopalia lignosa. The surface area of the chambers, especially the exhalant chamber, is noticeably greater than in the other two chitons studied. Beginning with the anterior-most gill the entire mantle cavity either develops numerous folds that extend back to the anal area or widens to form large, wide chambers. The girdle fold is developed into a definite flap (Fig. 31) and the osphradium extends almost to the last gill.

Mopalia hindsii possesses three of the mucous glands and lacks only the branchial mucous gland. Along the length of the gill series back to the anal area the cavity is almost entirely composed of mucous tracts. The pallial mucous gland originates opposite the anterior margin of the foot and halfway up the distal wall of the mantle cavity. Along the gill series the pallial mucous gland blends into the neural mucous gland which grades into the pedal mucous gland. The pedal mucous gland occupies about half of the proximal wall of the mantle cavity. In cross-section the mantle cavity along the gill region is composed of about two thirds mucous gland.

The cells of the pallial mucous tract, anterior to the gills,

is similar in structure to the cells of the pallial mucous tract in Mopalia muscosa. Along the gill series the cells become narrower and taller, and the tract becomes wider (Fig. 32). The cells of the other two tracts are similar to those found in the neural mucous gland of Mopalia lignosa except they are narrower (Fig. 33, 34). There is one exception noted in the cells of Mopalia hindsii. Located near the tip of the granule-containing secretory cells are isolated elongate goblet cells (Fig. 33, 34). These cells are similar in appearance to the cells about the osphradium that have retained their products (Fig. 25). Their secretory behavior is probably similar. The mucous gland cells along the gill series and back to the anal cone is about one-third to one-half taller than those in Mopalia lignosa.

The osphradial structure (Fig. 35) and gill structure is essentially identical to that of Mopalia muscosa and Mopalis lignosa. Cells outside the mucous tracts are similar in structure also with single goblet cells being more common along the two ventral edges of the mantle cavity.

is similar in structure to the cells of the pallial mucous tract in Mopalia muscosa. Along the gill series the cells become narrower and taller, and the tract becomes wider (Fig. 32). The cells of the other two tracts are similar to those found in the neural mucous gland of Mopalia lignosa except they are narrower (Fig. 33, 34). There is one exception noted in the cells of Mopalia hindsii. Located near the tip of the granule-containing secretory cells are isolated elongate goblet cells (Fig. 33, 34). These cells are similar in appearance to the cells about the osphradium that have retained their products (Fig. 25). Their secretory behavior is probably similar. The mucous gland cells along the gill series and back to the anal cone is about one-third to one-half taller than those in Mopalia lignosa.

The osphradial structure (Fig. 35) and gill structure is essentially identical to that of Mopalia muscosa and Mopalis lignosa. Cells outside the mucous tracts are similar in structure also with single goblet cells being more common along the two ventral edges of the mantle cavity.

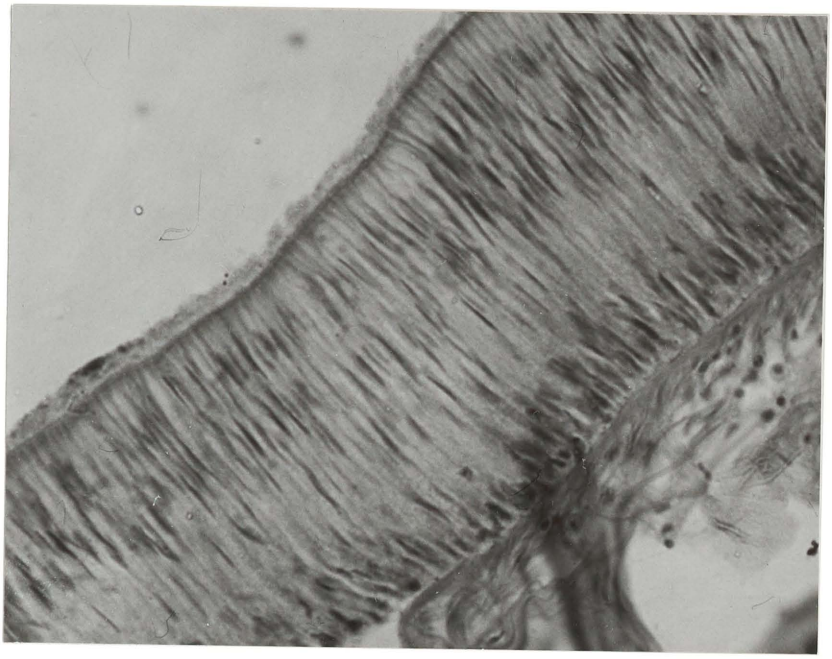


Fig. 32. Mopalia hindsii, pallial mucous gland along gill series. Ehrlich's haematoxylin and eosin. 205x.

