FINE STRUCTURE EVENTS IN MANTLE TISSUES OF THE PINTO ABALONE (*HALIOTIS KAMTSCHATKANA* J.H.JONAS, 1845) WHICH ARE ASSOCIATED WITH THE CULTURE OF MABÉ PEARLS

by

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ABSTRACT

The purpose of this research was to study the formation of nacre. The secretory system involved in shell mineralization was activated by the implantation of a nucleus between the mantle and the shell of northern abalone, *Haliotis kamtschatkana* Jonas 1845 (Gastropoda: Haliotidae). Histological and ultrastructural studies of the outer mantle epithelium of *H. kamtschatkana* were observed at intervals of various weeks.

The mantle epithelial cells secreted first the conchiolin in the formation of nacre. When the implanted nucleus was totally covered with conchiolin deposits, aragonite crystals which comprise the prismatic layer were deposited on the implanted nucleus. Subsequently, irregular nacre deposits which form the nacreous layer were secreted on the prismatic layer. The results suggest that during this deposition process, two different secretory cells types are responsible for producing and releasing conchiolin and nacre.

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No words are enough for my parents, Daniel Allende and Celina Ladoux for their constant love and moral support. And I must also mention my brother, sisters, brother in law, sister in law, nephew and nieces for their care and encouragement.

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LIST 0F ABBREVIATIONS

A	amoebocyte
BD	belt desmosomes
Bm	basal membrane
CT	connective tissue
EF	extrapallial fluid
GC	Golgi complex
GER	granular endoplasmic reticulum
GM	granular matrix
GR	glycogen rosettes
IS	intercellular spaces
M	mitochondria
MV	microvilli
N	nucleus
OME	outer mantle epithelium
Pm	plasma membrane
R	ribosome
SB	secretory blebs
SC	secretory cell
Si	secretory inclusion
SJ	septate junctions
SV	secretory vesicles
V	vacuoles
V1	type one vesicles
V2	type two vesicles

I. Introduction

The process of shell formation in Mollusca is of interest to biologists seeking to understand the nature of its composition (Gregoire *et al.*, 1955; Beedham, 1958a; Wilbur, 1964) or its response to injuries or inclusions (Tsujii *et al.*, 1958; Tsujii, 1968b&1968c). Recently, attention has been drawn to pearl formation in the northern abalone *Haliotis kamtschatkana* J.H. Jonas, 1845 for its obvious economic significance (Fankboner, 1988&1991) as well as for its potential to provide information on the basic mechanisms of shell secretion. The present study is an investigation of the role of the mantle in shell secretion and pearl formation in the abalone, *H. kamtschatkana*.

Information on shell structure and formation in molluscs has been derived largely from studies on bivalves and to a lesser extent, of gastropods. The main components of the molluscan shells are:

a) Periostracum: is the outer shell layer, and is composed of conchiolin (Beedham, 1958a; Timmermans, 1969). Conchiolin is a complex of proteins, polypeptides and a small amount of chitin (Beedham, 1958a; Wilbur, 1964; Cariolou and Morse, 1988; Samata, 1990). This periostracal protein is released by the basal cells of the inner surface of the outer fold of the mantle (Salueddin, 1976). It is changed to an insoluble compound and is hardened with quinone-tanned proteins containing phenolic residues (Beedham, 1958a; Bubel, 1973a&1973). After the secretion of the periostracum, the organic matrix is secreted by the mantle (Fretter and Graham, 1962; Wilbur, 1964).

b) Organic matrix: is composed of a water soluble component and a water insoluble component (Crenshaw, 1990; Samata, 1990). The soluble component is composed mainly of calcium-binding glycoproteins which are considered essential for the process of shell formation (Carioulou and Morse, 1988; Crenshaw, 1990; Samata, 1990). The insoluble component is composed of conchiolin which

forms the framework of the matrix (Wilbur, 1964; Beedham, 1965; Cariolou and Morse, 1988; Crenshaw, 1990; Samata, 1990). Conchiolin provides the sites for acidic macromolecules at which $CaCO_3$ crystals grow, serves to separate the individual crystals and binds them into a unified structure (Wilbur, 1964; Beedham, 1965; Crenshaw, 1990). The formation of calcareous layers begins when $CaCO_3$ crystals are deposited in the organic matrix, generally in the form of aragonite and calcite but also as vaterite (Wilbur, 1964; Salueddin, 1971).

c) Prismatic (outer) layer: is the first calcareous layer secreted. It develops from small rounded or elongated crystals randomly distributed in the organic matrix (Wilbur, 1964; Meenaski *et al.*, 1973; Wilbur and Salueddin, 1983). Each crystal is enclosed by a sheath of organic matrix (Tsujii *et al.*, 1958; Salueddin, 1971, Nakahara *et al.*, 1980&1982). The individual crystals elongate, thicken and unite to form larger crystals. As the crystals contact neighboring crystals, they form a prism shape (Tsujii *et al.*, 1958; Wilbur, 1964, Salueddin, 1971). The crystals grow perpendicular to the periostracum and it's growth is limited by the secretion of the nacreous layer (Wilbur and Salueddin, 1983).

d) Nacreous (inner) layer: is the second calcareous layer secreted. Initiation occurs by the deposition of mineral granules in the organic matrix (Watabe, 1965; Bevelander and Nakahara, 1969; Meenaski *et al.*, 1973; Nakahara *et al.*, 1980). Nacreous crystals grow and coalesce with others forming a lamella in bivalves (Tsujii *et al.*, 1958; Salueddin, 1971; Meenaski *et al.*, 1973; Nakahara *et al.*, 1980). In gastropods, growing nacreous crystals first form pyramidal stacks, and subsequently the crystals extend laterally in a continuous lamella (Nakahara, 1979; Nakahara *et al.*, 1982). The crystals of the nacreous layer form an uninterrupted lamella while those of the prismatic layer do not (Wilbur, 1964; Watabe, 1965; Salueddin, 1971; Meenaski *et al.*, 1973; Nakahara *et al.*, 1980). Nacreous crystals also are enveloped by organic matrix (Gregoire *et al.*, 1955; Watabe, 1965; Goffinet *et al.*, 1977).

Studies dealing with shell formation in terrestrial gastropods such as *Helix, Helisoma* and *Otala* have been performed in recent years (Durning, 1957; Salueddin, 1971; Wong and Salueddin, 1972; Meenaski *et al.*, 1973; Chan and Salueddin, 1974). Little, however, is known about the process of shell formation in marine gastropods and specifically in the Abalone (genus *Haliotis*). Abalone shell consists of the periostracum, the prismatic and the nacreous layers (Sakai, 1960). In *H. rufescens* shell (Nakahara *et al.*, 1982), the conchiolin that binds the calcite crystals in the prismatic layer is composed mainly of aspartic and cysteine acid. The nacreous layer consists of pyramidal stacks of hexagonal aragonite crystals. Alanine, glycine and aspartic acid are the main amino acids that form the conchiolin of this layer (Nakahara *et al.*, 1982).

Mutvei *et al.* (1985), carried out a mineralogical and microstructural study of the outer layer of the shell of eight species of *Haliotis*. They observed considerable variation in shell composition. The external layer in some species consisted entirely of aragonite or calcite, while in others it was composed of both minerals in different proportions. These authors described the capacity of the mantle epithelium to secrete both minerals simultaneously or alternately during growth. Dauphin *et al.* (1989) described the mineralogy, chemistry and ultrastructure of the external shell layer of ten species of *Haliotis*, and suggested that the prismatic layer is primarily composed of calcitic prisms in *H. kamtschatkana*. These prisms show distinct growth layers and decrease in size towards the outer surface. The union between the calcitic prisms and the nacreous layer show different patterns of development. Sometimes, aragonic rods are formed between the basal ends of the calcitic prisms in direct contact with the nacreous layer. These authors also suggest that the shells of *H. kamtschatkana* and *H. rufescens* resemble the fibrous calcite shell of bivalves.

Shell formation studies have led researchers to investigate the morphology and histochemistry of the mantle. The principal role of the mantle is the secretion of the shell but it also has sensory and

defensive functions (Fretter and Graham, 1962; Bevelander, 1988). The mantle is formed by two epithelial cell layers separated by connective tissue and covers the visceral mass (Fretter and Graham, 1962; Bevelander, 1988).

In most bivalves and some gastropods the mantle edge has three folds: outer, middle and inner (Fretter and Graham, 1962; Wilbur, 1964). The outer mantle epithelium is responsible for the deposition of both organic and inorganic substances in the extrapallial fluid (Durning, 1957; Wilbur, 1964; Beedham, 1965). The extrapallial fluid occupies the extrapallial space, the area between the outer mantle epithelium and the inner shell. Inorganic substances from the hemolymph cross the mantle epithelium by diffusion or active transport into the extrapallial fluid (Neff, 1972; Wilbur and Salueddin, 1983). The principle ions transported are Ca, Na, K, Mg, HCO_3, Cl and SO_4 for different marine and freshwater bivalves (Wada and Fujimuki, 1976). The organic substances of the extrapallial fluid are thought to be secreted by the outer mantle epithelium. The main organic compounds secreted are proteins, mucopolysaccharides, polysaccharides, organic acids and lipids which compose the organic matrix (Beedham, 1965; Wilbur and Salueddin, 1983; Samata, 1990). Bevelander and Nakahara (1969) suggested that the pallial fluid gives rise to the organic matrix responsible for crystal initiation and growth in the marines bivalves *Mytilus, Pteria* and *Anomnia*.

Most histochemical and histological studies of the mantle epithelial cells involved in shell regeneration have been carried out on bivalves such as *Anodonta*, *Pteria (pinctadata) martensii* and *Lymnea* and terrestrial gastropods, such as *Helix*. Higher rates of O_2 consumption were measured in the marginal zone of the mantle during periostracum formation in the snail *Cristia plicata spinosa* (Tsujii, 1962). O_2 consumption was lower in the central zone of the mantle during prismatic and nacreous layer formation (Tsujii, 1962). A moderate increase in RNA and alkaline phosphatase concentrations in mantle epithelial cells, has been reported to occur following damage of the shell in *Helix* (Durning, 1957), *Anodonta* (Salueddin,

1967) and *Lymnea* (Timmermans, 1973). High concentrations of carbonic anhydrase were observed in the mantle tissue during *Helisoma* shell regeneration (Kunigelis and Saluedin, 1983). Calcium salts, mucopolysaccharides and proteinaceous granules that are derived from the disintegration of cell inclusions induced by shell damage were observed (Abolins-Krogis, 1963; Kapur and Gibson, 1968). Glycogen and mucopolysaccharides were found in the outer mantle epithelium following shell damage to *Anodonta* (Salueddin, 1967).

Amoebocytes are implicated as calcium and protein carriers during shell formation for *Helix* (Wagge, 1951; Abolins-Krogis, 1976), for *Anodonta* (Beedham, 1965) and *Helisoma* (Kapur and Gupta, 1970). They have been found in the connective tissue and on the surface of mantle cells. According to these authors, amoebocytes enrich the repair membranes with various repair substances and cellular constituents. Amoebocytes have also shown evidence of pinocytosis activity in *Pinctadata radiata* and *Isognomous* (Beedham, 1965; Nakahara and Bevelander, 1967).

Several studies have shown that shell secretion is undertaken by different types of cells in special areas of the mantle epithelium. For instance, in Lamellibranchia *Macrocallista maculata* (Bevelander and Nakahara, 1967) and the bivalve, *Mytilus edulis* (Bubel, 1973a), the periostracum originates from the basal cells located in the base of the periostracal groove. These cells contain vacuoles, infolded plasma membrane, absence of microvilli and a less electron-dense cytoplasm than adjacent cells. Completion of the periostracum is achieved by cells of the mantle outer fold which contain numerous distended Golgi cisternae, ribosomes, microvilli and glycogen particles. Bubel (1973a) also describes lysosomes in these cells. Two types of columnar cells were described in the outer mantle epithelium of *Anodonta* by Machado *et al.* (1988). "A" cells containing desmosomes, glycogen particles, electron dense bodies and small cytoplasmic vesicles predominated in the epithelium. "B" cells contained a large number of mucous granules resembling chitin, with which they

secreted the organic matrix. Tall columnar cells containing numerous mitochondria and highly convoluted lateral cell membranes were observed in the outer mantle epithelium of *Mercenaria* (Neff, 1972) and *Cerastoderma* (Richardson *et al.*, 1981). These cell features suggest high rates of calcium transport from the mantle to the extrapallial fluid (Neff, 1972; Richardson *et al.*, 1981). Salueddin (1970) observed an increase in vacuoles, mitochondria with or without crystals, lamellar organelles and active basal endocytosis in the outer mantle epithelial cells during *Helix* shell regeneration. Periostracum formation in *Pteria (pinctadata) martensii* (Tsujii, 1968b&1968c) is accomplished by cells containing spherical granules, vacuoles, numerous lysosomes and slender nuclei. The prismatic and nacreous layers were secreted by cells with microvilli, few lysosomes, numerous mitochondria, well developed granular endoplasmic reticulum and a complex infolding in the basal cell membrane (Tsujii, 1968b&1968c). Outer mantle epithelial cells which normally produce the nacreous layer can be transformed to produce the periostracum, prismatic layer and nacreous layer in the bivalve *Musculus senhouisia* (Kawaguti and Ikemoto, 1962), the gastropods *Anodonta* (Beedham, 1965) and *Pteria (pinctadata) martensii* (Tsujii, 1968b&1968c). When cells changed from secretion of the periostracum to secretion of the nacreous layer, they increased in height and in number of mitochondria and the cytoplasmic particles they contained (Beedham, 1965; Kawaguti and Ikemoto, 1962; Tsujii, 1968b&1968c).

Although these studies provide information on changes in shell structure and mantle epithelium ultrastructure during shell formation, our understanding of these changes is incomplete. Also, most research has been directed towards the accessible terrestrial gastropods. Less is known about the process in marine gastropods. In the case of the genus *Haliotis*, a few studies of shell structure have been done (Sakai, 1960; Nakahara *et al.* 1982; Mutvei *et al.* 1985; Dauphin *et al.* 1989), but there is a lack of information on shell secretion. The northern abalone, *Haliotis kamtschatkana* is an appropriate representative for an investigation of the role of the

mantle in shell secretion and pearl formation. Pearl formation in this species is proposed by Fankboner (1991). It provides an opportunity to describe and study the function and interactions of the organelles in the outer mantle epithelium which regulate the formation of the calcareous layers in molluscs and at the same time to increase our understanding of the mechanisms of pearl formation.

Haliotis kamtschatkana is a marine snail that belong to the Phylum Mollusca, class Gastropoda. It is the only species of abalone that occurs on Canada's west coast, of approximately 70 species occurring world-wide (Fankboner, 1988). Fankboner (1988) reported that this species is locally known as the Japanese, pinto or northern ear shell, and occurs from the lower intertidal zone to 18 m. depth in semiprotected waters. In general, the haliotids are a gregarious species that fasten themselves to rocks with a strong foot. They graze on drifting algae. Sea stars and occasional sea otters are sources of predation. This abalone has an established commercial importance and is of potential value to the food and jewelry markets world wide (Fankboner, 1988).

The objectives of this study were to describe ultrastructurally the outer mantle epithelium in the northern abalone, *Haliotis kamtschatkana* and the fine structure events and interactions of the organelles in the outer mantle epithelium during Mabe pearl formation.

II. Materials and Methods

A- Collection sites

Specimens of *H. kamtschatkana* were collected by scuba diving at depths of 3 to 10 m. below 0 on the chart datum, in Barkley Sound on the West Coast of Vancouver Island (Lat. 48^0 50'N; Long. 125^0 8'W): the south shore of Sandford Is., the east shore of Helby Is. and at Kirby point.

B- Husbandry

The abalone were husbanded in 55 gal. polyethylene drums suspended from docking at 1.52 m. below zero tide in front of the Bamfield Marine Station. The plastic drums were pre-used for shipping fruit juice concentrate. The bottom and three windows in the sides of each drum were cut out and covered with polyethylene mesh (6.7mm.) to permit a constant flow of water through the container. The top of each drum was covered with a string-net closure to keep the abalones from escaping and to protect them from predators. The drums were suspended in the water by a rope bridle.

Each drum was cleaned and examined weekly for dead abalones, predators or any malfunction. Abalone were maintained on a diet of giant kelp, *Macrocystis integrifolia*. The noneaten residue of algal nematocysts, stipes and blades were removed weekly and replaced with fresh algae collected on the west shore of Helby Is..

Abalone are extremely sensitive to thermal variation (Sloan & Breen, 1988; Hahn, 1989). To monitor temperature variation, water surface temperatures were measured daily during low and high tides by means of a max.-min. thermometer hung from the dock and adjacent to the barrels. A second max.-min. thermometer was suspended from one of the suspended drums and was checked to determine temperature extremes every two weeks.

C- Nucleus Implantation

To induce nacre and pearl formation in abalone, 52 specimens of *H. kamtschatkana* were implanted with a single, 16 mm., hemispherical, polyester resin nucleus. The implanted nucleus was glued to the shell above the outer mantle epithelium that covered the gonads. The sample serial number, the name of the laboratory, and date of implant were printed on top of each shell. The sex, length and weight of each specimen were also recorded in a log..

D- Histology

D.1- Light Microscopy

Structural changes in the outer mantle epithelium during nacre and pearl formation were studied by fixing and embedding both normal and regenerating mantle tissues for histological and histochemical examination. Once a week for 14 weeks a single implanted abalone was relaxed with a solution of 7% magnesium chloride in tap water. After sacrifice, the mantle tissue enclosing the implanted nucleus was removed and fixed in 6% glutaraldehyde buffered with 0.2 M cacodylate (ph 7.4) for 15 min. at room temperature. Following a distilled water rinse, the tissues were post-fixed for 20 min in 2% osmium tetroxide buffered with Dorey's solution B, as described by Fankboner (1978). The tissues were rinsed again in distilled water and subsequently dehydrated in an increasing gradient of ethanol. This was followed by a 1:1 mixture of ethanol and propylene oxide, and finally 100% propylene oxide. The fixed material was embedded in Epon 812.

Thick sections of 0.5 - 1 μm. were cut with glass knives using a Reichert Ultramicrotome. The epoxy sections were stained in 1% toluidene blue buffered by 1% $Na_2B_4O_7$ (borax) followed by examination and photography using a Carl Zeiss microscope with attached camera. Epoxy sections for histochemical determination of mucoproteins and polysaccharides were stained by the Periodic Acid

Schiff (Pas) method (Humason, 1967) and observed under light microscopy. The average height and width of epithelial cells were measured from a sample size of 30 cells.

