

To Dr. Smith
with my compliments
Ph. L.

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COMPARATIVE HISTOLOGICAL STUDIES OF THE
CEMENT APPARATUS OF *LEPAS ANATIFERA*
AND *BALANUS TINTINNABULUM*

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Our earliest knowledge of glandular secretory structures in the Balanidae and the Lepadidae dates back to Darwin (1854) who erroneously described the unicellular cement glands as modifications of the ovarian wall. The location, function and morphology of the cement secreting glands and their associated canals were later described in more detail by Krohn (1859), Koehler (1888, 1889) Gruvel (1893, 1905, 1905a) and by Hoeck (1907), who observed structural differences in the cement complex in three genera: *Conchoderma*, *Lepas* and *Scalpellum*.

More recent studies on barnacles have been concerned chiefly with their taxonomy, life cycles, distribution, nutrition and ecology. However, histological investigations of the barnacle's cement apparatus have been stimulated by the current interest in natural adhesives (Lacombe, 1966, 1967). The purpose of this paper is to compare the histological characteristics of the cement glands and the conducting canals in *Lepas anatifera* and *Balanus tintinnabulum*, and to describe the pathways of glandular secretion in these representative species of the Pedunculata and Operculata.

MATERIALS AND METHODS

Lepas anatifera and *Balanus tintinnabulum* were collected in Guanabara Bay, Rio de Janeiro, Brazil. The animals were fixed in Bouin-Duboscq, Bouin prepared with sea water, Carnoy, Susa, Susa prepared with sea water, formalin containing 10% calcium phosphate, Gilson and Flemming's fixatives. Decalcification was accomplished by repeated treatment with Susa's fluid. After fixation and decalcification, the tissues were dehydrated according to the following schedule which was also used for the histological preparation of excised ovaries. The tissues were dehydrated in: 95% alcohol; two transfers through 100% alcohol; 100% alcohol + benzene (3:1) for approximately four hours; 100% alcohol + benzene (1:1) for four hours; 100% alcohol + benzene (1:3) for four hours; 100% alcohol + benzene (1:7) for six hours and benzene for ten hours before infiltration with paraffin. Tissue which was first fixed in Gilson's fluid for three hours was treated a second time in Susa's fixative followed by dehydration according to the above schedule. The same dehydration series was followed regardless of the fixative used in the study except that Bouin fixed tissue entered the dehydration series after transfer through 70% alcohol. Formalin and Flemming fixed material, after washing in running tap water for four hours, entered the dehydration series after transfers through 40% and 70% alcohol.