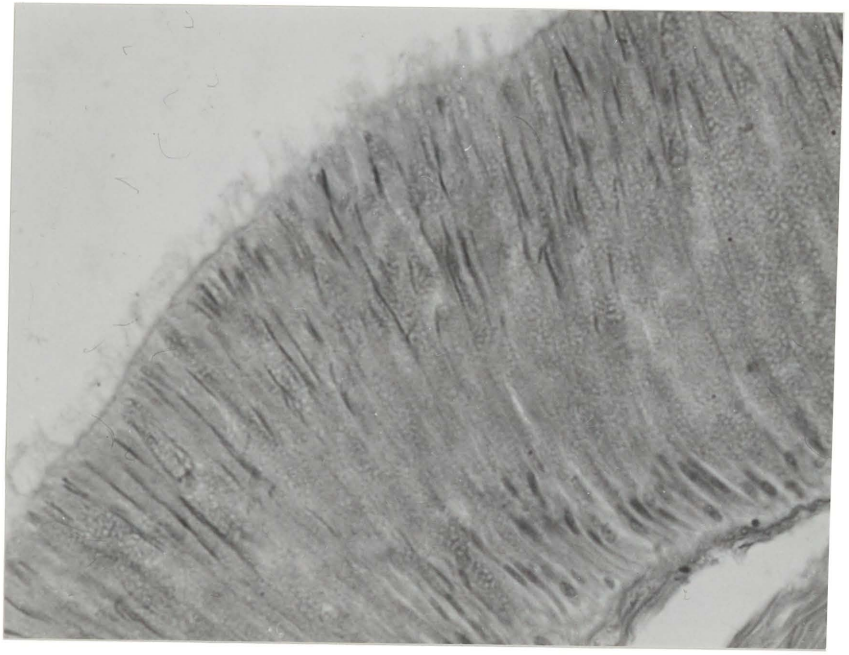


Fig. 33. Mopalia hindsii, neural mucous gland showing secretory cells with granules. Ehrlich's haematoxylin and eosin. 205x.

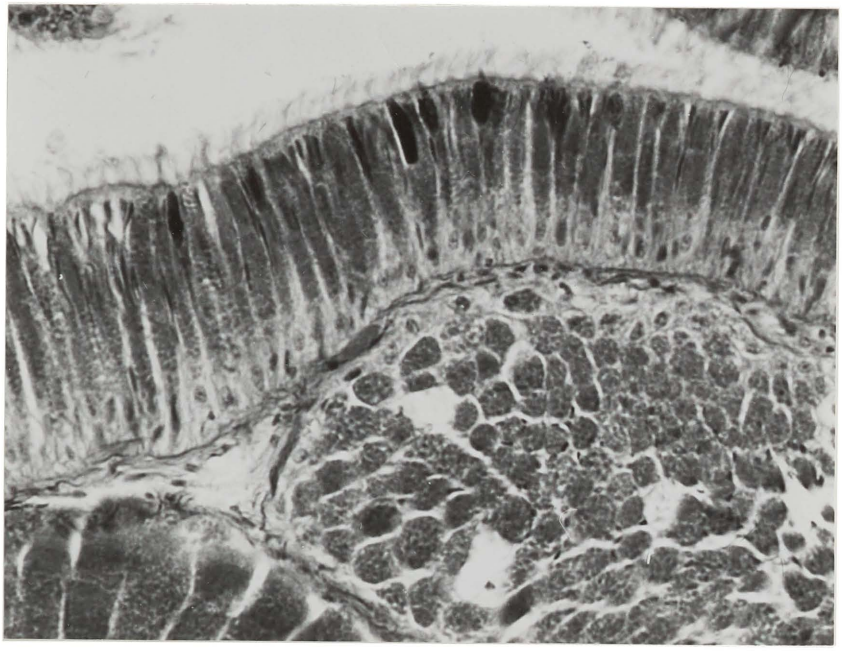


Fig. 34. Mopalia hindsii, pedal mucous gland, showing isolated goblet cells near its free edge. Ehrlich's haematoxylin and eosin. Photographed with green filter. 205x.