D.2- Transmission Electron Microscopy (TEM)

Normal and regenerating mantle tissues were fixed for TEM and dehydrated as described above. The fixed tissues were embedded in Epon 812 and thin sections of 50-70 nm. were cut and stained with uranyl acetate and lead citrate (Reynolds, 1963). Sections were then examined and photographed using a Philips 300 electron microscope.

D.3- Scanning Electron Microscope (SEM)

Normal mantle tissues were fixed for SEM as described above and dehydrated in a decreasing graded series of ethanol and in an increasing graded series of amylacetate followed by critical point drying. Mantle tissue preparations were stub-mounted, gold-coated and then photographed in a ETEC scanning electron microscope.

E- Terminology

Type one vesicles (V1): are usually localized above the nucleus in the upper cytoplasm. Size and shape of the type one vesicles varies. They can be round or ovoid and have an average length and width of 0.6-0.8 µm. and 0.4 µm. respectively. They contain amorphous and membranous masses of uneven electron density material. The contents can fill the vesicle. Eventually, a luminal space can exist between the material and the vesicle membrane. Occasionally these type one vesicles are fused into one large membrane bounded spherical body and have a mean diameter of 1.50-3.00 µm.. A luminal space surrounds the content and the vesicle membrane. The luminal space contains low electron dense particles. The content is amorphous with uneven electron density. These bodies are generally distributed in the apical cytoplasm.

Type two vesicles (V2): are spherical and have an average diameter of 0.6-0.8 μm. They have a very defined membrane and an even electron dense appearance. Sometimes they can contain a round or ovoid, dark electron dense inclusion. Occasionally, both inclusions are observed inside the vesicle. The vesicles are loosely packed and cytoplasmic material is seen dispersed between them.

III. Results

A- Histology and ultrastructure of the Outer Mantle Epithelium

The outer mantle epithelium (OME) which covers the gonads was formed by a single layer of columnar cells and a sheet of loose connective tissue (CT) (Fig. 1). The average height of the epithelium cells was 25 µm. and the average width was 5 µm. (Fig. 1). The cell surface area is increased by the presence of apical microvilli. The microvilli (MV) were numerous and closely packed, with a uniform diameter of 0.13 µm. (Fig. 2). Epithelial cells were apically joined by belt desmosomes (BD) (Fig. 3). Subsequent septate junctions (SJ) (Fig. 3), beneath the belt desmosomes were observed. Although the lateral cell membrane was fairly straight, occasionally a few narrow, intercellular spaces occured. The basal cell membrane (Bm) was moderately straight. Short and infrequent interdigitations occured (long black bold arrow) (Fig. 4). Below the basal membrane, the connective tissue was observed. Myofibrils (white bold arrow) were embedded in the granular matrix (GM) (Fig. 4) of the connective tissue and were oriented parallel to the epithelial cells.

The nucleus (N) was ovoid, elongated, centrally localized and occupied one third of the size of the cell (Fig. 1). One or two macronuclei were observed according to the orientation of the section. Heterochromatin was scattered in clusters in the karyoplasma and was also seen to adhere to the nuclear membrane. Ribosomes were unevenly distributed on the outer surface of the outer nuclear membrane.

Outer mantle epithelial cells had a cytoplasm dominated by mitochondria (M), type one vesicles (V1) and vacuoles (V) (Figs. 4-7). A few mitochondria were uniformly scattered throughout the apical, central and basal cytoplasm. These mitochondria (M) were mainly composed of tubular-shaped cristae and a granular matrix (Fig. 4). Occasionally, they were distributed among granular endoplasmic reticulum (black bold point) (Fig. 4).

Fig. 1- Longitudinal section of the outer mantle epithelium of *Haliotis kamtschatkana* showing the appearance of the mantle before nucleus implantation. Abbreviations used are: OME, outer mantle epithelium; N, nucleus; CT, connective tissue. Scale bar= 20 μm

Fig. 2- TEM-section of the apical packed microvilli of the outer mantle epithelial cells of *Haliotis kamtscahtkana*. Abbreviations used are: MV, microvilli; small vesicles, long black bold arrows. Scale bar= 1 μm.

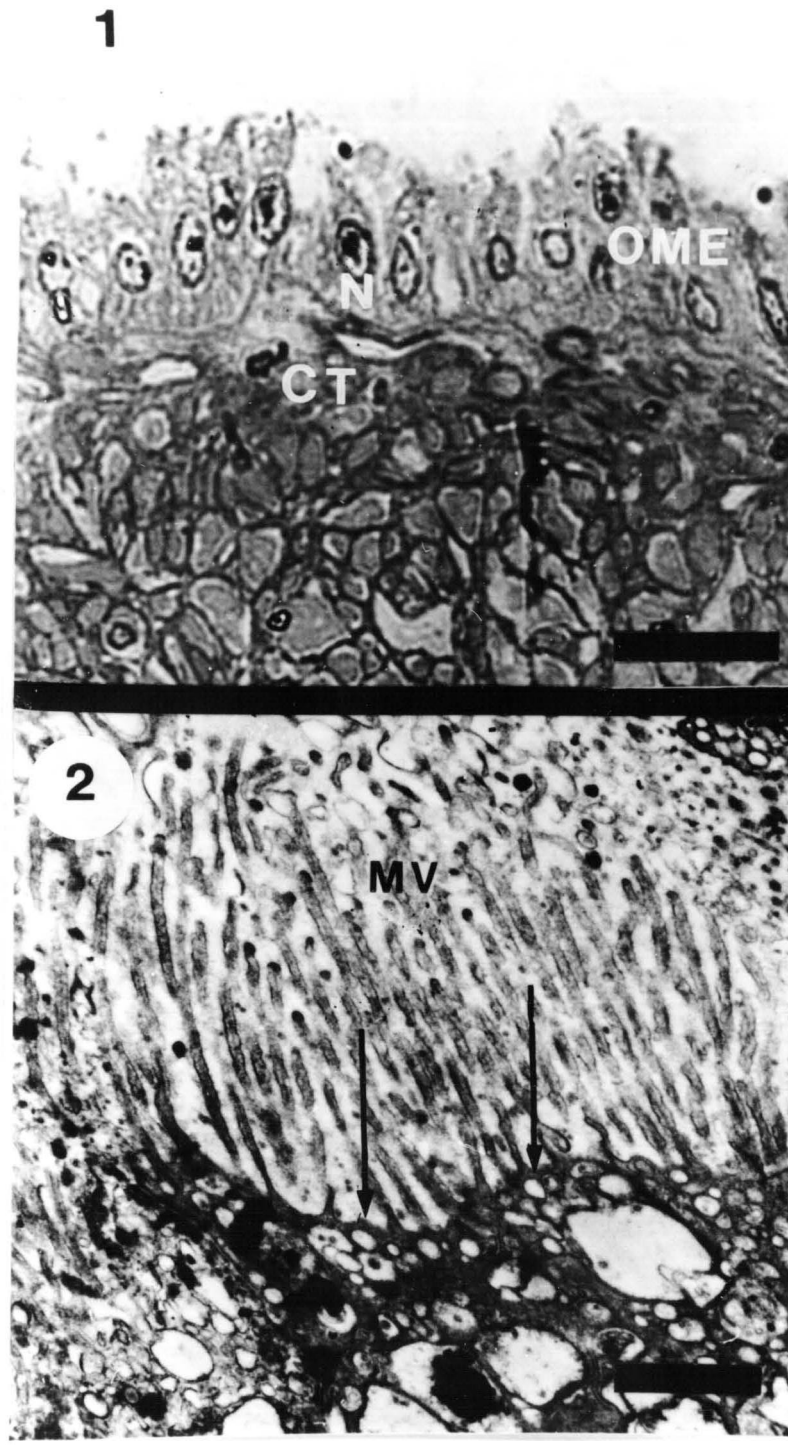

1
OME
N
CT
2
MV

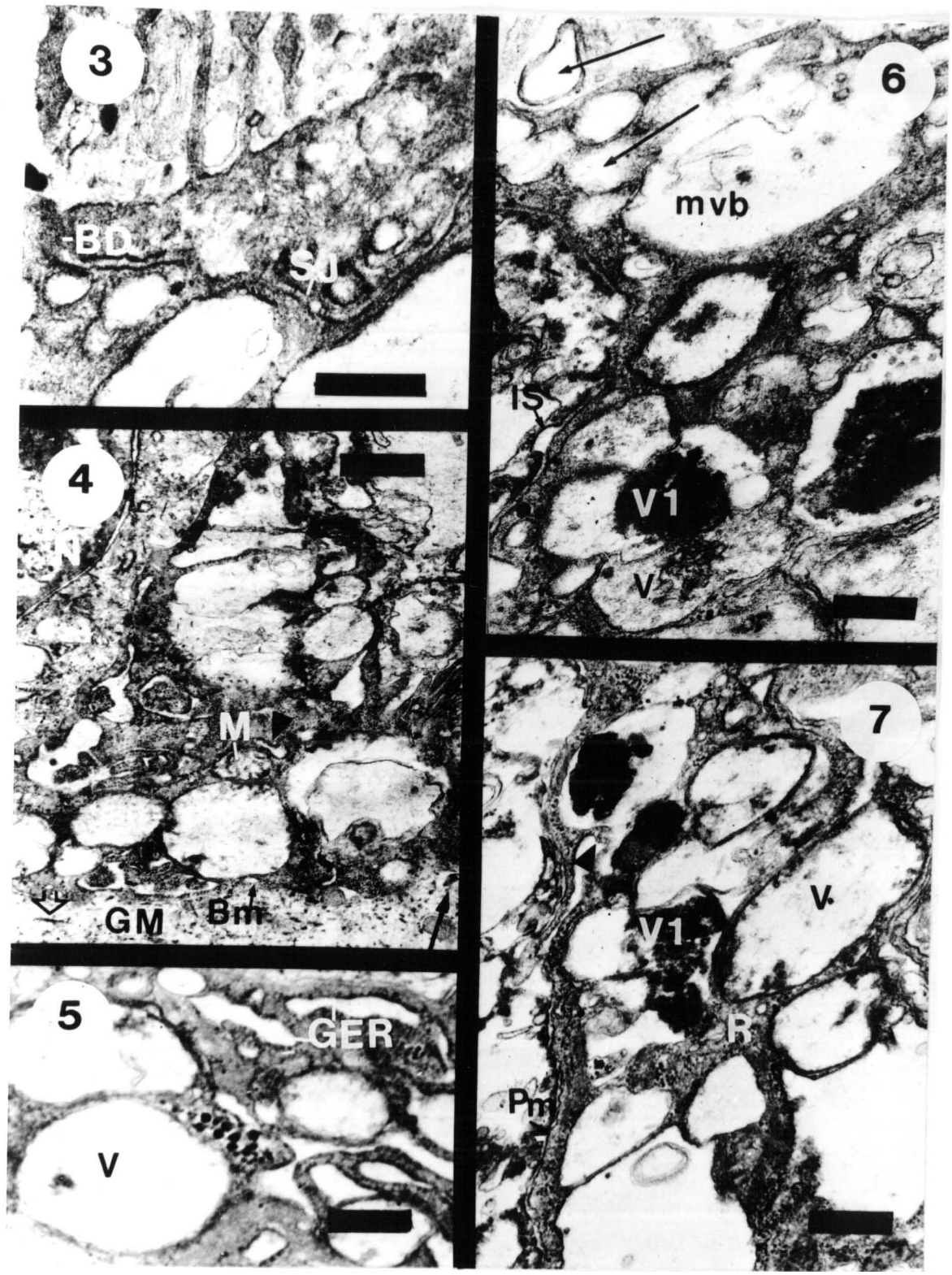
Fig. 3- TEM- section of apical junctions linking adjacent cells of the outer mantle epithelium. Abbreviations used are: BD, belt desmosomes; SJ, septate junctions. Scale bar= 0.3 μm.

Fig. 4- TEM-section of the basal cytoplasm of the outer mantle epithelium from *Haliotis kamtschatkana*. Note the granular endoplasmic reticulum distributed among the mitochondrion (black bold point). Abbreviations used are: N, nucleus; M, mitochondria; Bm, basal membrane; interdigitation, long black bold arrow; myofibrils, white bold arrow; GM, granular matrix. Scale bar= 0.4 μm.

Fig. 5- TEM-section of the infranuclear cytoplasm of the outer mantle epithelium from *Haliotis kamtschatkana*. Abbreviations used are: V, vacuole; GER, granular endoplasmic reticulum. Scale bar= 0.4 μm.

Fig. 6- TEM-section of the upper cytoplasm of the outer mantle epithelial cells from *Haliotis kamtschatkana*. Note type one vesicles (V1) among vacuoles (V). Abbreviations used are: mvb, multivesicular bodies; IS, intercellular space; small vesicles, long black bold arrows. Scale bar= 0.3 μm.

Fig. 7- TEM-section of the supranuclear cytoplasm of the outer mantle epithelial cells from *Haliotis kamtschatkana*. Note the straightness of the plasma membrane (Pm). Abbreviations used are: V1, type one vesicles, black bold point; R, ribosomes; V, vacuoles. Scale bar= 0.3 μm.


3
BD
SJ
4
M
GM
Bm
5
GER
V
6
mvb
IS
V1
V
7
V1
V
R
Pm

Other cytoplasmic organelles observed were type one vesicles (V1) (Figs. 6 and 7). These vesicles were usually localized above the nucleus in the apical cytoplasm. In light micrographs, the type one vesicles appeared very dark blue after staining with toluidine blue but the preparations failed to react with the Periodic Acid Schiff test. Occasionally, type one vesicles (V1) were distributed among vacuoles (V) (Figs. 6 and 7). Other organelles present in the cytoplasm were small, round or oval vacuoles (V) (Fig. 7). These vacuoles were scattered throughout the cytoplasm with the highest number occuring in the apical cytoplasm. The vacuoles (V) were either empty or contained light electron dense material (Fig. 6). Other cytoplasmic organelles present included multivesicular bodies. A few multivesicular bodies (mvb) were distributed below the microvilli in the apical cytoplasm (Fig. 6). They contained empty round vesicles in their luminal spaces.

The granular endoplasmic reticulum (GER) was localized under the nucleus (N) in the basal cytoplasm (Figs. 4 and 5). The cisternae of the granular endoplasmic reticulum were slightly distended (Fig. 5). The Golgi complex was not observed in any of the preparations. The cytoplasmic matrix was filled with free ribosomes (R) surrounding vacuoles, mitochondria and granular endoplasmic reticulum, either grouped or scattered (Fig. 7). Occasionally amoebocytes were present in preparations of the connective tissue.

The apical cytoplasm showed evidence of slight secretory activity. Small vesicles (long black bold arrows) occupied the upper cytoplasm adjacent to the base of the microvilli and were scattered between the type one vesicles, mitochondria and multivesicular bodies (Figs. 2 and 6). In contrast, the basal cytoplasm did not show evidence of secretory activity.

Scanning electron micrographs showed the surface of the outer mantle epithelium with the microvilli covered by an irregular layer of material. This material was dried extrapallial fluid (EF) which evenly covers the microvilli (Fig. 8). Sometimes the layer appeared in patches, corresponding to the surface cells; the junctions marking

adjacent cells were clearly visible (Fig. 9). Rarely, the complete microvilli (MV) surface was found to be clear of extraneous material. Occasionally a few secretory blebs (SB) were seen between the cells (Figs. 9 and 10).

B- Histological and ultrastructural changes in the outer mantle epithelium following nucleus implantation

The outer mantle epithelial cells were structurally modified during the secretory process of pearl and nacre formation. The primary modifications occured before nacre was deposited. Nacre deposition occured eight weeks following the implantation of the nucleus. After the 14th week of observation, epithelial cells did not show further major ultrastructural changes.

The mantle tissue was modified to the naked eye. The delineation of the implanted nucleus occured in the mantle epithelium. During the 1st week, this area was not conspicuosly defined but uniformly creamy yellowish. From the 2nd to the 14th week, the border of the implanted nucleus area was clearly delineated in the tissue by a golden brown circular line. This zone corresponded to the mantle membrane that lies underneath the zone of contact between the shell and the implanted nucleus. The inside area corresponded to the mantle tissue that covered the implanted nucleus and was yellowish brown in color.

B. 1- The first and second weeks

During the first two weeks following nucleus implantation the most obvious modification was the lengthening of the cells. The outer mantle epithelial cells became elongated but they retained their columnar shape. The mean height of cells was 40 µm. and the mean width 5 µm. (Figs. 11 and 12). A second morphological change was the appearance of additional mitochondria. Mitochondria (M) were concentrated particularly in the apical and basal cytoplasm (Figs. 13-15). These mitochondria (M) were often distributed among the granular endoplasmic reticulum and type one vesicles (Figs. 13-15).

Fig. 8- SEM of the outer mantle epithelium surface of *Haliotis kamtschatkana* covered by an irregular layer of extrapallial fluid (EF). Abbreviations used are: sp, sperm. Scale bar= 10 μm.

Fig. 9- SEM of the microvilli of the outer mantle epithelium surface. Note the presence of secretory blebs (long black bold arrows). Abbreviations used are: MV, microvilli. Scale bar= 10 μm.

Fig. 10- SEM of secretory blebs. Abbreviations used are: SB, secretory blebs; EF, extrapallial fluid; MV, microvilli. Scale bar= 10 μm.

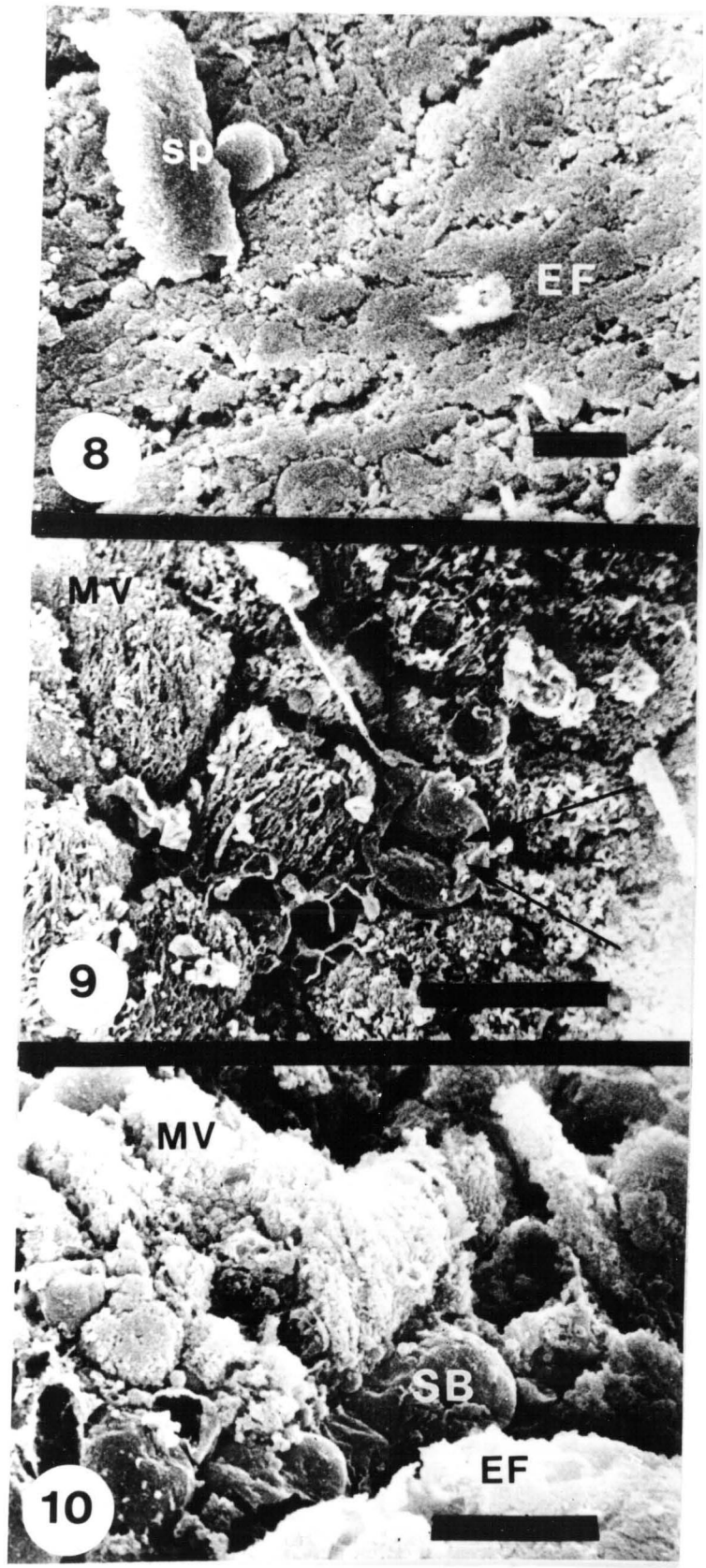

sp
EF
8
MV
9
MV
SB
EF
10

Fig. 11- Longitudinal section of the outer mantle epithelium of *Haliotis kamtschatkana* at the end of one week following nucleus implantation, showing the elongation of cells. Abbreviations used are: OME, outer mantle epithelium; N, nucleus; CT, connective tissue. Scale bar= 10 μm.

Fig. 12- Longitudinal section of the outer mantle epithelium of *Haliotis kamtschatkana* in the 2nd week following nucleus implantation. The cells surface shows an active secretion. Abbreviations used are: OME, outer mantle epithelium; CT, connective tissue; secretory blebs, white bold arrows. Scale bar= 10 μm.

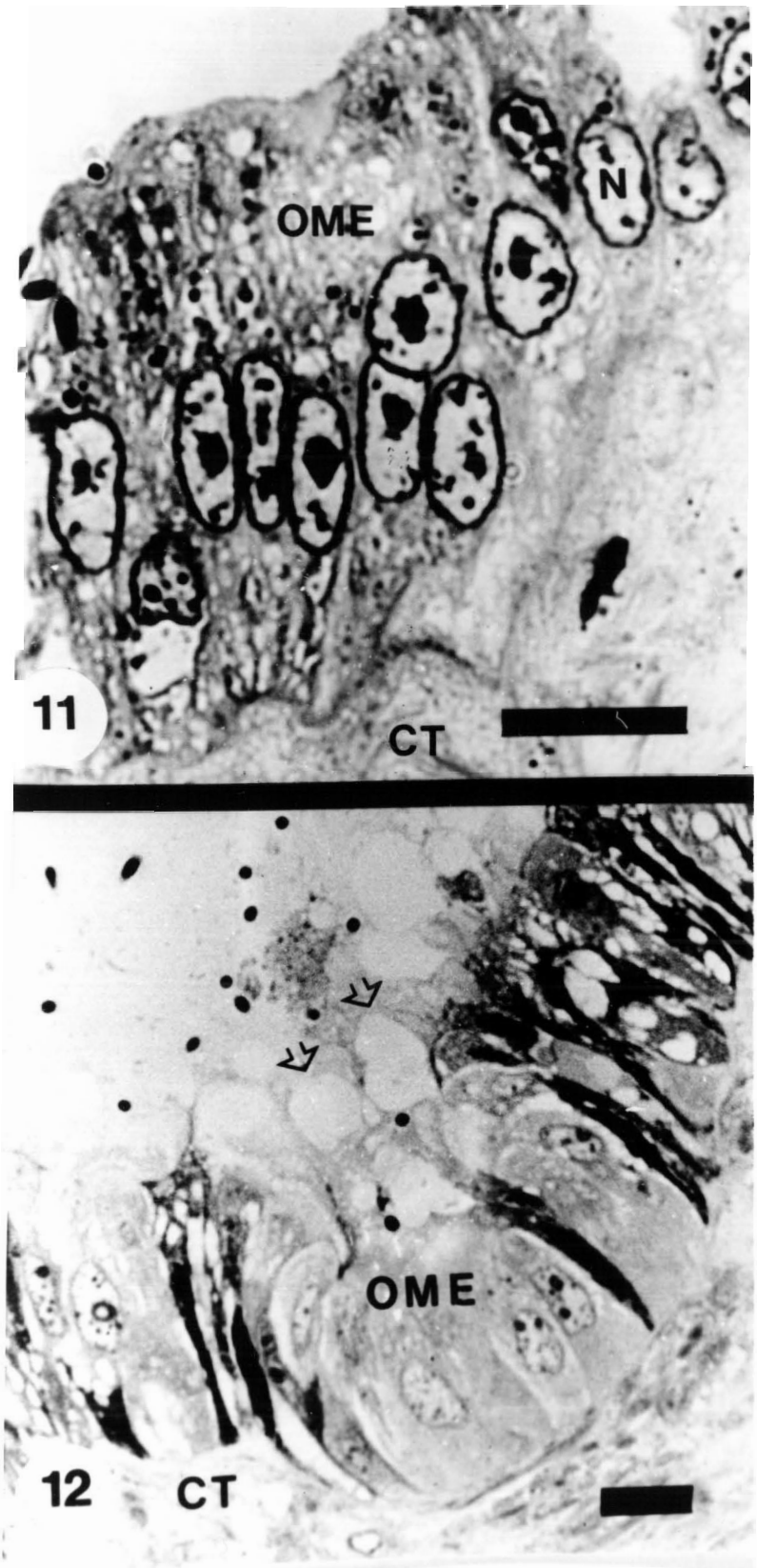
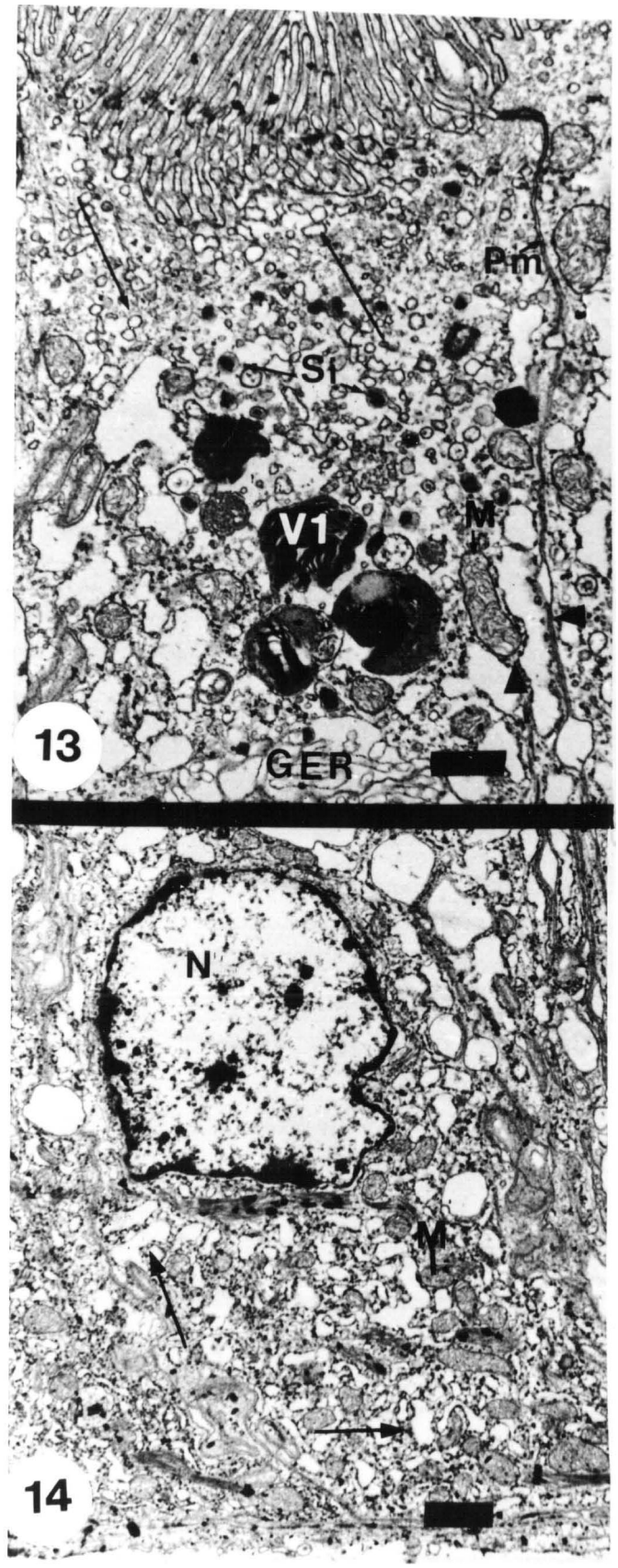

N
OME
11
CT
OME
12
CT

Fig. 13- TEM-section of the apical cytoplasm of the outer mantle epithelium of *Haliotis kamtschatkana* in the 1st week following nucleus implantation. Note the granular endoplasmic reticulum (GER) distributed near the plasma membrane (Pm; black bold point) and mitochondria M; black bold point). Abbreviations used are: small vesicles, long black arrows; Si, secretory inclusions; V1, type one vesicles. Scale bar= 1 μm.

Fig. 14- TEM-section of the basal cytoplasm of the outer mantle epithelium in the 1st week following nucleus implantation. Note the large amount of granular endoplasmic reticulum (long black bold arrows). Abbreviations used are: N, nucleus; M, mitochondria. Scale bar= 1 μm.


Pm
SI
V1
M
13
GER
N
M
14

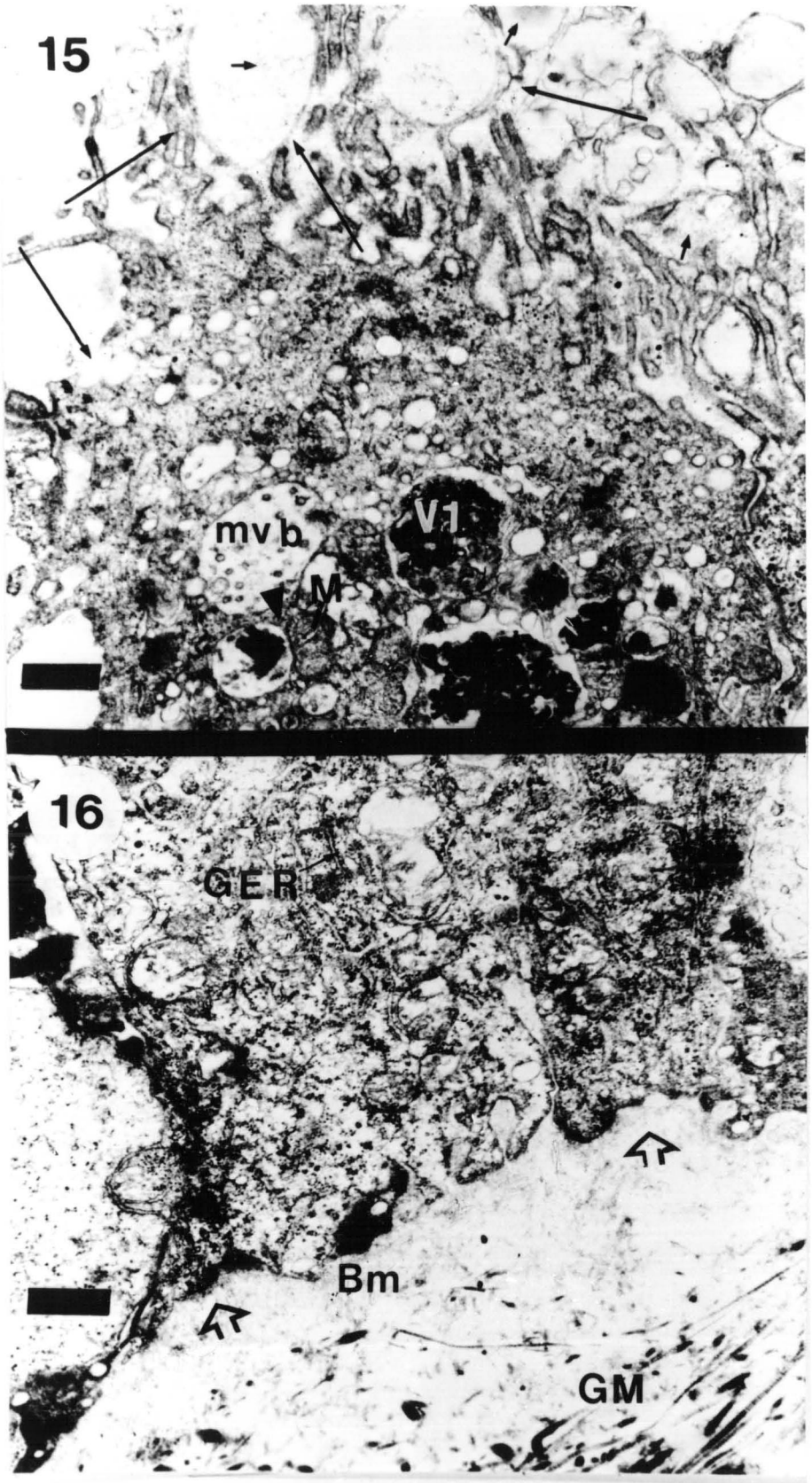
Additional granular endoplasmic reticulum (GER, long black bold arrows) (Fig. 13 and 14) appeared to occur in the cells. The cisternae of the granular endoplasmic reticulum were generally widened and convoluted (Figs. 13 and 14). They were distributed throughout the apical and basal cytoplasm. Occasionally, the granular endoplasmic reticulum cisternae were localized near the plasma membrane (Pm, bold point) (Fig. 13). Another obvious change was the appearance of secretory inclusions (Si) during the 1st week (Fig. 13). These secretory inclusions were sparsely scattered throughout the apical cytoplasm with the largest quantity situated below the microvilli. The inclusion had a dark, electron dense composition.

Light microscope preparations from week two revealed some golden brown vesicles above the nucleus in the apical cytoplasm. The observation of the same section in TEM showed type one vesicles (V1) (Fig. 13). Frequently, a few large type one vesicles (V1) were distributed among mitochondria and vacuoles (Fig.15). These vesicles had a mean diameter of 1.50-3.00 μm.. The type one vesicles content appeared amorphous and membranous with different dark electron density. Multivesicular bodies (mvb) were observed among the large type one vesicles and below the microvilli in the apical cytoplasm (Fig. 15). Another change in the mantle tissues was the presence of secretory cells in week two. Although, secretory cells were distributed rarely along the mantle epithelium.

Apparently, secretory activity was observed in the epithelial surface. Light micrographs displayed secretory blebs (white bold arrows) on the cell surface (Fig. 12). Electron micrographs revealed numerous swollen microvilli, secretory blebs and small vesicles fused with the cell membrane (long black bold arrows) (Fig. 15). Secretory blebs that contain light electron dense material (short black bold arrows) (Fig. 15) appeared to detach from the microvilli surface. Many small vesicles and secretory droplets which contained electron dense material were continuously present in later weeks. Electron photo-micrographs of the basal membrane revealed that this structure continued to be fairly straight, a few short interdigitations

Fig. 15- TEM-section of the apical cytoplasm of the outer mantle epithelial cells of *Haliotis kamtschatkana* in the 2nd week following nucleus implantation. Note the active secretion (long black bold arrows) and the different content (short black bold arrows) in the secretory blebs. Abbreviations used are: mvb, multivesicular bodies; M, mitochondria; mitochondrion distributed near type one vesicles, black bold point; V1, type one vesicle. Scale bar= 0.6 μm.

Fig. 16- Basal membrane of the outer mantle epithelium in the 2nd week following nucleus implantation. Abbreviations used are: Bm, basal membrane; GER, granular endoplasmic reticulum; basal interdigitations, white bold arrows; GM, granular matrix. Scale bar= 0.6 μm.


15
mvb
V1
M
16
GER
Bm
GM

(white bold arrow) (Fig. 16). Occasionally amoebocytes were observed in the connective tissue.

The materials secreted in the shell formation appeared to be deposited in the sequence described by Fankboner (1991). The first material deposited was a layer of calcium carbonate, followed by conchiolin, next a layer of aragonite crystals and a final sheet of nacreous aragonite. The shell areas covered by the different materials secreted appeared to follow a sequence. The first areas with deposits were the right forward edge and the spire of the inner shell, followed by the base of the implanted nucleus and finally the implanted nucleus itself.

During the 1st and the 2nd weeks a thin whitish layer of calcium carbonate was deposited. At the end of week two this layer was deposited over the right forward edge, the spire, the base of the implanted nucleus and the nucleus itself.

B. 2- The third, fourth and fifth weeks

During the 3rd, 4th and 5th weeks following nucleus implantation, there appeared to be active secretion. The mantle epithelial cells became further elongated with a mean height and width of 60 µm. and 5 µm. respectively (Figs. 17-20). During weeks three and four the cells were tightly packed which made the plasma membrane, plus belt desmosomes and septate junctions of adjacent cells difficult to resolve. In the 5th week however, these same junctions of the plasma membrane reappeared. The plasma membrane was slightly convoluted. Intercellular spaces were difficult to observe.

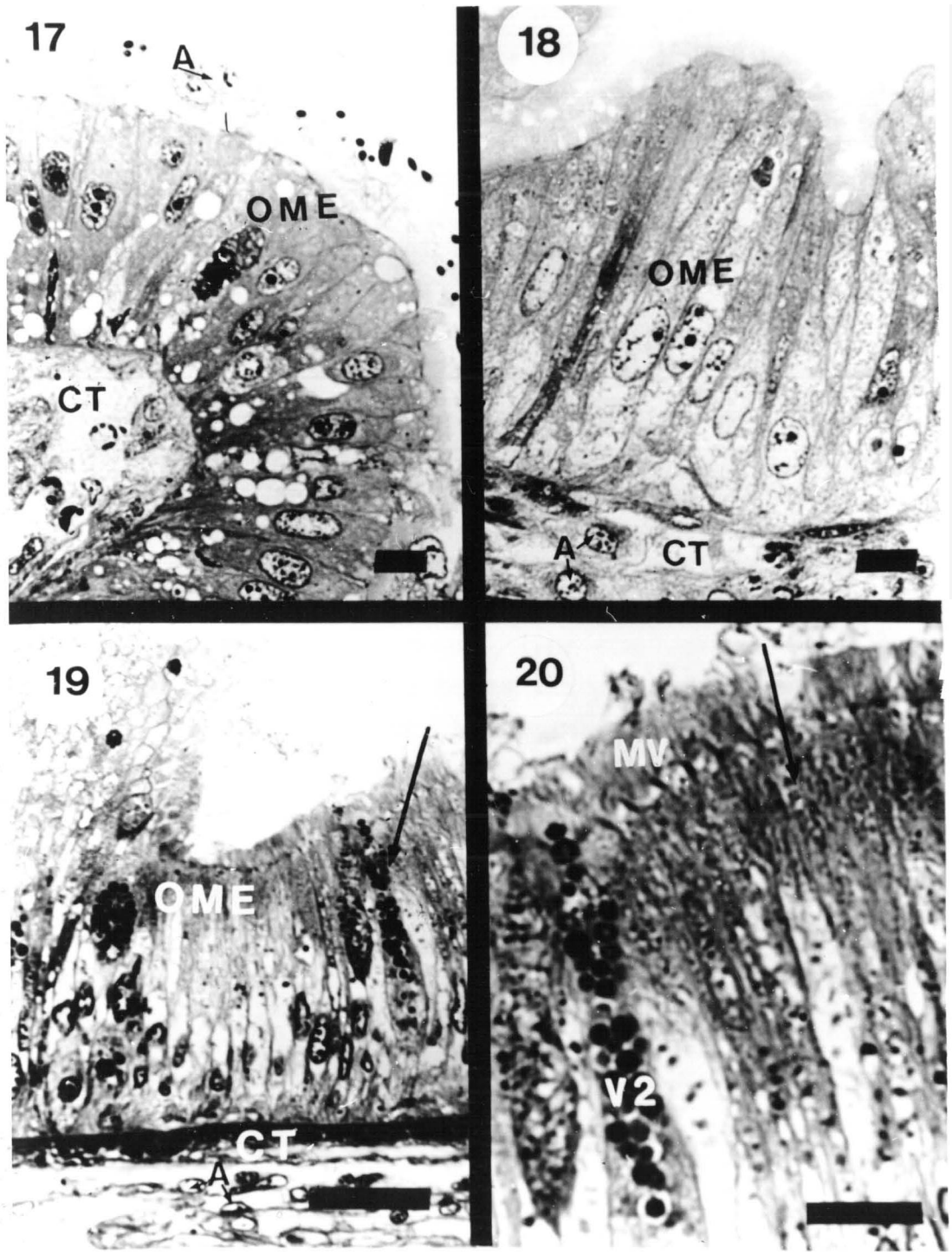
The first obvious modification compared with previous weeks was seen in the basal cell membrane (Bm), which appeared to have additional basal interdigitations (short black bold arrows) (Fig. 21). Numerous small vesicles (long black bold arrows) were densely distributed in the basal cytoplasm (Fig. 21).

Fig. 17- Light micrograph of the outer mantle epithelium of *Haliotis kamtschatkana* in the 3rd week following nucleus implantation. Note the elongation of the cells. Abbreviations used are: OME, outer mantle epithelium; CT, connective tissue; A, amoebocytes. Scale bar= 10 μm.

Fig. 18- Longitudinal section of the outer mantle epithelium of *Haliotis kamtschatkana* in the 4th week following nucleus implantation showing the tightness of the cells. Abbreviations used are: OME, outer mantle epithelium; CT, connective tissue; A, amoebocytes. Scale bar= 10 μm.

Fig. 19- Light micrograph of the outer mantle epithelium in the 5th week following nucleus implantation. Note the presence of cells containing type II vesicles (long black bold arrow) and the numerous amoebocytes (A) in the connective tissue. Abbreviations used are: OME, outer mantle epithelium; CT, connective tissue. Scale bar= 20 μm.

Fig. 20- Light micrograph of the outer mantle epithelium of *Haliotis kamtschatkana* in the 5th week following nucleus implantation. Higher magnification of cells showing type two vesicles (V2) and the high density of type one vesicles (long black bold arrow). Abbreviations used are: MV, microvilli. Scale bar= 10 μm.


17
A
OME
CT
18
OME
A
CT
19
OME
CT
A
20
MV
V2

A second noticeable change in the mantle cells is the appearance of additional vacuoles. Differently sized vacuoles (V) were widely distributed throughout the cytoplasm (Figs. 22-24). They were empty or contained two, or more superimposed lamellae (Fig. 24). Within the apical cytoplasm, vacuoles (V) were often distributed among mitochondria (M) (Fig. 22).

As in preparations found in earlier weeks, there were a large number of mitochondria. Mitochondria (M) were densely distributed throughout the cell (Figs. 22 and 25). Sometimes, extremely long slender mitochondria of 1.6 μm. or more in length were seen lying close to the lateral cell membrane, oriented parallel to the long axis of the cell (Fig. 25).

Another change in the cell cytoplasm was the appearance of additional type one vesicles especially in the 5th week. During the 3rd week, these type one vesicles were distributed above the nucleus. In the 4th and 5th weeks, type one vesicles were observed mainly in the apical cytoplasm below the microvilli. A few large type one vesicles were observed. Light micrographs of these weeks respectively showed a golden brown color due to the large quantity of type one vesicles (long black bold arrows) (Fig. 20). These type one vesicles (V1) were found among mitochondria (M, long black bold arrow) (Figs. 23 and 26). A few secretory inclusions (Si) were distributed in the apical cytoplasm between mitochondria and vacuoles (V) (Fig. 22). The secretion continued to be active. Secretory droplets were observed in the mantle surface and small vesicles below the microvilli in the apical cytoplasm.

A further change occuring in the cell cytoplasm was the presence of type two vesicles (V2) (Figs. 20 and 27). The type two vesicles displayed a light blue color after staining with toluidine blue and preparations were unstained with the Periodic Acid Schiff reaction. The type two vesicles were distributed in the apical cytoplasm below the microvilli or throughout the cytoplasm (Fig. 20). When the latter situation occurred, the nucleus was seen in a new location which was adjacent to the basal cytoplasm. Secretory cells

Fig. 21- Basal cytoplasm of the outer mantle epithelium from *Haliotis kamtschatkana* in the 3rd week following nucleus implantation. Note the numerous small vesicles (long black bold arrows). Abbreviations used are: Bm, basal membrane; basal membrane interdigitations, short black bold arrows. Scale bar= 1 μm.

Fig. 22- TEM-section of the apical cytoplasm of *Haliotis kamtschatkana* in the 3rd week following nucleus implantation. Note the appearance of additional vacuoles (V) and mitochondria (M). Abbreviations used are: Si, secretory inclusions; GR, glycogen rosettes. Scale bar= 1 μm.

Fig. 23- Central cytoplasm of the outer mantle epithelial cells in the 4th week following implantation. Abbreviations used are: GC, Golgi complex; M, mitochondria; V, vacuoles; V1, type one vesicles; R, ribosomes. Scale bar= 0.5 μm.

Fig. 24- Golgi complex and vacuoles containing inclusions of the outer mantle epithelial cells in the 4th week following nucleus implantation. Abbreviations used are: GC, Golgi complex; V, vacuoles; vacuoles containing lamellae, long black bold arrow. Scale bar= 0.5 μm

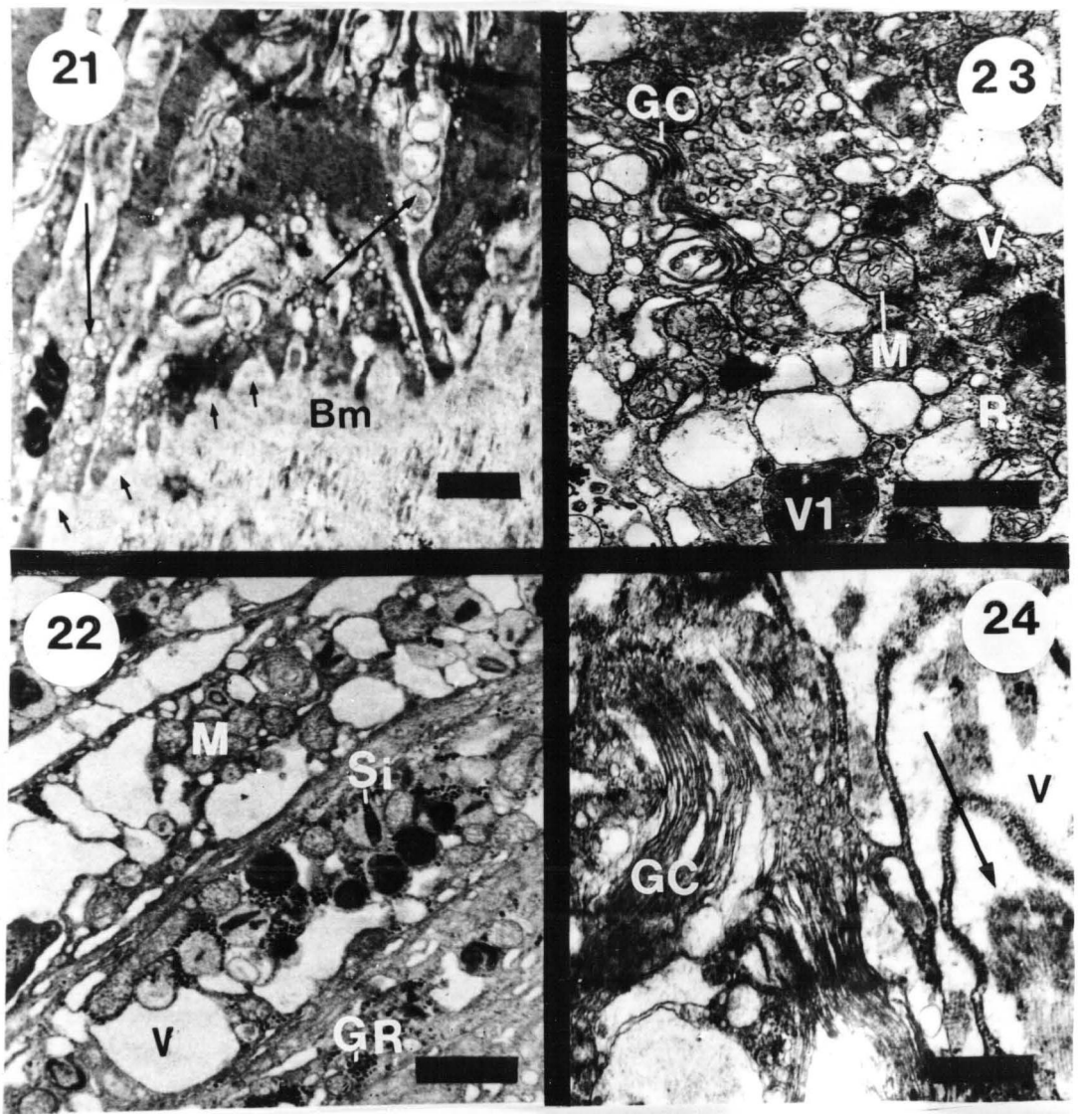
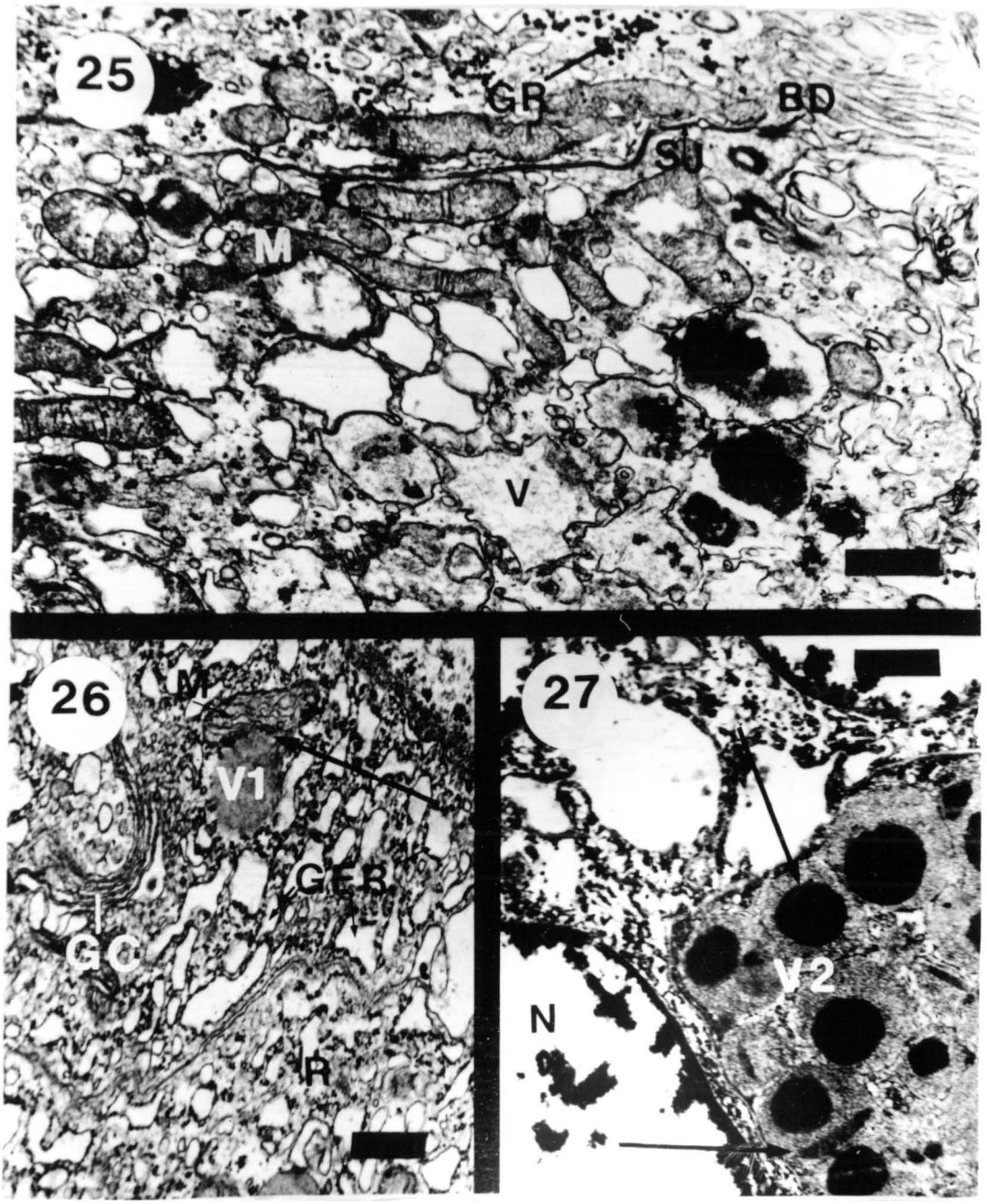

21
Bm
23
GC
V
M
R
V1
22
M
Si
V
GR
24
V
GC

Fig. 25- Apical cytoplasm of the outer mantle epithelial cells from *Haliotis kamtschatkana* in the 5th week following nucleus implantation. Note the elongated mitochondria (M) beside the plasma membrane. Abbreviations used are: V, vacuole; BD, belt desmosomes; SJ, septate junctions; GR, glycogen rosettes. Scale bar= 1 μm.

Fig. 26- TEM-section of the central cytoplasm of outer mantle epithelial cells in the 5th week following nucleus implantation. Abbreviations used are: GC, Golgi complex; GER, granular endoplasmic reticulum; mitochondria (M) distributed near type one vesicle (V1), long black bold arrow; R, ribosomes. Scale bar= 0.5 μm.

Fig. 27- TEM-section of type two vesicles of the outer mantle epithelium in the 5th week following implantation. Note the round and ovoid electron dense inclusions (long black bold arrows) in type two vesicle (V2). Abbreviations used are: N, nucleus. Scale bar= 1 μm.


25
GB
BD
SJ
M
V
26
M
V1
GER
GC
R
27
V2
N

were rarely observed in the mantle epithelium in the various preparations.

The appearance of multivesicular bodies remained unchanged, the same as week one. The granular endoplasmic reticulum (GER) (Fig. 26) appeared swollen and twisted when view in profile. It was concentrated in the perinuclear and basal cytoplasm. Fibrous material was present in several preparations, within their cisternae. The Golgi complex (GC) was normally located adjacent to the nucleus or in the apical cytoplasm (Figs. 23 and 26). The cisternae of the Golgi complex were oriented vertically, and the detached Golgi sacs were oriented towards the apical secretory surface. Numerous ribosomes (R) were densely clustered between the different organelles in the supranuclear, perinuclear and basal cytoplasm (Fig. 26). Large quantities of glycogen rosettes (GR) appeared in the cytoplasm particularly in preparations taken in the 5th week following nucleus implantation (Figs. 22 and 25). The distribution of these granules was clustered throughout the cytoplasm.

During the 3rd and 4th week, a very thin and nonhomogeneous layer of conchiolin appeared on the inner shell surface. This layer covered the base of the implanted nucleus and extended towards the spire and the right forward edge of the shell. By the 5th week, conchiolin was found deposited evenly over the spire, the right forward edge of the inner shell and the implanted nucleus.

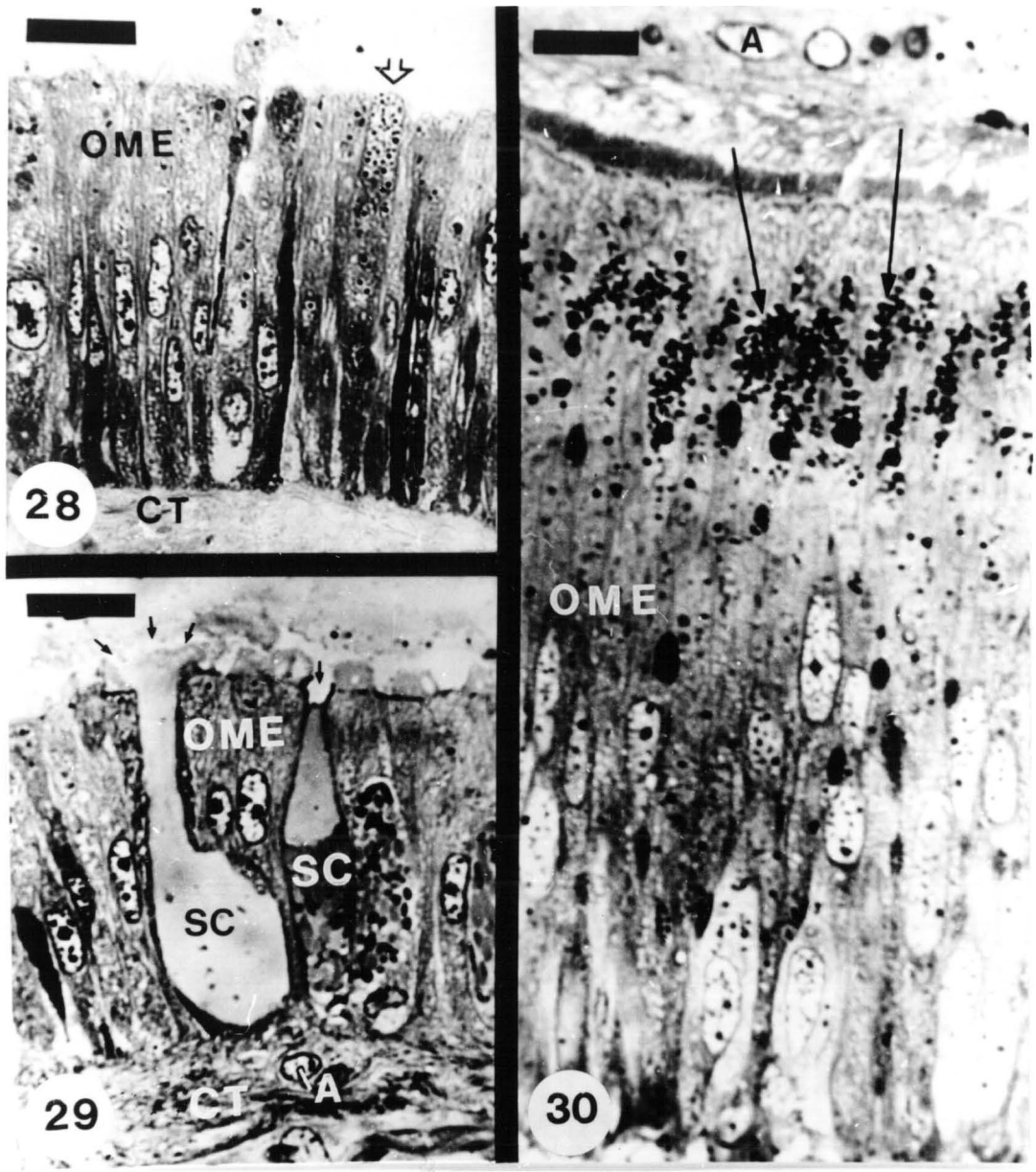
B. 3- The sixth and seventh weeks

During the 6th and 7th weeks following nucleus implantation, the epithelial cells appeared larger in height and the secretory product was larger (Figs. 28-30). The average height was of 80 µm. and an average width of 5 µm. A first change in the cell cytoplasm was the appearance of fewer mitochondria, vacuoles and granular endoplasmic reticulum. Mitochondria (M) (Fig. 31) were unevenly distributed in the apical and basal cytoplasm. Some vacuoles contained membranous inclusions and were distributed through all the cell cytoplasm, especially surrounding the nucleus. The granular

Fig. 28- Longitudinal section of the outer mantle epithelium from *Haliotis kamtschatkana* in the 6th week following nucleus implantation showing cells that contain type two vesicles. Abbreviations used are: OME, outer mantle epithelium; CT, connective tissue; cell containing type two vesicles, white bold arrow. Scale bar= 20 μm.

Fig. 29- Secretory cells of the outer mantle epithelium of *Haliotis kamtschatkana* in the 6th week following nucleus implantation. Note the rupture of the cell surface membrane and the release of secretory vesicles (short black bold arrows). Abbreviations used are: OME, outer mantle epithelium; CT, connective tissue; SC, secretory cell; A, amoebocytes. Scale bar= 20 μm.

Fig. 30- Longitudinal section of the outer mantle epithelium in the 7th week following nucleus implantation. Note the high density of type one vesicles in the upper cytoplasm (long black bold arrows). Abbreviations used are: OME, outer mantle epithelium; A, amoebocytes. Scale bar= 20 μm.


OME
28
CT
OME
SC
SC
29
CT
A
A
OME
30

endoplasmic reticulum was localized in the perinuclear and basal cytoplasm. As in previous weeks, the Golgi complex (GC) was found in the apical cytoplasm with the concave border of the cisternae apically oriented (Fig 32). As in earlier weeks, Golgi vesicles appeared to detach from the end of the cisternae and were oriented towards the surface (short black bold arrows) (Fig. 32). Several Golgi vesicles appeared to coalesce forming vacuoles.

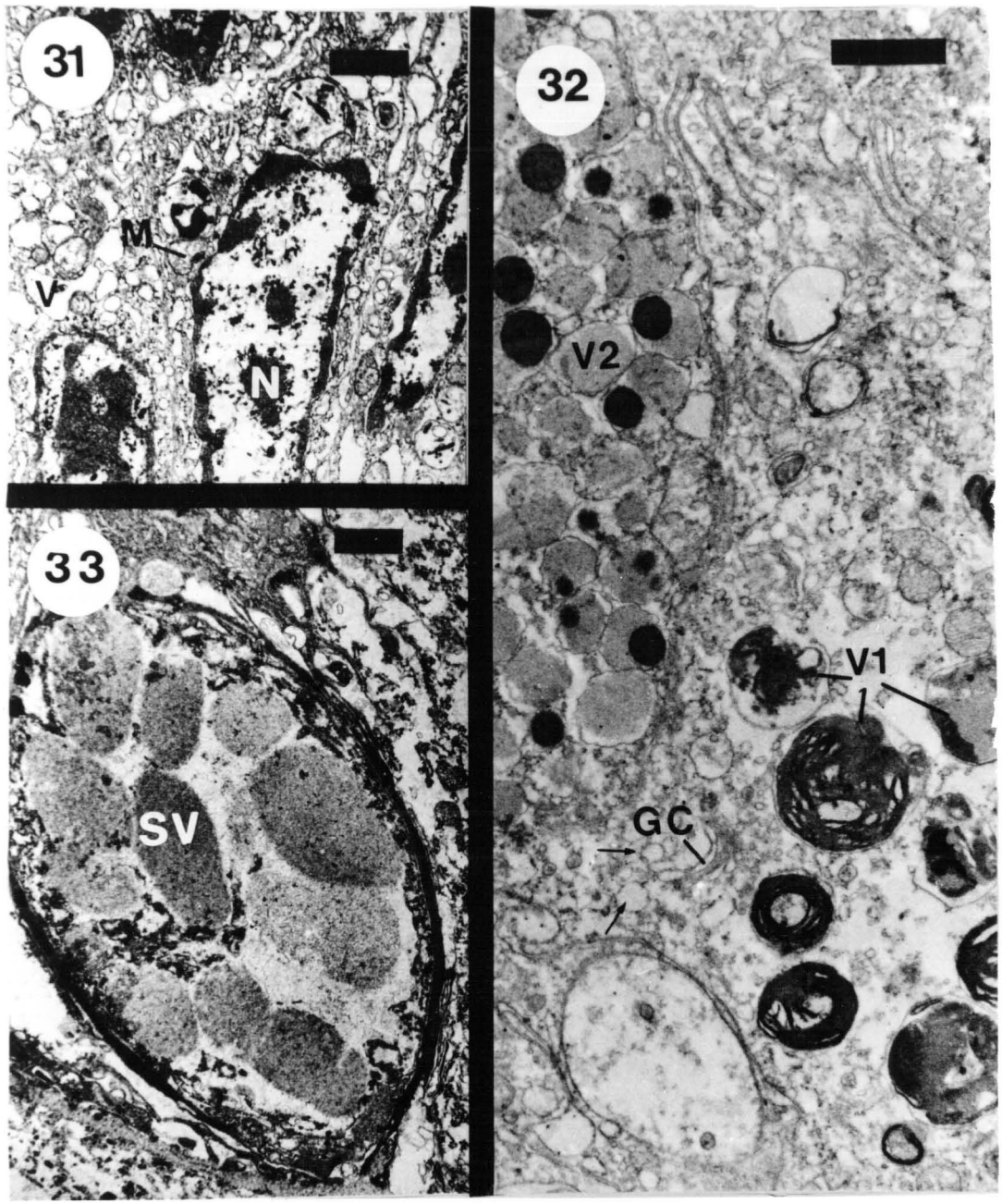
The second change in the mantle tissue was the appearance of additional cells that contained type two vesicles (white bold arrow) (Fig. 28). These cells were randomly distributed in the outer mantle epithelium. Cells which contained type one vesicles (long black bold arrows) (Fig. 30) were observed. These vesicles were localized in the apical cytoplasm. The vesicle content was membranous and resembled myelin figures (Fig. 32). Additional large type one vesicles were also observed in the apical cytoplasm. As seen weeks before, the epithelial cells showed a golden brown color when examined by light microscopy.

Another noticeable modification in the outer mantle epithelium (OME) was the appearance of additional secretory cells (SC) (Fig. 29). In the electron micrographs, secretory cells were usually seen to be tightly packed with slightly different electron dense secretory vesicles (SV) (Fig. 33). On higher magnification a membrane that surrounded them become visible. The ovoid secretory vesicles had a mean diameter of 1-3 μm (Fig. 33). Generally, the secretory vesicles occupied the entire cell in which the nucleus was basally localized. The secretory vesicles appeared dark pink after staining with toluidene blue and the preparations reacted positively with the Periodic Acid Schiff reaction. These results indicated the presence of polysaccharides. In light micrographs of the 6th week, the release of the secretory vesicles (short black bold arrows) (Fig. 29) was observed. The cell membrane appeared to rupture and the vesicles were poured to the exterior in a continues mass that covered the outer mantle epithelium surface (Figs. 29).

Fig. 31- Cross section of the outer mantle epithelial cells in the 6th week following nucleus implantation. Abbreviations used are: N, nucleus; M, mitochondria; V, vacuole. Scale bar= 1 μm.

Fig. 32- Golgi complex of the outer mantle epithelial cells in the 6th week following nucleus implantation. Golgi complex (GC) is apico-lateral oriented and small Golgi vesicles detach from the end (short black bold arrows). Abbreviations used are: V1, type one vesicles; V2, type two vesicles. Scale bar= 1 μm.

Fig. 33- TEM-section of a secretory cell of the outer mantle epithelium from *Haliotis kamtschatkana* in the 7th week following nucleus implantation. Abbreviations used are: SV, secretory vesicles. Scale bar= 1 μm.


31
M
V
N
32
V2
V1
GC
33
SV

During the 6th week there was no apparent modification of shell deposition. The implanted nucleus was covered completely with conchiolin. But in the 7th week a small white spot of aragonite crystals were deposited near the base of the implanted nucleus.

B. 4- The eighth and ninth weeks

During the 8th week and 9th week following nucleus implantation, the height of the cells had diminished. The mantle epithelial cells showed a mean height of 70 µm and width of 5 µm. respectively (Figs. 34 and 35). A first change in the epithelial cells was the appearance of additional type two vesicles (white bold arrow) (Fig. 34). The type two vesicles (V2) were distributed throughout the cytoplasm (Figs. 36 and 37). The round or elongate inclusion of type two vesicles had increased in size. Sometimes the inclusion appeared to occupy the whole vesicle or the central area. In the latter case, a luminal space with few electron dense particles was observed (V2; white bold arrow and bold point) (Fig. 37).

A second modification in the outer mantle epithelium (OME) was the appearance of additional secretory cells (SC) (Fig. 35). Secretory cells were observed one beside the other in groups of two or three cells (Fig. 35). Mantle epithelial cells still contained a large quantity of type one vesicles (long black bold arrow) (Figs. 34 and 35) that were mainly distributed in the apical cytoplasm below the microvilli. Electron micrographs showed various large type one vesicles. The content appeared to be tightly packed (long black bold arrow) (Fig. 38). These type one vesicles were often observed in the apical cytoplasm (black bold point) (Figs. 35).

There was no apparent change in the distribution of mitochondria (M) (Fig. 36), multivesicular bodies and granular endoplasmic reticulum in the mantle epithelial cells when compared with the preparations of the 6th and 7th weeks. The Golgi complex was not found. Glycogen rosettes were distributed in a large quantity throughout the cytoplasm, as were free ribosomes. The secretory

Fig. 34- Longitudinal section of the outer mantle epithelium of *Haliotis kamtschatkana* in the 8th week following nucleus implantation showing secretory cells, cells containing type one vesicles and cells containing type two vesicles. Abbreviations used are: SC, secretory cell; type one vesicle, long black bold arrow; large type one vesicle, black bold point; type two vesicles, white bold arrow; OME, outer mantle epithelium; CT, connective tissue; A, amoebocyte. Scale bar= 20 µm.

Fig. 35- Longitudinal section of the outer mantle epithelium in the 9th week following nucleus implantation showing the numerous distribution of type I vesicles and secretory cells. Abbreviations used are: OME, outer mantle epithelium; type one vesicle, long black bold arrow; SC, secretory cell; CT, connective tissue. Scale bar= 20 µm.

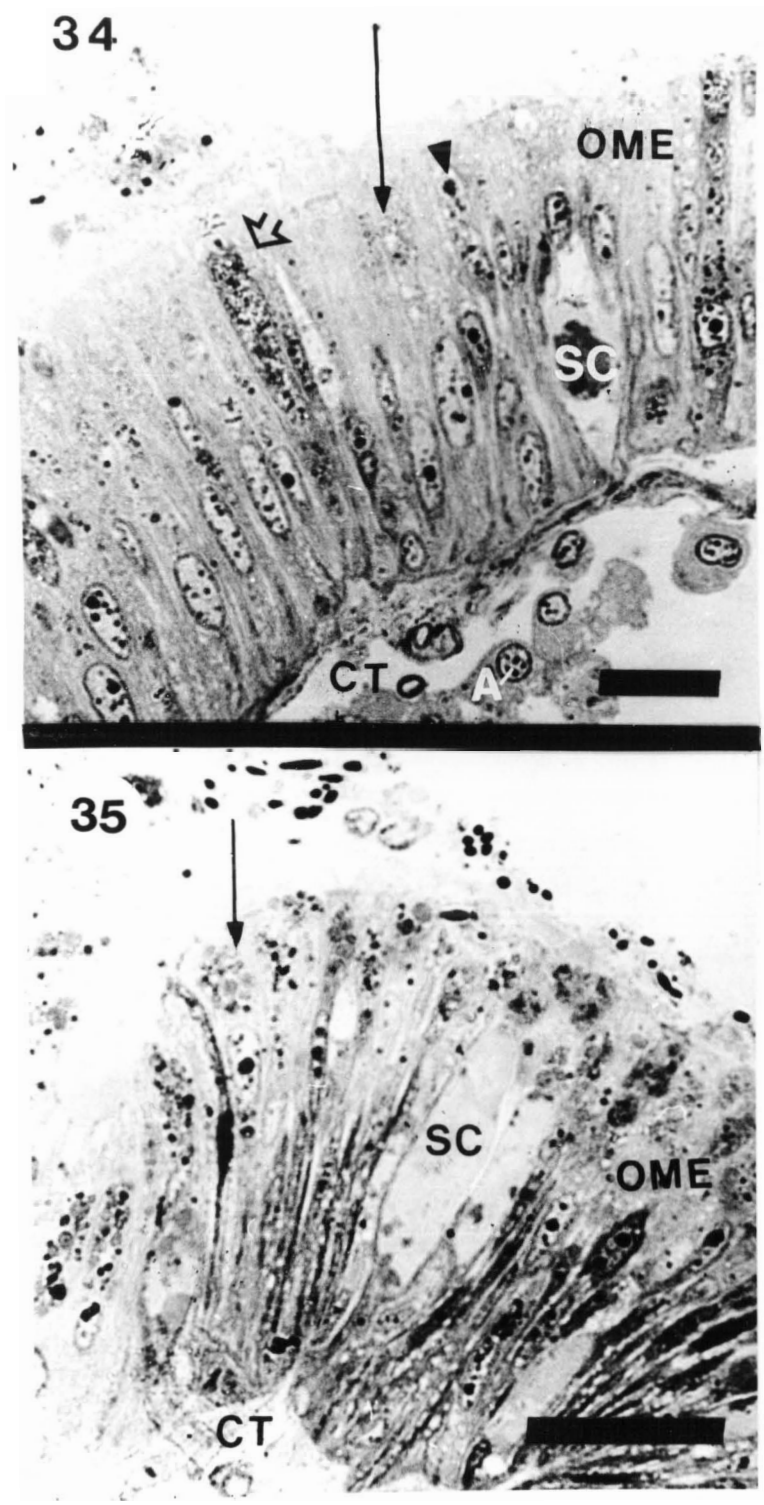
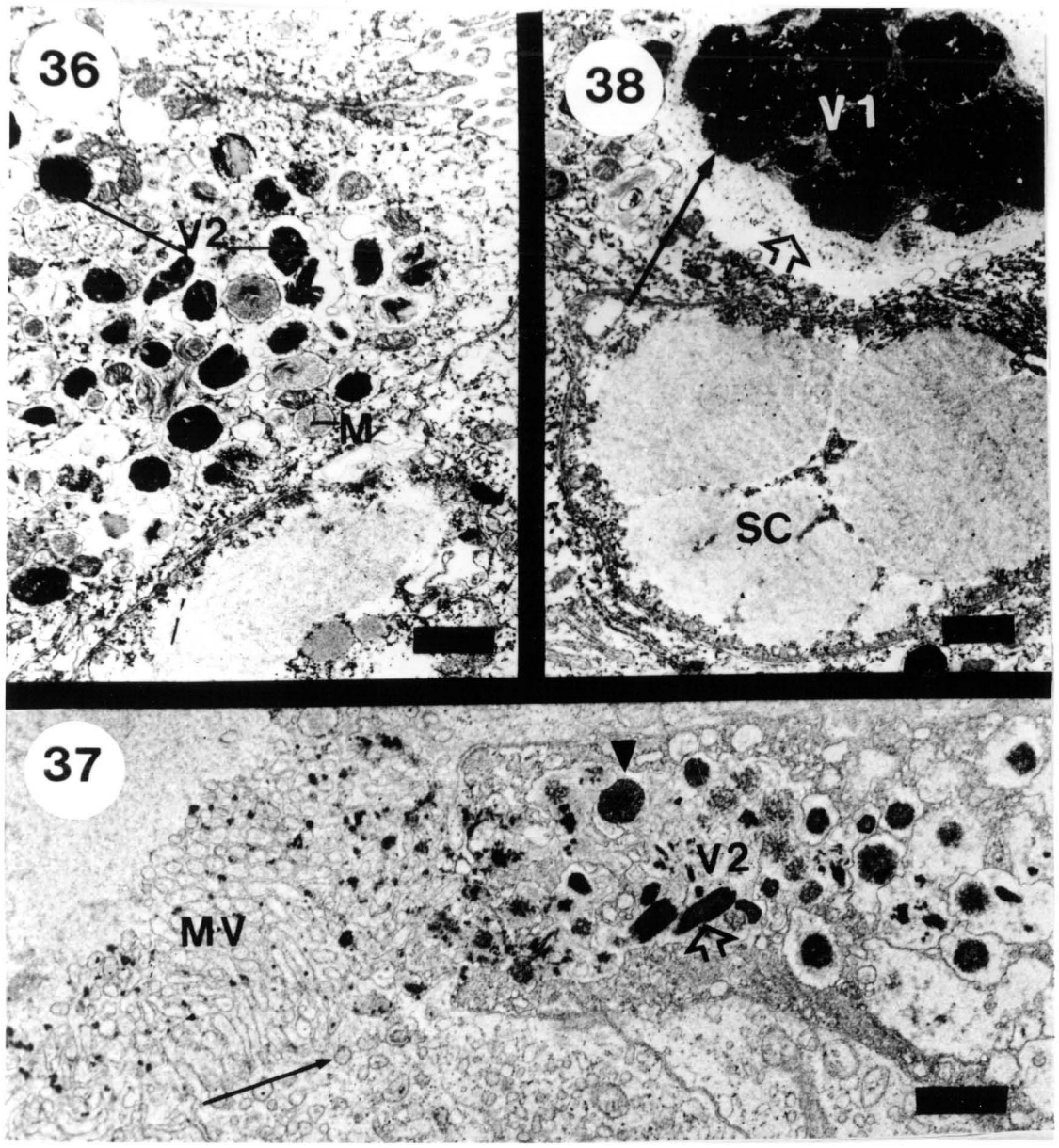

34
OME
SC
CT
A
35
SC
OME
CT

Fig. 36- TEM-section of the upper cytoplasm of the outer mantle epithelium in the 8th week following nucleus implantation. Abbreviations used are: V2, type two vesicle; M, mitochondria. Scale bar= 1 μm.

Fig. 37- TEM-section of the apical cytoplasm of the outer mantle epithelium in the 9th week following nucleus implantation. Note the circular (black bold point) or rod inclusion (white bold arrow) occupying the whole type two vesicle (V2). Abbreviations used are: MV, microvilli; small vesicles, long black bold arrow. Scale bar= 1 μm.

Fig. 38- TEM-cross section of the outer mantle epithelial cells of *Haliotis kamtschatkana* in the 8th week following nucleus implantation. Note the coalesce of several type one vesicles, (long black bold arrow) and the luminal space with electron dense particles (white bold arrow). Abbreviations used are: SC, secretory cell. Scale bar= 1 μm.


36
V2
M
38
V1
SC
37
MV
V2

activity in the apical surface continued. Numerous small vesicles were distributed in the apical cytoplasm.

The first deposition of nacre appeared on the 8th week on the right forward edge and the spire of the inner shell. In the 9th week, the conchiolin layer was covered with a thin homogeneous layer of aragonite crystals. Nacre depositions were secreted on the right forward edge of the inner shell.

B. 5- The tenth, eleventh, twelfth, thirteenth and fourteenth weeks

In the next five weeks from the 10th to the 14th week following nucleus implantation, the height of the cells remained as weeks eight and nine. The mantle epithelial cells height was about 60-70 µm (Figs. 39-42). At this stage additional secretory cells appeared in the mantle epithelium. The secretory cells (SC) were usually distributed side by side all along the mantle epithelium (Figs. 39-42). When this latter event did not occur, epithelial cells which contained type two vesicles (white bold arrow) and epithelial cells with type one vesicles (long black bold arrow) formed the rest of the outer mantle epithelium (OME) (Fig. 42). These cells were randomly distributed with the latter more numerous. Cross section electron micrographs showed the plasma membrane (Pm) highly convoluted (Fig. 43). As in previous weeks eight and nine, the basal cell membrane was remarkably convoluted with frequent basal interdigitations (black bold point) (Fig. 44). Several small vesicles were also observed above the basal cell membrane (long black bold arrow) (Fig. 44).

With regard to the cytoplasm morphology, no modification was observed from the 8th and 9th weeks. Mitochondria (M) were sparsely scattered throughout the apical and basal cytoplasm (Figs. 45 and 46). A few multivesicular bodies (mvb) were observed in the apical cytoplasm, below the microvilli (Figs. 45 and 46). The granular endoplasmic reticulum occured in the basal cytoplasm. Glycogen rosettes were distributed throughout the cytoplasm. Free ribosomes also appeared in the apical and basal cytoplasm. The Golgi complex

Fig. 39- Longitudinal section of the outer mantle epithelium of *Haliotis kamtschatkana* in the 10th week following nucleus implantation showing the appearance of additional secretory cells. Abbreviations used are: SC, secretory cell; OME, outer mantle epithelium; secreted secretory vesicles, short black bold arrows. Scale bar= 20 μm.

Fig. 40- Light micrograph of the outer mantle epithelium from *Haliotis kamtschatkana* in the 11th week following nucleus implantation. Abbreviations used are: OME, outer mantle epithelium; SC, secretory cell; type one vesicles, long black bold arrows; CT, connective tissue; A, amoebocytes. Scale bar= 20 μm.

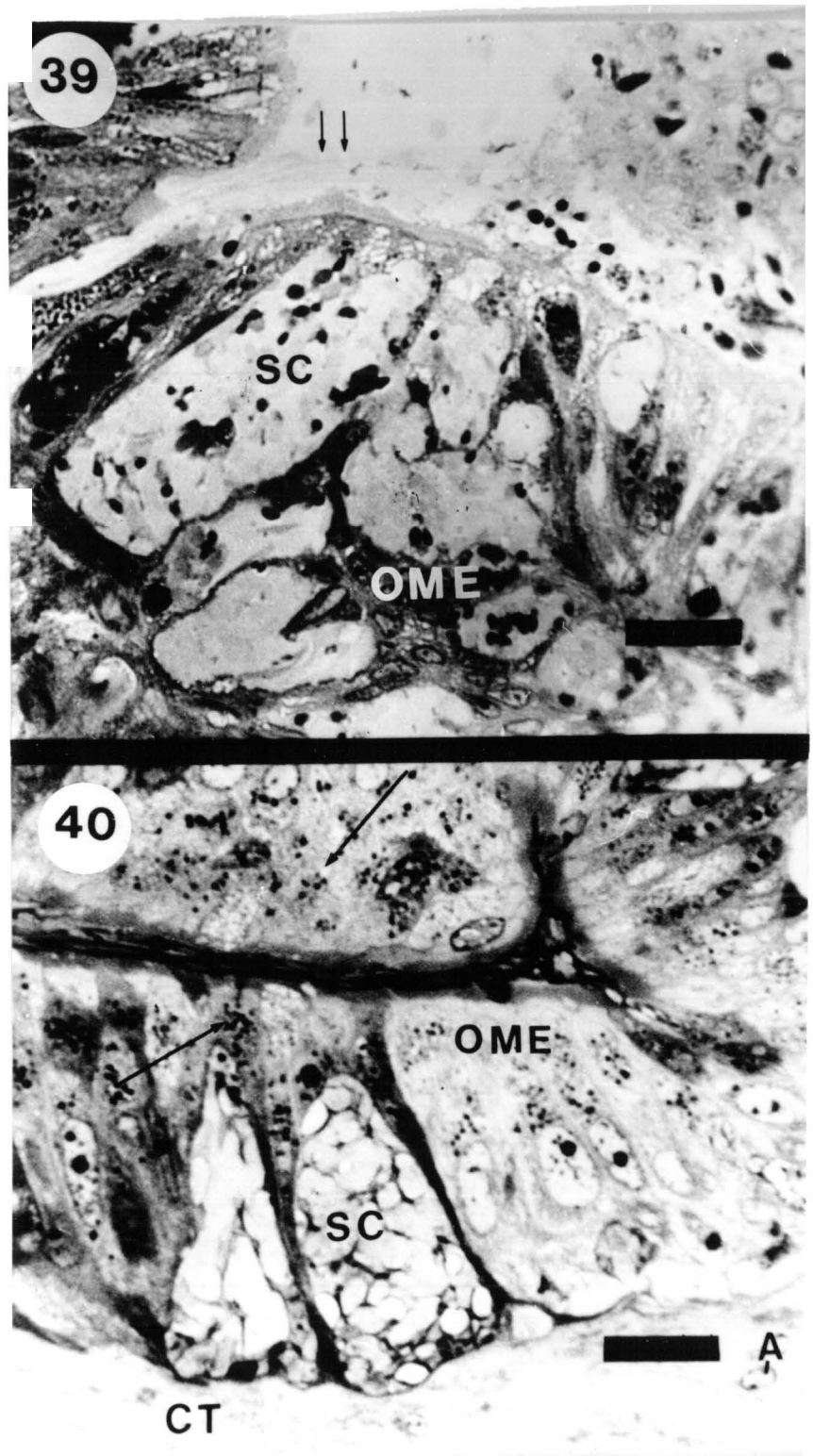

39
SC
OME
40
OME
SC
A
CT

Fig. 41- Longitudinal section of the outer mantle epithelium from *Haliotis kamtschatkana* in the 12th week following nucleus implantation. Abbreviations used are: OME, outer mantle epithelium; SC, secretory cell; release of secretory vesicles, black bold points; CT, connective tissue. Scale bar= 20 µm.

Fig. 42- Light micrograph of the outer mantle epithelium in the 13th week following nucleus implantation showing the distribution of secretory cells and cells containing type one vesicles and type two vesicles. Abbreviations used are: OME, outer mantle epithelium; SC, secretory cell; type one vesicles, long black bold arrow; type two vesicles, white bold arrows; CT, connective tissue; A, amoebocytes. Scale bar= 20 µm.

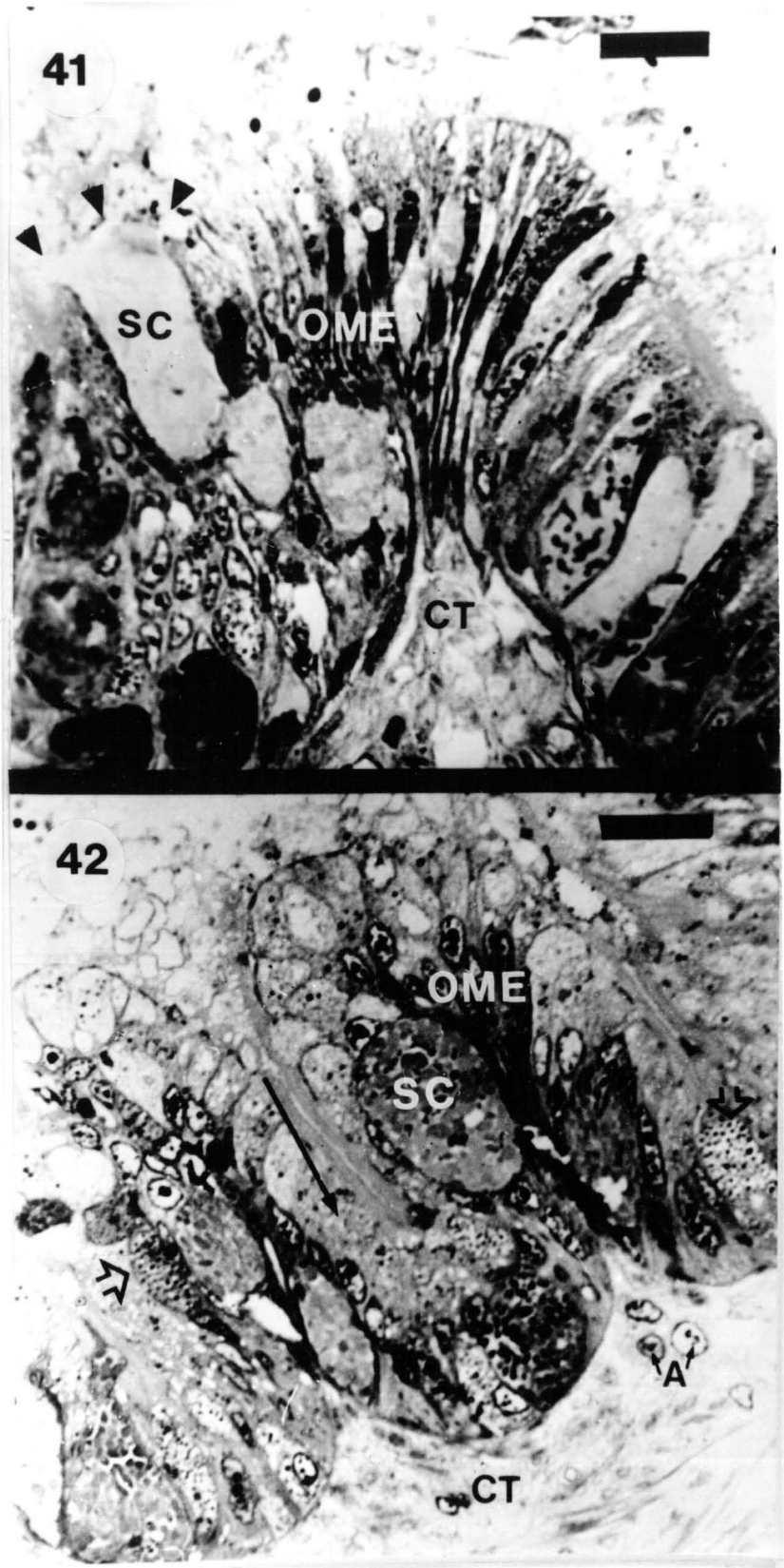
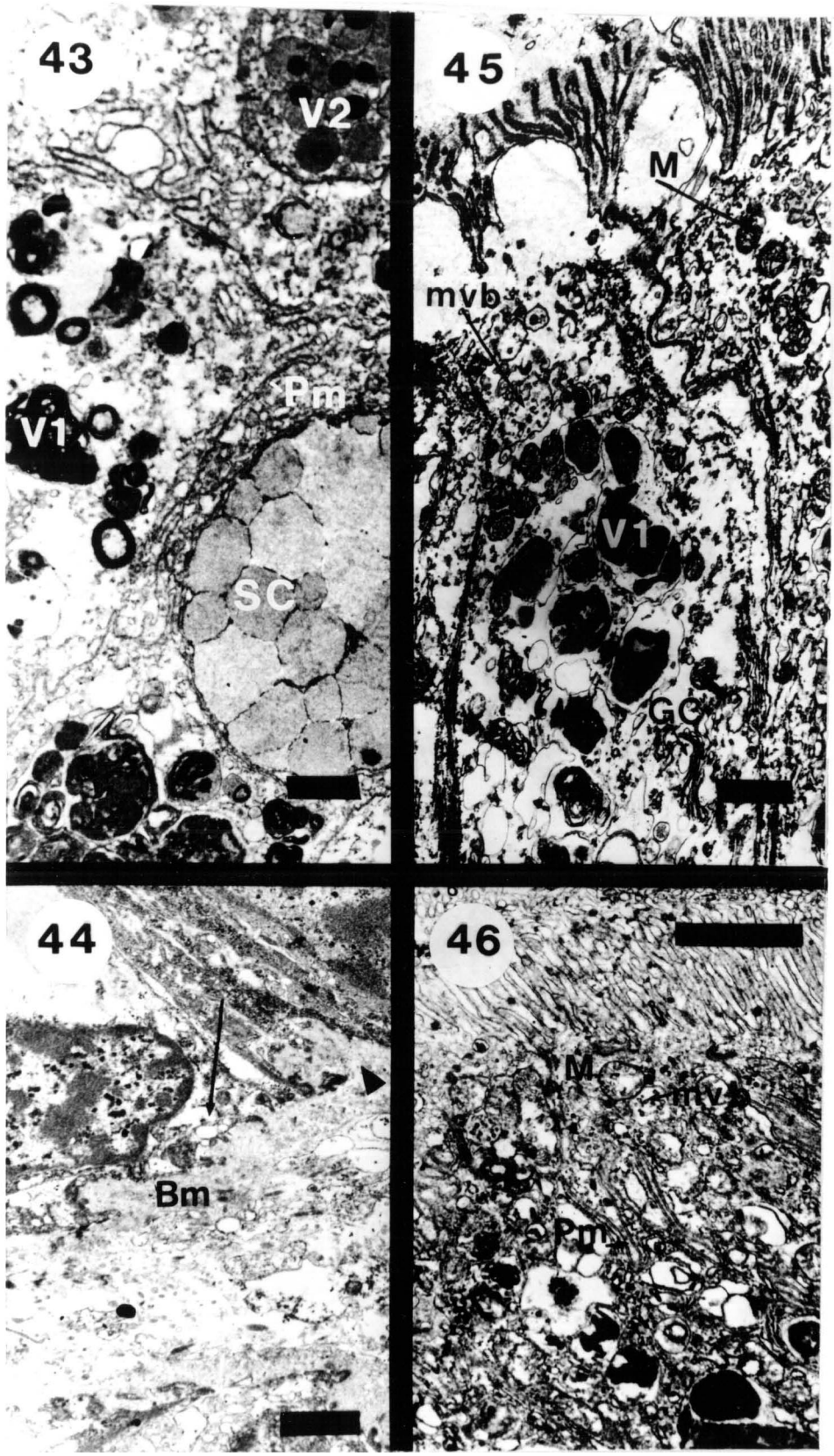

41
SC
OME
CT
42
OME
SC
A
CT

Fig. 43- TEM-cross section of the outer mantle epithelium of *Haliotis kamtschatkana* in the 10th week following nucleus implantation. Note the convoluted plasma membrane (Pm). Abbreviations used are: SC, secretory cell; V2, type two vesicle; V1, type one vesicle. Scale bar= 1 μm.

Fig. 44- Basal membrane of the outer mantle epithelium in the 11th week following nucleus implantation. Abbreviations used are: Bm, basal membrane; small vesicles, long black bold arrow, basal membrane interdigitations, black bold point. Scale bar= 1 μm.