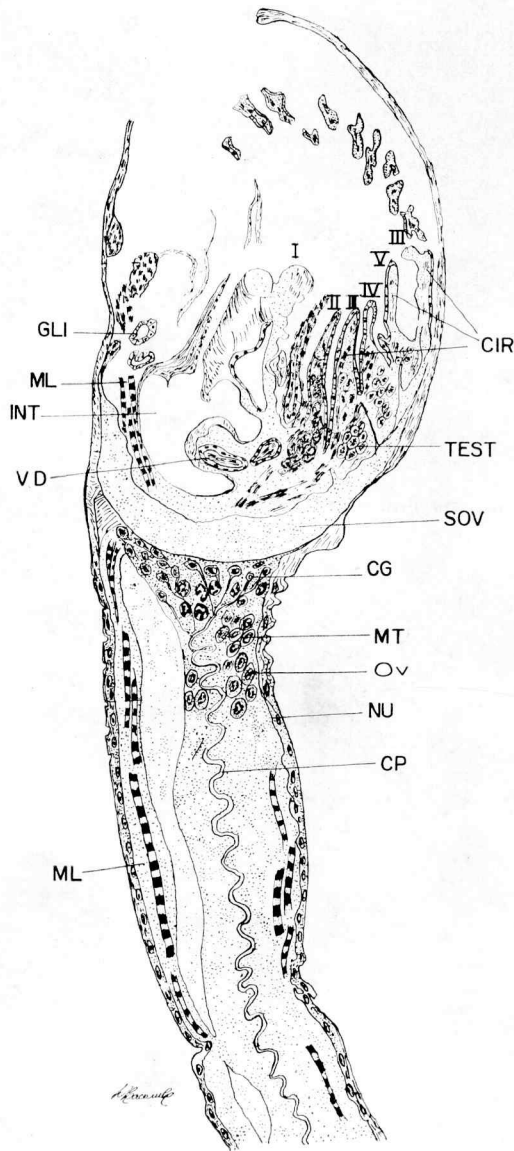


FIGURE 1. Schematic drawing of *Lepas anatifera* showing the gross morphology in cross section. CIR, thoracic appendages; TEST, testes; SOV, ovarian sac; CG, cement glands; MT, mantle tissue; Ov, ovaries; Nu, nucleus of cell in wall of peduncle; CP, principal canal; GLI, intestinal glands; ML, longitudinal muscle; INT, intestine; VD, vas deferens.

The paraffin embedded tissue was serially sectioned at 7 microns. For general histology the staining techniques of greatest value were found to be: Delafield's hematoxylin with Chromotrope 2R used as a counterstain; Ehrlich hematoxylin with