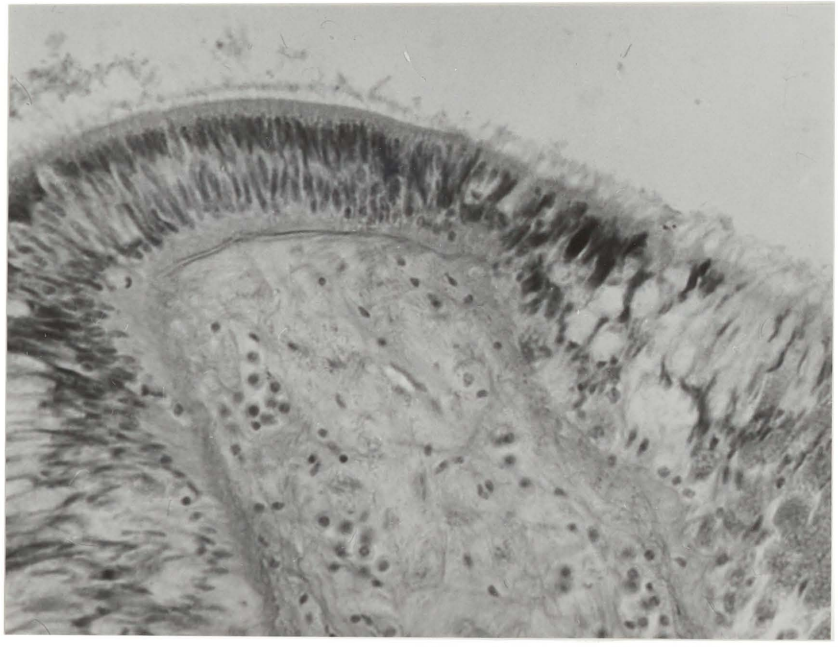


Fig. 35. Mopalia hindsii, osphradium. Ehrlich's haematoxylin and eosin. 205x.

DISCUSSION

The mantle cavity anatomy and currents found in Mopalia lignosa and Mopalia muscosa gives no clue as to why these two chitons have different ecological ranges. They are similar to one another and differ little from the mantle cavity anatomy and currents described and review for other chitons (Plate, 1897, 1899, 1901; Arey and Crozier, 1919; Hoffman, 1930; Yonge, 1939). Histological observation, however, demonstrated two areas where Mopalia lignosa and Mopalia muscosa differ. The length and distribution of cilia, and the structure, distribution and secretory behavior of the mucous glands are the two areas where the specimens studied showed the greatest comparable differences, especially when compared with similar structures in Mopalia hindsii, which help support the hypotheses put forth in this paper. These differences attempt to relate the investigated structural differences with the noted distributional differences.

Probably one of the key factors in controlling the distribution of the species studied is the presence or lack of the pallial mucous gland. The two chitons that are successful at exploiting the bay environment, Mopalia muscosa and Mopalia hindsii, possess the pallial mucous tract. The reaction of Mopalia muscosa in

experimentation to sudden increases in the concentration of suspended particles indicates a high degree of sensitivity. The pallial mucous gland is probably responsible for this sensitivity. The ability to release copious amounts of mucus in response to increases in suspended particles probably enhances the success of Mopalia muscosa and Mopalia hindsii. The lack of the pallial mucous gland in Mopalia lignosa in part helps to explain its limited range.

The efficiency of the two types of secretory cells is probably another factor helping to control the distribution of the species studied. The goblet cell, as found mainly in Mopalia muscosa, exhibits a wide range of activity. It can release its whole product rather quickly as is characteristic of rhythmically active cells and the restoration period is short. Tarao (1935) graphed the restitution times of cells of the hypobranchial gland of Haliotis japonica after secretion. He used a factor derived by dividing the sum of the height of glandular cells by the height of the fold of the hypobranchial gland. This gave him a measure of activity which he plotted against time. His graph showed that secretory cells (with granules) have a rather narrow range of activity when compared to mucous cells. The mucous cells exhibited a fairly short restitution time and a short secretion time. The mucous cells referred to in Tarao's paper correspond to the goblet cells of the chiton. Because of the activity of the goblet cell its efficiency is probably greater at handling suspended particles than that of the granule-containing