Fig. 45- TEM-section of the outer mantle epithelial cells of *Haliotis kamtschatkana* in the 11th week following nucleus implantation. Abbreviations used are: mvb, multivesicular body; M, mitochondria; GC, Golgi complex; V1, type one vesicles. Scale bar= 1.5 μm.

Fig. 46- Apical cytoplasm of the outer mantle epithelial cells of *Haliotis kamtschatkana* in the 12th week following nucleus implantation. Abbreviations used are: mvb, multivesicular body; M, mitochondria; Pm, plasma membrane. Scale bar= 1 μm.


43
V2
V1
Pm
SC
45
M
mvb
V1
GC
44
Bm
46
M
mv
Pm

(GC) was observed in the apical cytoplasm with the cisternae apico-laterally oriented (Fig. 45).

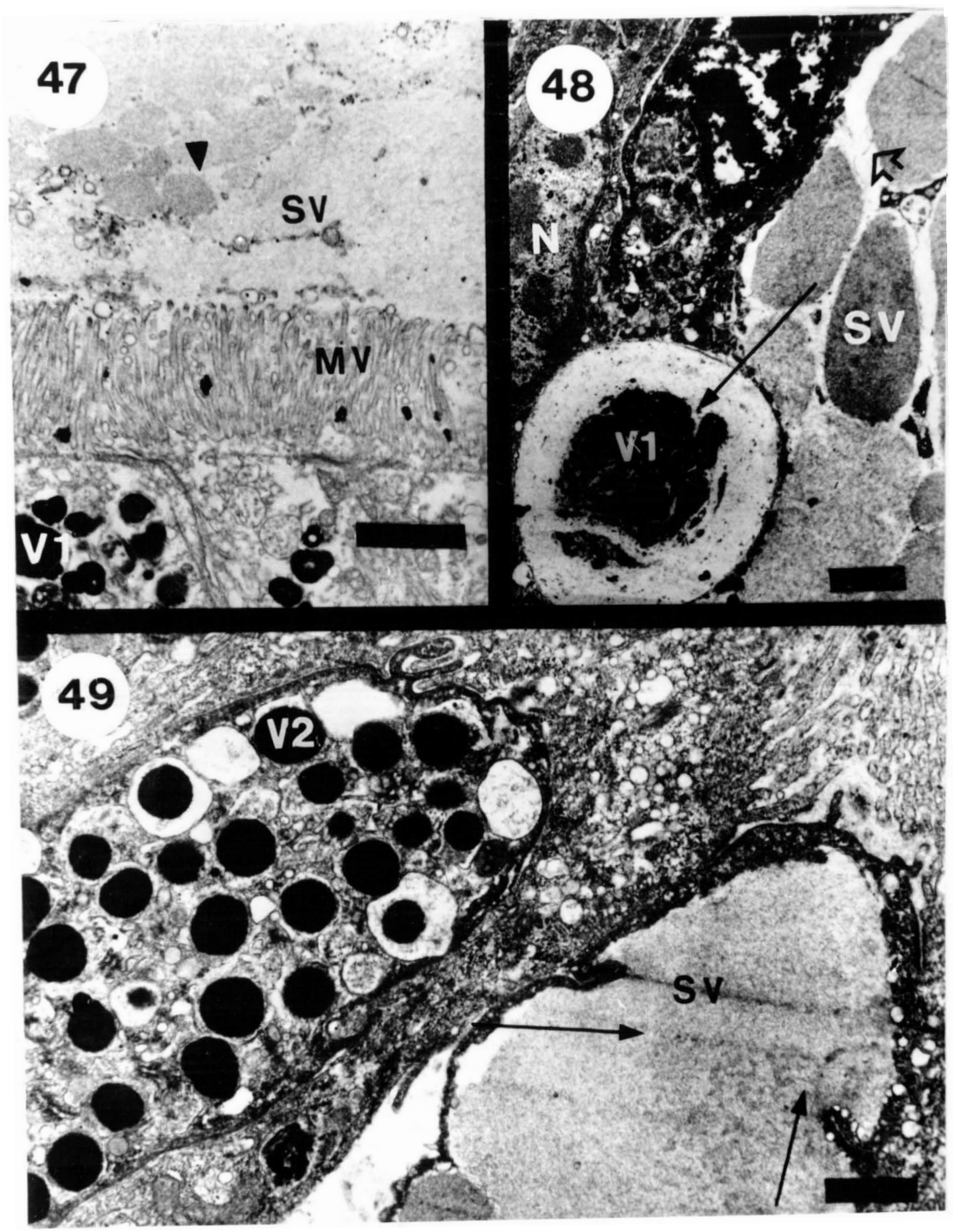
Although the epithelial cells had not elongated, the secretory activity continued (Figs. 39 and 41). Secretory cells (SC) were observed to expel the secretory vesicles (black bold point) in light micrographs of the 12th week (Fig 41). Examinations of electron micrographs indicated that secretory vesicles were secreted without their membranes (black bold point) (Fig. 47). The vesicles were observed fused in a mass or separate (Fig. 47). Secretory vesicles (SV) appeared to be surrounded by a membrane (white bold arrow) inside the cell (Fig. 48). However, secretory vesicles appeared to fuse before they were secreted (long black arrows) (Fig. 49).

During the 10th week, a nacre layer was secreted which covered the spire and the right forward edge of the inner shell surface, the base of the implanted nucleus and half of the nucleus itself. During the 11th to the 14th weeks nacre continued to be secreted in irregular deposits, covering the spire and the right forward edge of the inner shell, and the base of the implanted nucleus. The nacreous layer started to be deposited. During these fourteen weeks the implanted nucleus was not completely covered with nacre so that the formation of the complete pearl was not observed.

Fig. 47- Secreted secretory vesicles on the outer mantle surface of *Haliotis kamtschatkana* in the 12th week following nucleus implantation. Note that secreted secretory vesicles (SV) are not enclosed by a membrane (black bold point). Abbreviations used are: MV, microvilli; V1, type one vesicle. Scale bar= 1 μm.

Fig. 48- TEM-section of the basal cytoplasm of the outer mantle epithelial cells of *Haliotis kamtschatkana* in the 12th week following nucleus implantation. Note that the nucleus (N) is pushed to one side. Abbreviations used are: SV, secretory vesicles; secretory vesicles membrane, white bold arrow; V1, type one vesicle. Scale bar= 1 μm.

Fig. 49- Apical view of a secretory cell and type two vesicles of the outer mantle epithelial cells of *Haliotis kamtschatkana* in the 13th week following nucleus implantation. Note the fusion of secretory vesicles (SV) before release (long black bold arrows). Abbreviations used are: V2, type two vesicles. Scale bar= 1 μm.


47
SV
MV
V1
48
N
SV
V1
49
V2
SV

IV- Discussion

Boutan (1923) suggested that the pearl nucleus provokes a chronic inflammation in the mantle epithelium evident by the elongation of the pearl sac cells. An increase in the height of the mantle epithelial cells after shell damage has been reported in a number of molluscs including the bivalves *Musculus senhouisia* (Kawaguti and Ikemoto, 1962), *Pteria (pinctadata) martensii* (Tsujii, 1968a) and the gastropod, *Lymnea stagnalis* (Timmermans, 1973). In addition, Timmermans (1973) observed that the portion of the mantle in which the cells were elongated was considerably larger than the mantle area in contact with the shell defect. In the present study the initiation of the process of nacre formation in *Haliotis kamtschatkana* starts with cellular enlargement in the outer mantle epithelium. The maximum cellular elongation occurs when the implanted nucleus is covered completely with conchiolin. Several studies have reported that cells of the outer mantle epithelium are more elongated during the secretion of the periostracum and the prismatic layer than the secretion of the nacreous layer for different molluscs (Kawaguti and Ikemoto, 1962; Abolins-Krogis, 1963; Beedham, 1965; Tsujii, 1968a&1968b; Salueddin, 1970; Tsujii, 1976). My results support these observations. The mantle epithelial cells of *H. kamtschatkana* reach their maximum height during the secretion of conchiolin and prismatic layer. The cell enlargement is necessary to provide the cell space to contain the various organelles involved in secretion. These changes in height suggest a peak of cellular activity before the nacreous layer is laid down. Therefore, it confirms the production and secretion of organic compounds and inorganic ions necessary for the formation of the organic matrix where calcification occurs. .

The implanted nucleus in *H. kamtschatkana* shell was covered first by a layer of $CaCO_3$ followed by conchiolin. Crenshaw (1990) reported that what Fremy originally designated as conchiolin is the residue of molluscan shells after treating the shell in HCl. This residue was insoluble in water, alcohol and ether. Conchiolin is the

extracrystalline insoluble matrix which encloses the crystals and binds crystals and layers of crystals into an integrated structure (Meenaski *et al.*, 1971; Crenshaw, 1990; Samata, 1990). It is mainly composed of proteins and polysaccharides (Gregoire, 1955; Goffinet, 1977). In the present study TEM micrographs show the cell cytoplasm dominated mainly with granular endoplasmic reticulum, mitochondria and vacuoles. These organelles suggest that the main activity of the mantle epithelial cells is the secretion of conchiolin.

Beedham (1958a) reported that the outer mantle epithelial cells of Lamellibranchs which secretes a nacreous layer has lower concentrations of ribonucleic acids than epithelial cells which secreted the periostracum suggesting that ribonucleic acids were involved in the secretion of conchiolin. The appearance of additional granular endoplasmic reticulum followed by a diminution of it has been observed in the outer mantle epithelium of *Musculus senhouisia* (Kawaguti and Ikemoto, 1962), *Pteria (pinctadata) martensii* (Tsujii, 1968a&1968b) and *Helix* (Salueddin, 1970) during the deposition of conchiolin following shell damage. Well developed granular endoplasmic reticulum is found in cells actively engaged in protein synthesis (De Robertis, 1965; Hopkins, 1978). Thus the appearance of additional granular endoplasmic reticulum with wide cisternae and the deposition of conchiolin suggest evidence of active protein secretion. Richardson *et al.* (1981) suggested a transportation sequence between the plasma membrane, Golgi complex and the granular endoplasmic reticulum in the secretion of periostracum on *Cerastoderma edule*. Similar to Richardson *et al.* (1981) observations, in this study the localization of granular endoplasmic reticulum near the plasma membrane was observed in the cells of *H. kamtschatkana*, which suggests the possible transfer of ions through these two organelles and probably with the Golgi complex in the secretion of organic compounds.

One of the main functions of mitochondria is to produce ATP (De Robertis, 1965; Hopkins, 1978). Mitochondria also have the capability to accumulate calcium and phosphate very efficiently

especially in tissues undergoing calcification (De Robertis, 1965; Shapiro and Granspan, 1969; Hopkins, 1978). Shapiro and Granspan (1969) suggested that mineralizing tissues can increase the levels of mineralization by means of mitochondrial activity and a matrix which is capable of being mineralized. According to Salueddin (1970) a high number of mitochondria loaded with crystals were seen in *Helix* during shell regeneration. In the bivalves *Mercenaria mercenaria* (Neff, 1972) and in *Cerastoderma edule* (Richardson *et al.*, 1981), a large number of mitochondria were in close association with the plasma membrane as a characteristic of ionic transport in the outer mantle epithelial cells. According to these authors, mitochondria play a role as calcium ion pumps, however no deposition of intercellular calcium was detected. These authors suggested that the main route of calcium was by the intercellular spaces and a smaller fraction moved in a soluble ionized form through the mantle. In fact in the present study, mitochondria appeared to be distributed near granular endoplasmic reticulum and plasma membrane. This nearness between organelles of synthesis and ion exchange functions suggests a possible transport of ions and molecules agreeing with the latter authors. Another event that suggests high mitochondrial activity is the presence of long mitochondria during the weeks when the implanted nucleus was being covered by conchiolin. The length of the mitochondria infers a high capacity to produce ATP or accumulate calcium and phosphate, suggesting calcification, a process that occured a week after the prismatic layer was deposited on the implanted nucleus. Studies of shell regeneration in the bivalves *Pteria (pinctadata) martensii* (Tsujii, 1968a&1968b), *Mytilus*, *Cardium* and *Nucula* (Bubel, 1973a) and the pulmonate gastropod *Helix* (Salueddin, 1970) revealed an association between wound repair and an increase in the number of mitochondria in the outer mantle epithelium. Salueddin suggested that the outer mantle epithelial cells resembled the mantle edge cells that normally secrete the prismatic layer. My results with *H. kamtschatkana* confirm this. Mitochondria are numerous during the conchiolin deposition and previous deposition of the prismatic layer.

Another feature observed in the mantle of *H. kamtschatkana* during conchiolin secretion is the additional appearance of vacuoles which coincide with observations done on *Helix* by Salueddin (1970). Fournie and Zylberberg (1987) observed dictiosomes containing lamellae. These lamellae composed the periostracum of the slug *Deroceras reticulata*. In this study, several vacuoles containing lamellae appeared near the Golgi complex. These vacuoles are probably derived from an amalgamation of Golgi vesicles. The lamellae may be conchiolin although they were not seen to be secreted as Fournie and Zylberberg reported it.

The Golgi complex has rarely been observed in normal outer mantle epithelium of molluscs (Neff, 1972). In inactive secretory cells, Golgi complexes are inconspicuous (Bubel, 1989). Several studies have revealed Golgi complexes in the outer mantle epithelium of different molluscs after shell damage (Kawaguti and Ikemoto, 1962; Tsujii, 1968a&1968b; Bubel, 1973b; Tsujii, 1976). One of the main functions of the Golgi is the packaging of polysaccharides, proteins, glycolipids and glycoproteins for export from the cell (Whaley, 1975). In the present study the observations confirm that the Golgi apparatus is not found in the mantle epithelium without nucleus implantation. Although the Golgi complex was not observed in the first and third weeks following nucleus implantation, the large number of vacuoles present in the cells during those weeks suggests its presence. Moreover, subsequent to the 4th week following nucleus implantation it was always present. This phenomenon is also correlated with the contiguous augmentation of type one vesicles, type two vesicles and secretory cells. This suggests that in epithelial cells the Golgi complex secretes conchiolin and type one and type two vesicles. In the case of secretory cells, the main function may be to form secretory vesicles.

Lysosomes are a morphologically heterogeneous group of membrane-bound organelles containing acid hydrolase enzymes for intracellular digestion (De Robertis, 1965; De Duve, 1983). Although cytochemical tests to recognize lysosomes were not done in this

study, the organelles named as type one vesicles and type two vesicles are probably secondary lysosomes. Tsujii (1968a) suggested that lysosomes control the secretory function of mantle epithelial cells in *Pteria (pinctadata) martensii*. Bubel (1973b) agreed and reported that they control periostracum secretion and engulf surplus intracellular material utilized to secrete the organic layer. Bubel (1973c) cited various authors who suggest that autophagic vacuoles are formed within cells which are under metabolic stress because of extensive remodelling or injury. This author also states that during periostracum repair, autophagic vacuoles digest the cytoplasmic components for reutilization in the synthesis of periostracal material. According to Salueddin (1976), lysosomes may digest cellular material and remove the excess periostracal units during periostracum formation in *Helisoma*. Material digested by lysosomes is able to diffuse through the limiting lysosomal membrane into the cytoplasm for use by the cell (De Robertis, 1965; Hopkins, 1978; De Duve, 1983). Autophagocytosis, is a process by which worn out or damaged cytoplasmic organelles are engulfed, isolated and broken down within a lysosome (Hopkins, 1978; De Duve, 1983). Bubel (1989) suggests that in active secretory cells lysosomes and autophagic vacuoles appear to regulate oversecretion. In fact, the additional appearance of type one vesicles and type two vesicles suggests regulation of conchiolin secretion by autophagocytosis of the organelles related to its production. Although the organelles related to secretion are diminished in number, these cells can continue to secrete conchiolin because they still maintain the histological characteristics for it's secretion. Another observation that suggests the existence of lytic enzyme activity in the type one and type two vesicle content is the different electron density and membranous texture. This autophagocytosis may also occur to reutilize the chemical constituents of the organelles for further synthesis. Though Bubel (1973) reported the secretion of autophagic vacuoles content in periostracum formation but in the present study no secretion of type one vesicles was seen. It may also be possible that the content of the large type one vesicles probably is indigestible material and may form residual bodies. This event of autophagocytosis and lysosomal

digestion suggests that the amount of conchiolin needed for the deposition of the calcium crystals is less than the layer deposited in previous weeks. The height of the mantle epithelial cells diminished during the deposition of the prismatic and nacreous layer. This change is probably due to a reduction in the amount of organelles in the cytoplasm.

Beedham (1958*a*) suggested that gland cells in the outer mantle epithelium of *Mytilus* and *Ostrea* may play a role in calcification. Mucous cells were not observed in the outer mantle epithelium during shell regeneration in *Musculus* (Kawaguti and Ikemoto, 1962) and in *Helix* (Salueddin, 1970). In contrast, Machado *et al.* (1968) described a "B" cell in the outer mantle epithelium of *Anodonta*. These "B" cells contained a mucous protein similar to chitin that contribute in the formation of the organic matrix (Machado *et al.*, 1968). Furthermore, sulfated mucopolysaccharides were measured in the mantle edge of *Helisoma* by (Kapur and Gibson, 1968) According to these authors, the mucopolysaccharides were derived from mucous glands which surrounded each plate of the nacreous layer and bound the calcium ions during calcification. The secretory cells observed in the present study resemble the mucous cells found by Bubel (1973a) in the outer mantle epithelium of *Cardium edule* that were involved in calcification. In the mantle epithelium of *H. kamtschatkana*, secretory cells appeared in larger numbers during the period of deposition of the prismatic and nacreous layer, suggesting that secretory cells are involved in the process of calcification.

Glycoproteins, polypeptides and polysacharides are the principal compounds of the soluble fraction of the organic matrix (Simkiss and Wilbur, 1989). In addition, Samata (1990) reported that glycoproteins play an important role in Ca-binding and that the amino acids are slightly different between the nacreous and prismatic layer. Furthermore, he stated that aspartic acid was responsible for promoting the nucleation of $CaC0_3$ crystals. In my study, secretory cells were positive to mucoglycoprotein and

polysaccharide reactions. This suggests that these cells can contain mucoglycoproteins. In addition, the presence of numerous secretory cells during the deposition of the prismatic and nacreous layers suggests that secretory cells may be responsible for secreting the material that nucleates calcium carbon crystals and binds them together. This material would be the soluble component of the organic matrix.

Carr and Toner (1960) cited as holocrine secretion the process of discharge associated with sebaceous glands of skin. The sebaceous cells accumulate their fatty product within the cytoplasm and finally are destroyed entirely, pouring out the residual cytoplasmic organelles along with the secretory products. In the present study, secretory cells discharge their products but no residual cytoplasmic organelles were seen in the mantle surface. This suggests that secretory cells follow a compound exocytosis (Dahl *et al.*, 1979) whereby the contents of several vesicles are ejected through an opening at the cell membrane. According to Dahl *et al.*, the process involves the vesicles that fuse with the cell membrane or with each other. However, in my study the secreted secretory vesicles may be fused or isolated inferring that the intracellular fusion of the vesicles can be incomplete.

Glycogen particles, together with soluble enzymes and ribosomes constitute the internal medium of a cell (Bubel, 1989). In the case of *Lymnea stagnalis*, (Timmermans, 1969), *Mytilus mytilus*, (Bubel, 1973a) and *Pteria (pinctadata) martensii*, (Tsujii, 1976) studies indicated that mantle cells contain large quantities of glycogen in regenerating shells and this was related to calcification. All of these observations were made following the suggestion of Wilbur (1964) that carbonate necessary for calcification was derived from metabolic carbon dioxide. Metabolic carbon dioxide is generated by the glycogen metabolism. In the present study, glycogen rosettes were augmented continuously with the largest quantity occurring during secretion of the nacreous layer.

Endocytosis is the uptake of extracellular materials, such as proteins, metabolic end products and foreign particles within membrane-limited vacuoles or microvesicles (Allison and Davies, 1974). The endocytotic process involves the invagination of the plasma membrane forming small vesicles (Hopkins, 1978). Exocytosis is the release of secretory products from cells within membrane limited vesicles or granules (De Robertis, 1965; Allison and Davies, 1974). Organelles such as Golgi, GER, lysosomes and vesicles are considered to be pathways of secretory activity (Tartakoff, 1987). Tartakoff indicates that in continuously secreting cells, the carriers that intervene between the Golgi and the cell surface are small vesicles. Infoldings, endosomes, vesicles and lysosomes are considered to be endocytotic pathways. Tartakoff further indicates that endosomes intervene between the moment of the vesicle closure from the cell basal surface to the delivery to the lysosomes. The present study involves several ultrastructural features that implicate both exocytosis and endocytosis in the outer mantle epithelium during nacre formation. Apical small vesicles, secretory blebs and secretory vesicles suggest an active exocytotic activity. Meanwhile, small vesicles in the basal cytoplasm suggest the utilization of ions from the connective tissue for calcification.

Several authors have suggested that amoebocytes play a role in shell repair (Boutan, 1923; Wagge, 1955; Abolins-Krogis, 1963; Beedham, 1965; Tsujii, 1968a; Bubel, 1973a). Wagge (1955) indicated that amoebocytes carried calcium crystals and proteins. Abolins-Krogis suggested that amoebocytes contribute in shell repair by ingesting material or releasing it. The release of the material was done by the disorganization of the cell membrane (Kapur and Sen Gupta, 1976). In the present study, the largest number of amoebocytes were observed in the stages before the prismatic and nacreous layer were deposited. Amoebocytes were observed in the connective tissue near the basal membrane and on the cell surface, and are presumably involved in shell repair as suggested by Wagge (1955). However, no direct observation was made of amoebocytes releasing or ingesting material.

V. Conclusions

My results agree in part with previous studies on different molluscs (Tsujii, 1969a&1969b; Salueddin, 1970; Kawaguti and Ikemoto, 1962; Bubel, 1973a, 1973b&1973c; Fournie and Zlberberg, 1987) and have provided information on nacre formation and shell regeneration of northern abalone, *Haliotis kamtschatkana*. The implanted nucleus activates the secretory system involved in calcification. Consequently the outer mantle epithelium is capable of secreting the organic matrix and prismatic and nacreous layers. The most noticeable changes that occur in the outer mantle epithelial cells following nucleation are:

a) an elongation of the epithelial cells followed by a diminution of cell height, suggesting a peak of cellular activity. The cellular space is apparently augmented in order to contain the organelles related to secretion,

b) epithelial cells contained a larger amount of granular endoplasmic reticulum, vacuoles and mitochondria while the epithelium is secreting conchiolin, the insoluble component of the organic matrix,

c) a greater control of the conchiolin secretion by type one vesicles and type two vesicles, organelles proposed as lysosomes.

d) a larger number of secretory cells while the epithelium is forming the prismatic and nacreous layer, and apparently responsible for secreting the soluble component of the organic matrix that nucleates and binds calcium crystals in biomineralization.

These observations collectively suggest that the outer mantle epithelium of *H. kamtschatkana* has the potential to produce all of the components of the organic matrix essential for calcification.

VI. Literature Cited

Abolin-Krogis, A. 1963. The histochemistry of the mantle of *Helix pomatia(L)* in relation to the repair of the damaged shell. Ark. Zool. 15: 461-464.

Abolin-Krogis, A. 1976. Ultrastructural study of the shell repair membrane in the snail *Helix pomatia (L).* Cell Tiss. Res. 172: 455-476.

Allison, A. and Davies, P. 1974. Interactions of membranes, microfilaments and microtubules in endocytosis and exocytosis. In: Advances in Cytopharmacology. Raven Press, New York, pp. 237-248.

Beedham, G. 1958a. Observations on the mantle of the Lamellibranchia. Quart. J. Microsc. Sci. 99: 181-197.

Beedham, G. 1965. Repair of the shell in species of *Anodonta.* Proc. Zool. Soc. Lond. 14: 107-125.

Bevelander, G. and H. Nakahara. 1967. An electron microscope study of the formation of the periostracum of *Macrocallista maculata.* Cal. Tiss. Res. 1: 55-67.

Bevelander, G. and H. Nakahara. 1969. An electron microscope study of the formation of the nacreous layer in the shell of certain bivalve molluscs. Cal. Tiss. Res. 3: 84-92.

Bevelander, G. 1989. Abalone: Gross and Fine Structure. The Boxwood Press, pp.1-78.

Boutan, L. 1923. Nouvelle etude sur les perles naturelles et sur les perles de culture. Ann. Des. Sc. Nat. Zool. (10) 6: 1-91.

Bubel, A. 1973a. An electron microscope study of periostracum formation in some marine bivalves.I.The origin of the periostracum. Mar. Biol. 20: 213-221.

Bubel, A. 1973b. An electron microscope study of periostracum formation in some marine bivalves.I.The cells lining the periostracal groove. Mar. Biol. 20: 222-234.

Bubel, A. 1973c. An electron microscope study of the periostracum repair in *Mytilus edulis*. Mar. Biol. 20: 235-244.

Bubel, A. 1973d. An electron microscope investigation of the cells lining the outer surface of the mantle in some marine molluscs. Mar. Biol. 20: 245-255.

Bubel, A. 1973. An electron microscope investigation into the distribution of polyphenols in the periostracum and cells of the inner face of the outer fold of *Mytilus edulis*. Mar. Biol. 23: 3-10.

Bubel, A. 1989. Microstructure and Function of Cells. John Wiley & Sons, pp.1-271.

Cariolou, M.A. and D.E. Morse. 1988. Purification and characterization of calcium binding conchiolin shell peptides from the molluscs, *Haliotis rufescens*, as a function of development. J. Comp. Physiol. B 157: 717-729.

Carr, K. and Toner, P. 1982. Cell Structure. Third edition. Churchill Livingstone, Edimburgh, pp.1-388.

Chan, W. and A.S.M. Salueddin. 1974. Evidence that *Otala lacta* (Müller) utilized calcium from the shell. Proc. Malac. Soc. Lond. 41: 195-200.

Crenshaw, M.A. 1990. Biomineralization. In: Skeletal Biomineralization: Patterns, Processes and Evolutionary Trends. Volume 1, Joseph G. Carter (eds.), Van Nostrand Reinhold, N.Y., pp.1-9.

Dahl, G., R. Ekerdt and M. Gratzl. 1979. Models for exocytotic membrane fusion. In: Secretory mechanisms. Symposia of the Soc. for Exp. Biology, number XXXIII, Cambridge Univ. Press, pp. 349-368.

Dauphin, Y., J. Cuif and D. Mutvei. 1989. Mineralogy, chemistry and ultrastructure of the external shell layer in ten species of *Haliotis* with reference to *Haliotis tuberculata* (Mollusca: Archaesgastropoda). Bull. Geol. Inst. Univ. Uppsala 15: 7-17.

De Duve, C. 1983. Lysosomes revisited. Europ. J. Biochem. 37: 391-397.

De Robertis, E.D.P., F.A. Saez and E.M.F. De Robertis. 1975. Cell Biology. Sixth edition, W.B. Saunders Company, pp.1-615.

Durning, C. 1957. Repair of a defect in the shell of the snail *Helix aspersa*. J. Bone J. Surg. 39: 377-393.

Fankboner, P.V. 1978. Suspension feeding mechanisms of the armoured sea cucumber, *Psolus chitinoides* Clark. J. Exp. Mar. Biol. Ecol. 31: 11-25.

Fankboner, P.V. 1988. The Canadian Encyclopedia. Second edition, volume I A-EDU, Hurtig Publishers. pp.1.

Fankboner, P.V. 1991. Pearl Culture in Abalone. INFUFISH International 4: 52-55.

Fretter, V. and A. Graham. 1962. British Prosobranch Molluscs. Bartholomew Press, pp. 1-755.

Fournie, J. and Zylberberg, L. 1987. Ultrastructural organization, and regeneration of the periostracum in the slug *Deroceras reticulatum* (Mollusca). Can. J. Zool. 65: 1935-1941.

Goffinet, G., Ch. Gregoire and M. Voss-Foucart 1977. On the ultrastructure of the trabeculae in the interlamellar membrane of nacre conchiolin of the *Nautilus* shell. Arch. Internat. Physiol. Bioch. 85: 849-863.

Gregoire, C., G. Duchateau and M. Flokin. 1955. La trame protidique des nacres et des perles. Ann. Institut Oceanogr. 31: 1-36.

Hahn, K. 1989. Handbook of Culture of Abalone and Other Marine Gastropods. CRC-Press, pp.1-348.

Hopkins, C.R. 1978. Structure and Function of Cells. W.B. Saunders Company, pp.1-266.

Humason, G.L. 1967. Animal Tissues Techniques. Freeman & Company, pp.1-569.

Kapur, S.P. and M.A. Gibson. 1968. A histochemical study of the development of the mantle edge and shell in the freshwater gastropod, *Helisoma duryi eudiscus* (Pilsbry). Can. J. Zool. 46: 481-491.

Kapur, S.P. and A. Sen Gupta. 1970. The role of amoebocytes in the regeneration of shell in the land pulmonate, *Euplecta indica* (Pfieffer). Biol. Bull. 139: 502-509.

Kawaguti, S. and N. Ikemoto. 1962. Electron microscopy on the mantle of a bivalve, *Musculus senhousia* during regeneration of the shell. Biol. J. Okayama Univ. 8: 31-42.

Kunigelis,S. C. and A.S.M. Salueddin. 1983. Shell repair rates and carbonic anhydrase activity during shell repair in *Helisoma duryi* (Mollusca). Can. J. Zool. 61: 397-402.

Machado, J., F. Castilho, J. Corimbra, E. Monteiro, C. Sa and M. Reis. 1988. Ultrastructural and cytochemical studies in the mantle of *Anodonta cygnea*. Tissue & Cell 20: 797-807.

Meenaski, V.R., P.E. Hare and K.M. Wilbur. 1971. Aminoacids of the organic matrix of neogastropod shells. Comp. Biochem. Physiol. 40 B: 1037-1043.

Meenaski, V.R., P.C. Blackweldder and K.M. Wilbur. 1973. An ultrastructural study of the shell regeneration in *Mytilus edulis* (Mollusca: Bivalvia). J. Zool. (London) 171: 475-484.

Mutvei, H., Y. Dauphin and J.P. Cuif. 1985. Observations sur l'organisation de la couche externe du test des *Haliotis* (Gastropoda) un cas exceptionel de variabilite mineralogique et microstructurale. Bull. Mus. Nat. Hist. Paris 4 Ser. 7: 73-91.

Nakahara, H. and G. Bevelander. 1967. Ingestion of particulate matter by the outer surface cells of the mollusc mantle. J. Morph. 122: 139-146.

Nakahara, H. 1979. An electron microscope study of the growing surface of nacre in two gastropod species *Turbo cornutus* and *Tegula pfeifferi*. Venus, Jap. J. Malac. 38: 205-211.

Nakahara, H., M. Kakei and G. Bevelander. 1980. Fine structure and amino acid composition of the organic envelope in the prismatic layer of some bivalves shell. Venus, Jap. J. Malac. 39: 167-177.

Nakahara, H., G. Bevelander and M. Kakei. 1982. Electron microscopic and amino acid studies on the outer and inner shell layers of *Haliotis rufescens*. Venus, Jap. J. Malac. 41: 33-46.

Neff, J.M. 1972. Ultrastructure of the outer epithelium of the mantle in the clam *Mercenaria mercenaria* in relation to calcification of shell. Tissue & Cell 4: 591-600.

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17: 208.

Richardson, C.A., N.W. Runhaw and D.J. Crisp. 1981. A histological and ultrastructural study of the cells of the mantle edge of a marine bivalve, *Cerastoderma edule*. Tissue & Cell 13: 715-730.

Sakai, S. 1960. On the formation of the annual ring on the shell of the abalone, *Haliotis discuss var. Hannai ino*. Tohoku J. Agricult. Res. 3: 239-244.

Salueddin, A.S.M. 1967. The histochemistry of the mantle during the early stage of shell repair. Proc. Malac. Soc. Lond. 37: 371-379.

Salueddin, A.S.M. 1970. Electron microscopic study of the mantle of normal and regenerating *Helix*. Can. J. Zool. 48: 409-416.

Salueddin, A.S.M. 1971. Fine structure of normal and regenerated shell of *Helix*. Can. J. Zool. 49: 37-41.

Salueddin, A.S.M. 1976. Ultrastructural studies on the structure and formation of the periostracum in *Helisoma* (Mollusca). In: Watabe,N. and K.M. Wilbur (eds). The Mechanisms of Mineralization in the Invertebrates and Plants. Univ. of South Carolina Press, pp.309-337.

Samata, T. 1990. Ca-binding glycoproteins in molluscan shells with different types of ultrastructure. The Veliger 33: 190-201.

Shapiro, I. and Greenspan, J. 1969. Are mitocondria directly involved in biological mineralization? Cal. Tiss. Res. 3, pp. 100-102.

Simkiss, K. and K.M. Wilbur. 1989. Biomineralization. Cell Biology and Mineral Deposition. Academic Press, pp.1-337.

Sloan, N.A. and P.A. Breen. 1988. Northern Abalone *Haliotis kamtschatkana* in British Columbia:Fisheries and Synopsis of Life History Information. Dep. of Fish. and Oceans, Ottawa, pp. 1-46.

Tartakoff, A. 1987. The Secretory and Endocytic Paths, volume 6. John Wiley & Sons, New York, pp. 1-235.

Timmermans, L.P.M. 1969. Studies on shell formation in molluscs. Neth. J. Zool. 19: 417-523.

Timmermans, L.P.M. 1973. Mantle activity following shell injury in the pond snail *Lymnaea stagnalis*. Malac. 14: 53-61.

Tsujii, T., D.G. Sharp and K.M. Wilbur. 1958. Studies on shell formation.VII. The submicroscopic structure of the shell of the oyster *Crassosterea virginica*. J. Biophys. Biochem. Cytol. 4: 275-280.

Tsujii, T. and O. Isono. 1962. Studies on the mechanisms of shell and pearl formation. VII. The respiratory metabolism of the mantle in the normal and in the regenerating shell formation. J. Facul. Fish., Prefec. Univ. Mie 5: 371-377.

Tsujii, T. 1968a. Studies on the mechanism of shell and pearl formation. Electron microscopical determination of calcium in pearl sac and mantle epithelium in the pearl oyster *Pteria (pinctadata) martensii* (Dunker). J. Facul. Fish., Prefec. Univ. Mie 6: 432-442.

Tsujii, T. 1968b. Studies on the mechanism of shell and pearl formation. X.The submicroscopic structure of the epithelial cells on the mantle of pearl oyster, Pteria (pinctadata) martensii (Dunker). J. Facul. Fish., Prefec. Univ. Mie 6: 59-65.

Tsujii, T. 1968c. Studies on the mechanism of shell and pearl formation.XI.The submicroscopal observations on the mechanism of formation of abnormal pearls and abnormal shell. J. Facul. Fish., Prefec. Univ. Mie 6: 69-77.

Tsujii, T. 1976. An electron microscopic study of the mantle epithelial cells of *Anodonta sp.* during shell regeneration. In: Watabe, N. and K.M. Wilbur (eds.) The Mechanism of Mineralization in the Invertebrates and Plants. Univ. of South Carolina Press, pp. 339-353.

Wada, K. and T. Fujimuki. 1976. Biomineralization in bivalve molluscs with emphasis on the chemical composition of the extrapallial fluid. In: Watabe, N. and K.M. Wilbur (eds.) The Mechanisms of

Mineralization in the Invertebrates and Plants. Univ. of South Carolina Press, pp. 175-190.

Wagge, L.E. 1951. The activity of amoebocytes and of alkaline phosphatases during the regeneration of the shell in the snail, *Helix aspersa*. Quart. J. Microsc. Sci. 9: 307-321.

Wagge, L.E. 1955. Amoebocytes. In: Bowne, G.H. and Danielli, J.F. (eds.) International Review of Cytology. Volume IV. Academic Press Inc. New York, pp. 31-78.

Watabe, N. 1965. Studies on the shell formation.XI.Crystal-matrix relationships in the inner layers of molluscs shells. J. Ultrastr. Res. 12: 351-370.

Watabe, N. 1983. Shell repair. In: Salueddin, A.S.M. and K.M. Wilbur (eds.) The Mollusca: Physiology. Volume 4 part I, Academic Press, pp. 235-287.

Whaley, W.G. 1975. The Golgi apparatus. Volume 2. Springer Verlag, New York, Wien, pp. 1-190.

Wilbur, K.M. 1964. Sheli formation and regeneration. In: Wilbur, K.M. and C.M. Yonge (eds) Physiology of Mollusca VI, Academic Press, pp. 243-277.

Wilbur, K.M. and A.S.M. Salueddin. 1983. Shell formation. In: Salueddin A.S.M. and K.M. Wilbur (eds.). The Mollusca: Physiology. Volume 4 part I, Academic Press, pp. 235-287.

Wong, V. and Salueddin, A.S.M. 1972. Fine structure of normal and regenerated shell of *Helisoma duryi duryi*. Can. J. Zool. 50: 1563-1568.