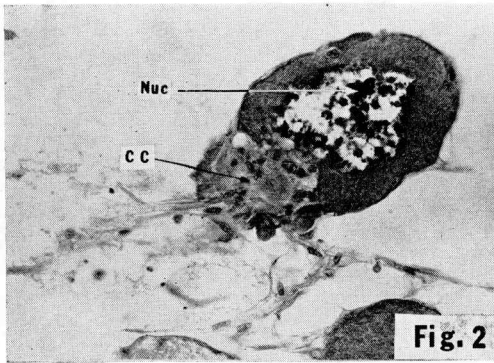


Fig. 2

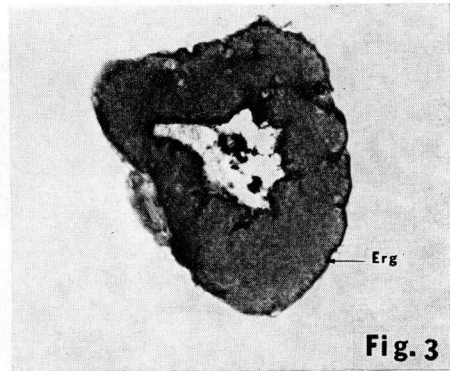


Fig. 3

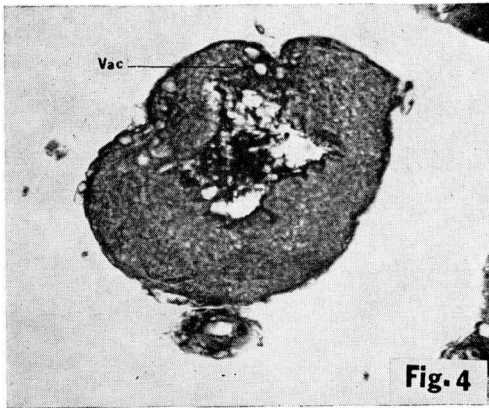


Fig. 4

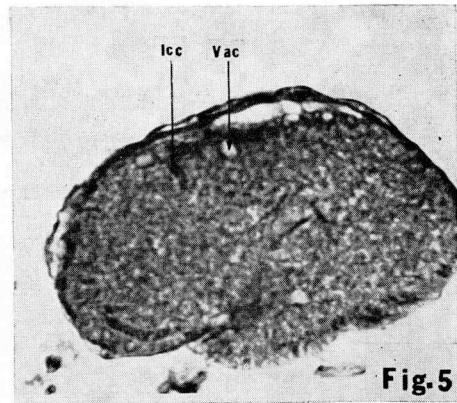


Fig. 5

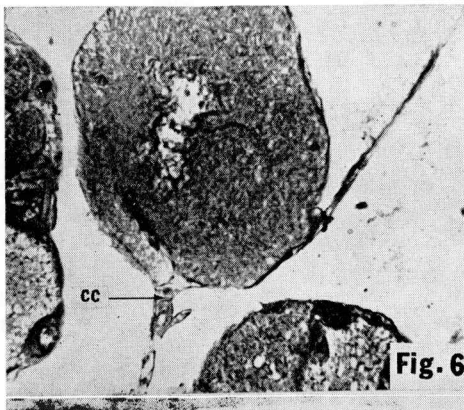


Fig. 6

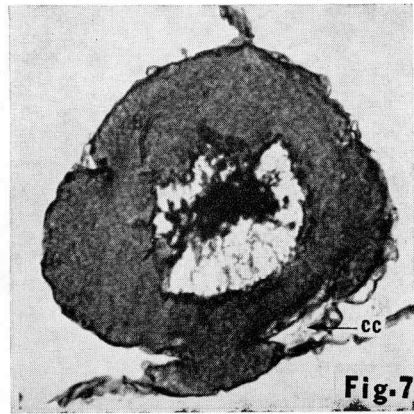


Fig. 7

FIGURE 2. Photomicrograph of unicellular cement gland of *Lepas anatifera* showing the large numbers of nucleoli (Nuc) and the collector canal (CC) at one pole of the cell.

FIGURE 3. *Lepas anatifera* cement gland showing a small zone of ergastoplasm (Erg) near the cell membrane.

FIGURE 4. Large cytoplasmic vacuoles (Vac) of *Lepas anatifera* cement gland cell.

FIGURE 5. Branches of intracellular canal (ICC) in the cytoplasm of *Lepas anatifera* cement gland.

FIGURES 6 and 7. Collector canals (CC) in the cement glands of *Lepas anatifera*, limited to a small region at one pole of the cell.

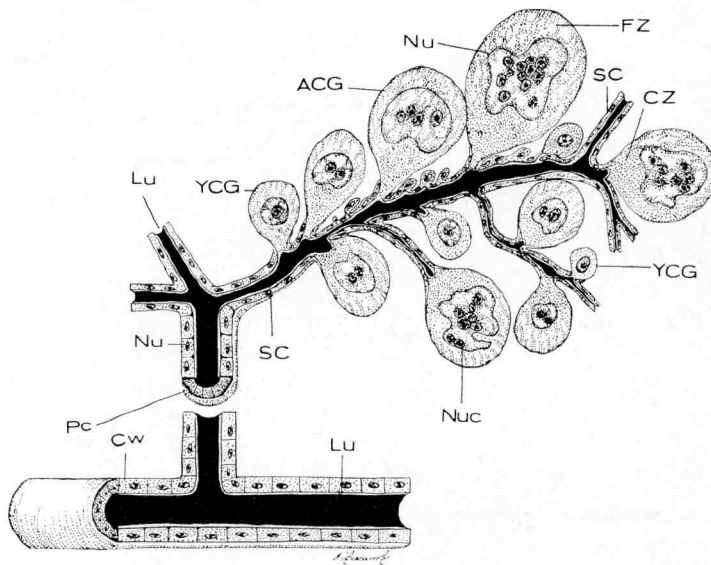


FIGURE 8. Diagrammatic representation of the cement glands and their associated canals in *Balanus tintinnabulum*. YCG, young cement gland; Nuc, nucleolus of cement gland; Lu, lumen of a cement conducting canal; CW, canal wall; PC, principal canal; Nu, nucleus; ACG, mature cement gland; FZ, formation zone for intracellular secretion; SC, secondary canal; CZ, accumulation zone for intracellular secretion.

eosin as a counterstain; nuclear fast red counterstained with naphthol green; Heidenhain's iron hematoxylin; nuclear fast red counterstained with azan and Weigert hematoxylin with Congo Red and Orange GG (1:1) used as counterstain.

RESULTS

The cement apparatus in *Lepas anatifera* and *Balanus tintinnabulum* is composed of unicellular glands and a series of connecting tubular canals which communicate from the glands to the area of barnacle attachment. Cement formed in the glands is conducted through the canals to the basal area where it hardens to form a bond with the substrate. In the present study, the terminology applied to the canals corresponds primarily to their location and function in the cement conducting system.

The principal canals are large, tubular structures which receive the cement from the smaller secondary canals. The walls of the principal canals are thick and are lined by a chitinous-like cuticle which is visible under polarized light. The principal canals of *Lepas anatifera* consist of two simple ducts that extend from the juncture they make with the secondary canals to the base by following a course parallel to the walls of the peduncle (Fig. 1).

Balanus tintinnabulum has many principal canals that conduct the cement received from the secondary canals to circular canals in the basal plate (Lacombe, 1966). In both species, the cement gland cells arise from the squamous epithelia of the walls of the secondary canals. It is assumed that the secretory products

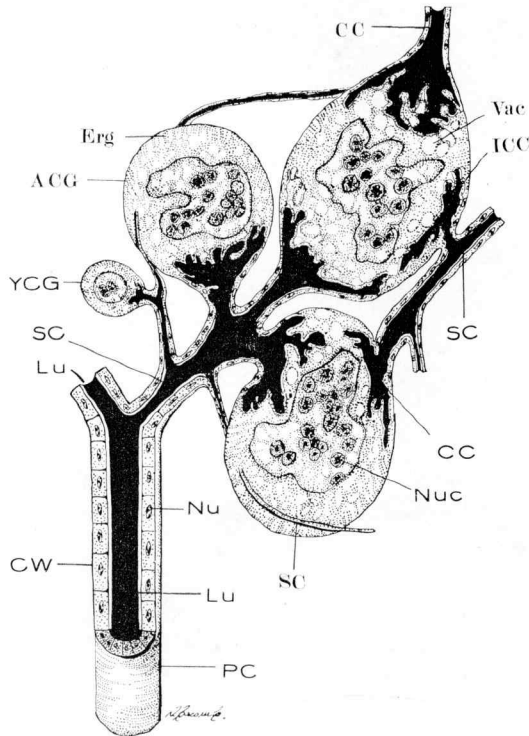


FIGURE 9. Schematic diagram of the cement glands and their canals in *Lepas anatifera*. YCG, young cement gland; ACG, mature cement gland; Erg, ergastoplasm; CC, collector canal; Vac, vacuole; ICC, intracellular canal; SC, secondary canal; Nuc, nucleolus; PC, principal canal; Lu, lumen; CW, cell wall; Nu, nucleus.

pass into the collecting canals which have direct continuity with the secondary canals from which they arise. In *Lepas anatifera* the collecting canals branch to form the intracellular canals. *Balanus tintinnabulum* lacks the intracellular canals.

The cement glands of *Lepas anatifera* (Fig. 1, CG) are found near the ovaries (Ov) in the peduncle, just beneath the capitulum or that portion of the animal which contains the major body parts enclosed in calcareous plates. These glands, measuring 30 to 40 microns in diameter in an animal 8 to 10 centimeters in length, have numerous nucleoli (Nuc) usually concentrated near the nuclear membrane and are surrounded by fine chromatin granules (Fig. 2). Each gland cell has a small zone of ergastoplasm (Erg) located near the cell membrane (Fig. 3). Vacuoles, varying in size, are found throughout the cytoplasm but in some instances the larger vacuoles (Vac) accumulate near the intracellular canals (Figs. 4 and 5, ICC). Collecting canals (CC) are limited to a small region at the cell pole nearest the secondary canal (Figs. 6 and 7). The cement glands for *Balanus tintinnabulum* and their canals are shown schematically in Fig. 8. The gland cells are dispersed among the interfollicular spaces of the ovary and are characterized histologically by a marked polarity in their staining reactions. When

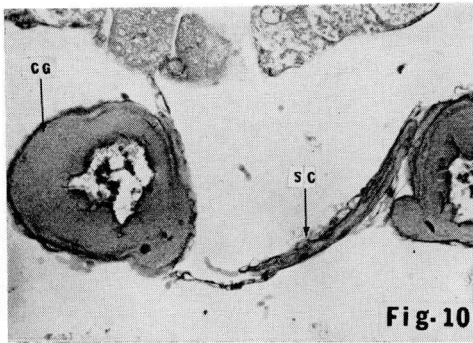


Fig-10

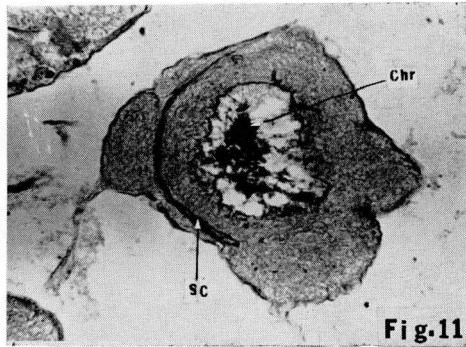


Fig-11

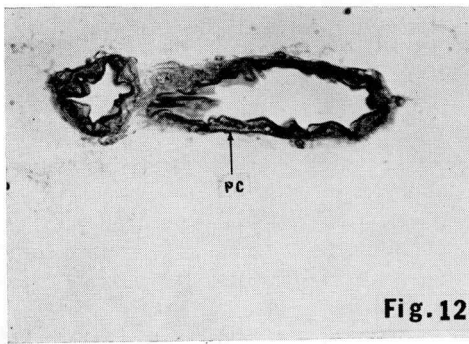


Fig-12

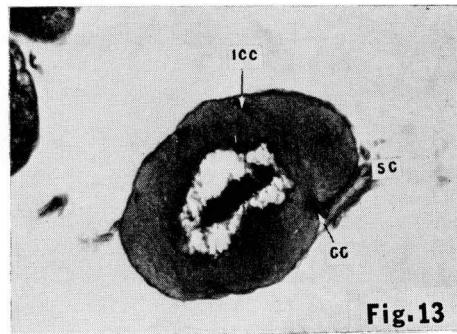


Fig-13

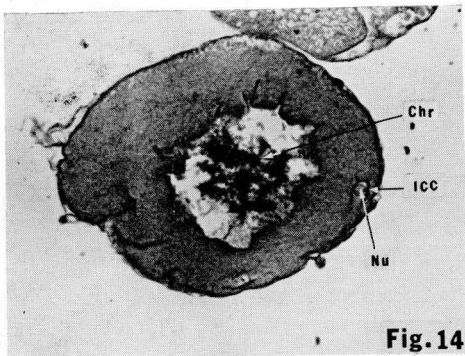


Fig-14

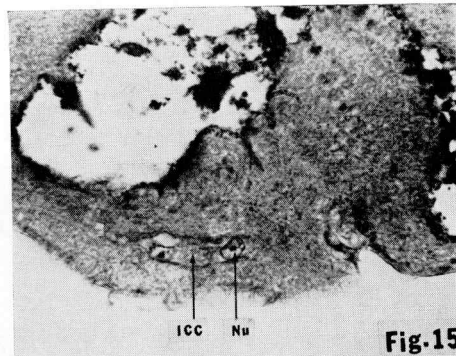


Fig-15

FIGURE 10. SC, a secondary canal of *Lepas anatifera* shown between two cement glands (CG).

FIGURE 11. SC, a secondary canal in *L. anatifera* shown partially encircling a cement gland whose nucleus is rich in chromatin material (Chr.).

FIGURE 12. PC, a principal canal of *L. anatifera* at a point where the canal bends providing a transverse and a longitudinal view.

FIGURE 13. Canals closely associated with the cement glands of *Lepas anatifera*. ICC, intracellular canals; SC, secondary canal; CC, collector canal.

FIGURES 14 and 15. Cement gland of *Lepas anatifera*. Chr, chromatin; ICC, intracellular canals; Nu, nucleus in wall of intracellular canal.

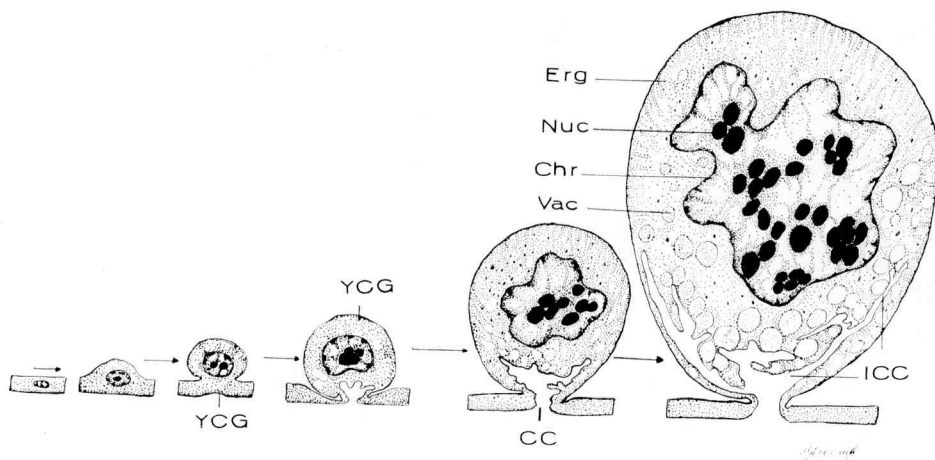


FIGURE 16. Diagrammatic representation of the development of a cement gland and its canals in *Lepas anatifera*. YCG, young cement gland; CC, collector canal; ICC, intracellular canal; Nuc, nucleolus; Chr, chromatin; Erg, ergastoplasm.

the tissue is stained with nuclear fast red using naphthol green as a counterstain, the more dense zone of the cell (CZ), stains green while the less dense zone at the opposite pole (FZ), stains red. The gland cells vary in size but at maturity they may reach 40 microns in diameter. They appear singly or in groups of twenty or more cells that possess a filamentous-like cytoplasm apparently rich in mitochondria. The nucleus of a small cell (YCG) is usually round but in larger or more mature cells (ACG) the nuclei show increasing degrees of polymorphism. Chromatin is dense in the small cells but is dispersed in the large cells. Twelve or more nucleoli may be seen clearly in larger cells when fixed in Flemming's fluid and stained with Heidenhain's iron hematoxylin.

The gland cells of *Lepas anatifera*, and their association with the canal system, are shown schematically in Figure 9. Other aspects of the canal system are shown in photomicrographs in Figures 10-15. These figures show:

1. A secondary canal (SC) between two cement gland cells (CG) (Fig. 10),
2. a secondary canal partially encircling a cement gland cell which contains a large amount of chromatin (Chr), (Fig. 11),
3. the cellular structure of a principal canal (PC) in a section where the canal bends upward providing both a transverse and a longitudinal view (Fig. 12),
4. the position of a collecting canal (CC) within the cytoplasm of a cement gland (Fig. 13),
5. and the nuclei (Nu) of the walls of the intracellular canals (ICC) within the gland (Figs. 14 and 15).

The development of a cement gland (YCG), its intracellular canals (ICC) and collecting canal (CC) are diagrammatically depicted in Figure 16. A squamous epithelial cell, destined to give rise to a cement secreting cell, enlarges on the

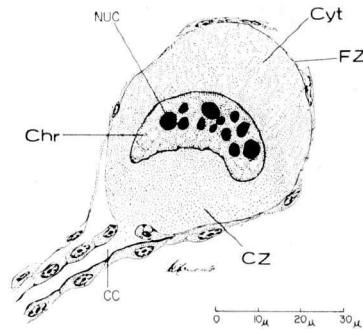


FIGURE 17. Schematic drawing of a cement gland and its canals in *Balanus tintinnabulum*. Chr, chromatin; CZ, accumulation zone for intracellular secretion; CC, collector canal; Cyt, filamentous-like cytoplasm; FZ, formation zone for intracellular secretion; Nuc, nucleolus.

wall of the secondary canal. As the cell grows the nucleus enlarges with a concomitant increase in chromatin (Chr), possibly the result of endomitosis. With continued growth, the walls of the secondary canal penetrate the cytoplasm, to form the collecting canal (CC) which branches into the tubular intracellular canals (ICC).

An individual gland cell of *Balanus tintinnabulum* is presented schematically in Figure 17 showing the two dense cytoplasmic zones (FZ and CZ), in which the cytoplasm at one pole is filamentous-like (Cyt). The other pole contains the collecting canal (CC) at the area where the glandular secretion accumulates. The nuclei, which may contain twelve or more nucleoli (Nuc), are polymorphic and have a villous surface.

An individual cement gland of *Lepas anatifera* is shown diagrammatically in Figure 18. The cytoplasm is not differentiated into two distinct zones but does contain many vacuoles (Vac). The nuclei are also polymorphic but unusually large. A fine connective tissue membrane (CT) is visible around the gland cells. Individual counts indicate the presence of more than 32 nucleoli (Nuc) of varying size surrounded by chromatin granules.

DISCUSSION

The more primitive barnacles, represented by *Lepas anatifera*, have the cement gland cells and canal system in the peduncle. In this species, cement formed in the gland cells is conducted through the primary canals to the base of the peduncle where it apparently hardens after contact with the substrate. Among the acorn barnacles, especially those members of the Balanidae with a calcareous basal plate like that of *Balanus tintinnabulum*, cement conducted through the primary canals passes into the circular canals at the base before it makes contact with the substrate.

Among closely related species of the Lepadidae, the distribution and the morphology of the cement glands and their canals may vary. Thus, Koehler (1889) reported that the cement glands of *Lepas* are limited to an area in the peduncle 2 to 3 millimeters beneath the capitulum. In *Scalpellum* the glands extend for a greater distance in the peduncle but in *Pollicipes* they are distributed

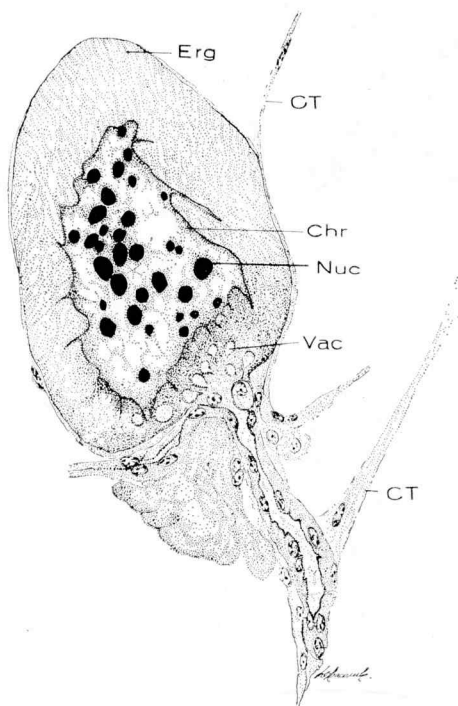


FIGURE 18. Schematic diagram of *Lepas anatifera* cement gland. Erg, ergastoplasm; CT, connective tissue membrane; Chr, chromatin; Nuc, nucleolus; Vac, vacuole.

along its entire length to the base. The cement glands of *Conchoderma*, another pedunculate barnacle, were found by Krohn (1859) to be situated in the mantle.

The distribution of the cement glands also varies with the species in the Balanidae (Lacombe and Liguori, unpublished results). For example, the cement glands of *Balanus tintinnabulum* are distributed among the interfollicular spaces of the ovaries and the connective tissue surrounding the ovarian follicles. In related species such as *Balanus nubilis*, the cement glands and their canals are not closely arranged in the interfollicular area.

Basic cytological differences in the cement gland cells of *Lepas anatifera* and *Balanus tintinnabulum*, indicate differences in the secretory mechanism. The large numbers of nucleoli, the presence of a zone of ergastoplasm and vacuoles distributed throughout the cytoplasm of the glands of *Lepas anatifera*, are characteristics of cells having a high level of metabolic activity. In this case, the glands correspond to the apocrine type in which secretory function is accompanied by a loss of some cytoplasm. The histological evidence indicates that the cellular secretion is transported to the intracellular canals in vacuoles formed in the cytoplasm. The walls of the intracellular canals, collecting canals and secondary canals have been found to be syncytial, a property which possibly enhances the transport of the soluble secretion across the cell membranes. The cement glands of *Balanus tintinnabulum* also have many nucleoli. However, they correspond to the

merocrine type gland. The difference in the staining reaction at opposite poles, indicates a difference in pH. The cytoplasm is filamentous-like rather than vacuolated suggesting that the secretion formed in the zone FZ, (Fig. 17), is transported in the cytoplasm to the opposite pole CZ, where it accumulates. The change in pH between these zones may account for an increase in solubility and passage across the membrane of the collecting canal.

Examination of the histological and cytological characteristics of barnacle tissues has helped to clarify the cement forming and conducting mechanisms in these animals. Details concerning the fine structure of the cement apparatus reported by Lacombe (1968) and under investigation in this laboratory, may further clarify the mechanisms of cement secretion and conduction. It is hoped that these studies, in conjunction with histoenzymological investigations of the cement apparatus (Arvy and Lacombe, 1968; Arvy and Liguori, 1968; Arvy, Lacombe and Shimony, 1968) may lead to the chemical identification of the adhesive material secreted by barnacles.

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SUMMARY

1. The histological characteristics of the cement apparatus of barnacles have been compared in *Lepas anatifera* and *Balanus tintinnabulum*.
2. In both species the cement is formed in unicellular glands and conducted to the points of attachment through a series of tubular canals.
3. The cement gland cells of *L. anatifera* differ from those of *B. tintinnabulum* in several cytological details which may indicate differences in the secretory mechanisms.

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