secretory cells. Barnawell (1954) while considering Mopalia hindsii as the most successful exploiter of the entire bay environment indicates that Mopalia muscosa is more successful in areas where the concentration of suspended sediment particles is especially high. Another observation that tends to support this hypothesis is the expanded mantle cavity area exhibited by Mopalia hindsii. The neural mucous gland in Mopalia muscosa, composed entirely of goblet cells, is apparently more successful at consolidating sediment particles than the combined pedal, neural, and pallial mucous glands of Mopalia hindsii which are composed primarily of granule-containing secretory cells.

The differences in cilia length and distribution are probably due to several factors. The efficiency of the goblet cells at handling sediments in the mantle cavity probably precludes the presence of long cilia in the mantle cavity. The viscosity of the secretion is probably greater in the goblet cells. Long cilia would be inefficient in a highly viscous medium and could also incumber the actual release of the secretion. In a less viscous medium the long cilia could function properly and would not interfer with secretion, especially in a cell with continuous activity.

One area that has not been investigated is the determination of particle load capacity of the chitons studies. This would involve subjecting chitons to varying concentrations of known particle sizes and observing their ability to handle these loads. Sediment samples

collected from Boiler Bay, Oregon and Mission Beach Jetty, San Diego, California, showed considerable differences in percentage of the various sizes of particles by weight. The sample from Boiler Bay had 13% of its particles smaller than 60 microns whereas the sample from San Diego had 50% of its particles smaller than 60 microns. This indicates that the concentration of suspended particles would be higher at the San Diego station than the Boiler Bay station. Further study will probably show that the limit in particle size that Mopalia lignosa can successfully handle is in the 60-140 microns size range.

The function of the mucous glands is to consolidate sediment particles that enter the mantle cavity with the respiratory currents (Yonge, 1939). Yonge (1939) suggests that the mucous glands are probably primitive in their function of cleansing the mantle cavity in chitons with holobranch gill series. The increased currents produced by the added gills would be sufficient to keep the mantle cavity clean (Yonge, 1939). The distribution of the mucous glands in chitons with a holobranch gill series is probably due in part to the environment inhabited. The currents created by the additional gills obviously are not sufficient in cleansing the mantle cavity of chitons inhabiting environments with high concentrations of suspended particles making it necessary for the mucous glands to be retained to insure success. This becomes evident when one examines the mantle cavity of Mopalia muscosa and Mopalia hindsii.

The role of the osphradium in chitons is another area of uncertainty. Plate (1897, 1899, 1901) considered the osphradium of chitons to be olfactory as did Arey and Crozier (1919). Yonge (1939) suggests that, as in the prosobranch gastropods, the osphradium might be used to estimate suspended particles in the respiratory currents. Purchon (1968) suggests that the osphradium might be a chemo-receptor organ capable of detecting the presence of spermatozoa or ova of the same species to insure co-ordination of spawning. From observations on the currents of the chitons studied herein, it is apparent that they have the ability to sense varying environmental conditions in the anterior portion of the mantle cavity. The chitons reacted to chemicals and high concentrations of suspended particles before the chemicals or particles had reached the osphradium. The pallial mucous tract in Mopalia muscosa and Mopalia hindsii is apparently an efficient tester of the respiratory currents. Observations on Mopalia lignosa indicate that the gills are also sensitive to varying physical factors of the environment. The presence of neurosensory cells in the mucous tracts also indicate a degree of sensitivity. In chitons with holobranch gill series it is important to detect sediments or adverse chemicals before they pass over the gills, not after, when the damage could have already been manifested. The structure of the osphradium indicates that it is sensory, but because of its position posterior to the gills and in the exhalant chamber it is probably a remnant of the ancestoral form being retained as a secondary sensory structure.

SUMMARY

1. The anatomy and currents of the mantle cavity of the chiton's Mopalia lignosa and Mopalia muscosa are similar.
2. The histological structure of the mantle cavity of Mopalia lignosa and Mopalia muscosa differ in the distribution and length of the cilia present and also in the structure, distribution and secretory behavior of the mucous glands.
3. Mopalia lignosa possesses long cilia which are present throughout most of the mantle cavity, whereas Mopalia muscosa possesses short cilia.
4. Mopalia muscosa possesses a pallial and a neural mucous tract. Goblet cells principally form these mucous tracts with neurosensory cells being present.
5. Mopalia lignosa possesses only a neural mucous tract, subdivided into inhalant and exhalant portions. Cells of this tract are secretory with medium to coarse granules.
6. Goblet cells are able to secrete ther entire contents rapidly and have a short restoration period. Their secretion is probably more viscous than the secretion of the granule-containing cells and this probably accounts for the relative lack of cilia in Mopalia muscosa. Secretion of these cells is efficient at

accumulating suspended particles and facilitating their exit.

7. Secretory cells are of a merocrine type. Their secretion is less viscous than the secretion of goblet cells and is easily handled by the long cilia. These cells are inefficient at handling high concentrations of suspended particles.

8. Mopalia hindsii possesses both types of cells in its mucous tracts. The expanded mucous tracts contain mostly granule-containing secretory cells which indicates the inefficiency of these cells compared to the goblet cells in handling high concentrations of suspended sediment particles.

9. The pallial tracts, when present, and the gills are efficient testers of respiratory currents. The osphradium is secondary in importance.

10. The development of mucous glands in chitons with a holobranch gill series is partly a result of the environment inhabited.

LITERATURE CITED

Arey, L. B. and W. J. Crozier. 1919. The sensory responses of Chiton. J. Exp. Zool. 29:157-260.

Barnawell, E. B. 1954. The biology of the genus Mopalia in San Francisco Bay. M.A. Thesis, Univ. of California, Berkeley.

Cowden, R. 1963. Histological observations of the foot of Chiton. Trans. Amer. Microsc. Soc. 82:406-416.

Gabe, M. and L. Arvy. 1961. Gland cells. In Brachet, J. and A. E. Mirsky (ed.) The Cell. Volume V. Academic Press, New York: 1-88.

Hoffmann, H. 1930. Amphineura und Scaphopoda. In H. G. Bronn (ed.) Klassen und Ordungen des Tierreichs. 3, Abt 1.

Hyman, L. H. 1967. The Invertebrates: Volume VI: Mollusca I. McGraw-Hill Book Co., New York.

Plate, L. 1897, 1899, 1901. Die Anatomie und Phylogenie der Chitonen. Zool. Jahrb. suppl. 4, 5.

Purchon, R. D. 1968. The Biology of the Mollusca. Pergamon Press, London.

Tarao, S. 1935. Microscopical study on the hypobranchial gland of Haliotis japonica Reeve with a note on the restitution of the secretion. J. Fac. Sci. Hokkaido Imp. Univ., Ser. VI (Zool.)

4:103-114.

Ricketts, E. F. and J. Calvin. 1968. Between Pacific Tides. Stanford University Press, Stanford, California.

Yonge, C. M. 1939. On the mantle cavity and its contained organs in the Loricata (Placophora). Quart. J. Micros. Sci. 81:367-390.

Yonge, C. M. 1947. The pallial organs in the aspidobranch Gastropoda and their evolution throughout the Mollusca. Phil Trans. B, 232:443-518.

COMPARATIVE ANATOMY AND HISTOLOGY OF THE MANTLE CAVITY OF THE CHITONS (POLYPLACOPHORA) MOPALIA MUSCOSA AND MOPALIA LIGNOSA

Theodore P. Winfield Jr.

Department of Zoology

M.A. Degree, August 1971

ABSTRACT

The chiton Mopalia muscosa ranges from the open rocky coast to the bay environment, whereas the related chiton Mopalia lignosa is restricted to the outer rocky coast. The mantle cavity currents, anatomy and histology were investigated.

Mucous gland cells of Mopalia muscosa are goblet type secretory cells. Their apparent ability to deliver their secretion rapidly together with having a short restitution period probably accounts for Mopalia muscosa having success at invading the bay environment. The mucous glands of Mopalia lignosa consist primarily of cells with medium to coarse secretory granules. These cells apparently are not as efficient at handling suspended sediments thereby limiting in part the range of Mopalia lignosa. The distribution of cilia in the mantle cavity is correlated with secretory cell type. Mantle cavity and gill anatomy along with their currents are similar in the two chitons.

COMMITTEE APPROVAL: