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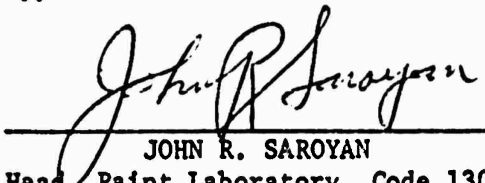
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ATTACHMENT MECHANISM OF BARNACLES
Fouling Prevention
The Study of the Adhesion of Calcareous Types
Attaching Marine Organisms
by
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FIRST PROGRESS REPORT

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Approved:


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ABSTRACT

The initial attachment of the barnacle is shown to be a purely mechanical hold by the suction cups of the cyprid antennae. An adhesive cement may be secreted for reinforcement but is not essential for permanent attachment. The Balanidae have permanent, periodically functioning glands which are located in the living mantle tissue. These glands develop directly from the cyprid cement glands. The cement glands and the rest of the cementing apparatus of the Balanidae are basically identical with those of the Lepadidae. The cementing apparatus is flushed after each cement secretion. In this way, old ducts are kept open for emergency repair or reattachment. This emergency secretion is expected to be chemically identical to the cyprid and the normally secreted adult cement.

SUMMARY

Problem

To study the attachment mechanism of the sessile barnacle with the goal of determining the chemical nature of the attaching cement. A knowledge of the chemical composition of the cement would make possible the following two objectives:

1. To develop a powerful adhesive for underwater or wet surface applications based on a hardening mechanism similar to that of the barnacle cement.
2. To develop improved antifouling coatings based on the principle of preventing the formation or hardening of the attaching cement or of reducing the adhesive strength.

Findings

The following new concepts of the attachment mechanism of the barnacles are demonstrated, explained and proposed:

1. The initial attachment of the barnacle is only a mechanical adhesion by means of the hydraulic suction cups at the tip of the cyprid antennae. There is a rigid chamber with one elastic membrane in the second and one in the third antennal segment. These chambers are connected. The muscles attached to the elastic membrane of the second segment can increase the volume of the chamber, which will result in fluid moving from the third to the second segment. This, in turn, will pull in the elastic membrane of the third segment which acts as a suction cup if the membrane is pressed on a substratum.
2. Calculations show that the adhesive force of the suction cups is strong enough to hold the cyprid in place against currents up to 186.4 cm/sec (3.6 knots).
3. The cyprid completes the attachment by folding the antennae, resulting in sealing the passage between the two chambers. Rigid prong and socket formations

of the second and third segments lock the antennae in this position; therefore, the suction of the cups can be maintained without muscle exertion and even after death.

4. The initial attachment is usually reinforced by an adhesive cement secretion, but the cement is not essential for achieving permanent attachment.

5. The pair of cyprid cement glands is retained by the adult and, in the *Balanus*, they are located in the living mantle tissue near the lateral part of the baseplate perimeter.

6. The cement glands are permanent organs throughout the life of the barnacle; they function periodically in relation with the moulting or growing period; and they do not degenerate after the secretory period.

7. During growth, as the demand for cement increases, new glands develop on each side, joining the older ones in a cluster.

8. The individual glands are connected by means of ducts. These ducts join on each side in a cell cluster from which the secretion is conducted toward the baseplate by one main channel.

9. The chemical properties of the secretion are altered in the cell cluster, probably indicating the secretion of a catalyst or crosslinking compound necessary for the cement hardening.

10. The two main channels are connected to the remnants of the second antennal segment of the cyprid at the point of initial attachment.

11. Both families of Cirripedia: the Balanidae and the Lepadidae have an anatomically identical cementing apparatus: (1) periodically functioning permanent glands on both sides in the mantle, (2) cement glands consisting of smaller units, which are connected by ducts at one point, at which the chemical properties of the secretion are altered and (3) main channels which conduct the secretion toward the initial attachment point.

12. The Balanidae develop a new duct network in each moulting period. Each network conducts the cement to the newly enlarged baseplate perimeter. After development, the duct network is moulted and becomes an integral part of the baseplate. These developments indicate that the shell-growing periods correspond with the moulting periods.

13. The originating points for the duct networks are the nodules, which form around the main channel. The nodules have no secretory function; they are only distributing chambers and in conjunction with the main channel, which goes through the nodules as a continuous tube, the nodules function as regulating valves.

14. A unique flushing mechanism removes the cement from the nodules, ducts, and from the vicinity of the orifices before the cement hardens. As a result, the older portions of the duct network are kept open and ready to be used if the baseplate detaches from the substratum or is injured.

15. Barnacles are also capable of developing new ducts leading into the injured or detached areas.

16. Since the cyprid cement gland is the basis of the adult cementing apparatus, and the same apparatus is used for secondary secretion, the cyprid cement, the primary or adult cement, and the emergency or secondary cement are chemically similar or identical substances.

17. The secondary cement, which is available in larger quantities than either the cyprid or the primary cement, can be collected for chemical analysis.

18. Furthermore, now that the true cement glands in the Balanus have been located, extraction of these glands makes the collection of the cement components or monomers possible for further studies.

Recommendations

In view of the encouraging results of the preliminary phase of the project, results which, in themselves, represent a basic contribution to the Biological Sciences on barnacle attachment, the following recommendations are made:

1. Continue studies to
 - a. Determine the chemical and physical nature of barnacle cement.
 - b. Determine the chemical mechanism of cement hardening.
 - c. Synthesize a "barnacle cement type" adhesive for underwater and wet surface conditions.
 - d. Produce improved antifouling coatings with antiadhesion properties.
2. Authorize funds for further study under the NAVSHIP RDT&E Program.

ADMINISTRATIVE INFORMATION

The study of the attachment mechanism of barnacles is the biological phase of the Mare Island Paint Laboratory project: Fouling Prevention, The Study of the Adhesion Mechanism of Calcareous Types of Attaching Marine Organisms. This project is part of the In-House Research/Independent Exploratory Development Program, NAVSHIPS Project No. SF020-99-02, Task 11906, authorized by NAVSHIPS letter 3900 Serial 03421-92 of 30 September 1966.

The work was initiated by Mr. John R. Saroyan, Head of the Mare Island Paint Laboratory, and was accomplished by Mr. Elek Lindner, Research Chemist, and Mrs. Carol A. Dooley, Chemist.

REPORT OF INVESTIGATION

INTRODUCTION

An investigation of the attachment mechanism of the sessile barnacles has been pursued to aid future studies on fouling problems with an emphasis on the determination of the chemical and physical properties of the cement. The attachment apparatus of cyprids and attached adult barnacles was studied in wholemounts, partial wholemounts, and tissue sections. The anatomy and development of the attachment apparatus were reconstructed and the concepts of its function were revised. Detached adult barnacles were successfully reattached, serving as models for studies of the attachment mechanism.

ATTACHMENT MECHANISM OF BARNACLES

MATERIALS AND METHODS

Attached adult barnacles were collected on glass, plexiglass, and rubber panels for studies of attachment. Barnacles removed from these panels served as sources of secretions for histochemical investigations and of embryos for rearing. The embryos were hatched and reared through the attaching cyprid stage by a modification of the techniques developed by Freiburger and Cologer (17, 18). Studies were made on the attachment organs and on the attachment mechanism of these cyprids in vivo, in whole mounts and in tissue sections.

The attaching cyprids and attached adult barnacles of the *Balanus crenatus*, *B. glandula*, and *B. improvisus* species were collected on standard glass microslides for microscopic study of the baseplate and attachment. The specimens were killed, fixed, decalcified, and stained on the original slides. Ten percent neutral buffered formalin by Lillie (1954) (27), Baker's (1944) formol-calcium (27), and Zenker's fluid were used for fixation. Specimens fixed in Baker's formol-calcium for 24 hours, decalcified in Jenkin's fluid (28) for 24 hours, and refixed in Baker's formol-calcium for 48 hours provided the most satisfactory results. For general histology, Mallory's Trichrome technique was adapted, but some special purpose stains and histochemical reactions were also employed for additional information.

For studying barnacles in microtome sections, solid paraffin blocks were exposed at the fouling sites. Barnacles attached to the paraffin were allowed to reach 3 to 6 mm in diameter before they were fixed in Baker's formol-calcium and decalcified in Jenkin's fluid, while still attached to the paraffin substratum. During the later steps of dehydration, the substratum was dissolved to leave the specimen with all substances between its baseplate and the substratum intact for imbedding and sectioning. These sections were treated with Mallory's Trichrome for general histology, and with some additional more specific stains and reagents.

INITIAL ATTACHMENT

BACKGROUND

In the literature, we find only a few investigations of the attachment mechanism and organs of barnacles, and the majority of these observations were made on stalked, rather than sessile cirripedia. There are even fewer references on the initial attachment mechanism of the cyprid larva. Darwin (13) observed that the disc at the end of each cyprid antenna becomes cemented to the substratum and suggested that the cement is secreted at the edge of the disc. Darwin's concept is generally accepted in the literature. Scattered literature references suggest that an adhesive is secreted at the tip of the antennae and that eventually this adhesive hardens to trap the cyprid on the substratum. Recently, Crisp (7) stated that the first antennae are provided with sucker-like discs and that the cyprid pours cement through the sucking discs to provide the initial fixation.

The behavior of the planktonic cyprid form of the barnacle at the time of the attachment was described in detail by several authors. As Visscher (34) noted, the cyprids "walk" by alternating the antennae on the substratum and "test" different areas before final attachment. The testing process can probably be explained by experiments of Pomerat and Weiss (29), Weiss (35), Gregg (20), Crisp and Meadows (9, 10), Knight-Jones (23), Crisp and Barnes (8), and Smith (30,31), which demonstrated that cyprids exhibit definite preferences for certain physical or chemical characteristics of the substratum. Visscher also observed that the antennae adhere so firmly that the cyprid can free them only with a violent struggle. Bernard and Lane (2) suggest that the cyprid eventually becomes unable to detach itself, and that this completes the attachment.

EXPERIMENTAL

Microscope preparations of the antenna of the *Balanus crenatus* cyprid (Fig. 1) show that the third segment is bell-shaped, having a flat, circular base with

a thin, flexible rim. The main body of the third segment is about 30 microns in diameter and 30 microns high; the rim is about 35-40 microns in diameter. The base membrane is an average of 5 microns thick. The 5 micron thick walls of the upper two-thirds of the third segment are composed of a rigid tissue (Fig. 2), probably chitinous, which does not shrink or change shape under the influence of aging, drying, or the chemicals used during preparations. These rigid walls form a chamber of constant shape leaving only the flat, circular base flexible. A similar rigid chamber is found in the tip of the second segment directly adjacent to the third segment. A thinner, elastic membrane stretching diagonally between the rigid ribs forms the wall of this chamber inside the antenna. The inner space of these chambers appears to be lacking in solid tissues. On the outside, the rigid walls of the second and third segment form prong-like projections.

Bernard and Lane (2) found that the antennae are folded to attain the proper position for final attachment. This is confirmed by examining the cyprid attachment and baseplates of adult *Balanus crenatus* and *B. glandula*, which always show both the third and part of the second segment touching the substratum (Fig. 3). Once this position of the antennae is assumed, the cyprid does not seem to be able to straighten the antennae. Even if the recently attached cyprid is detached by force, the antennae remain folded (Fig. 4). The folding of the antennae seems to complete the process of permanent attachment. We found no adhesive cement secretion up to this point. In fact, we found normally developed adult *B. glandula* specimens without any trace of cyprid cement (Fig. 3), which indicates that the cement secretion is not essential for achieving permanent attachment. In most cases, however, the permanent attachment is reinforced by an adhesive cement. This cement is believed to be secreted only after the antennae are folded into position, since it is shown that the third segment imbedded in the cement patch is always accompanied by part of the second segment (Fig. 5).

DISCUSSION

The anatomy of the third segment of the antenna indicates that this bell-shaped segment with its elastic baseside functions as a suction cup. The rigid chambers of the second and third segment are probably connected by a passageway, and they are filled with some fluid of low viscosity. Some of the powerful muscle bands of the antennae are probably connected to the thin membrane forming the top of the chamber in the second segment and increase or decrease the volume of this chamber by their action (Fig. 6). An increase in the volume of this chamber draws in some fluid from the third antennal segment. As a result, the volume of liquid within the chamber of the third segment is reduced, which causes the flexible circular baseside to be pulled inward. If the flexible rim is pressed on a substratum and provides a tight seal, a vacuum is created under the base membrane as it is contracted and the baseside becomes concave. The relaxation of the muscles results in fluid flowing back into the third segment, thus releasing the vacuum under the base. By this arrangement, the third antennal segment functions as a hydraulic suction cup and the chamber of the second segment controls the operation by an action similar to that of the ampoule of the starfish suction cup.

When the antenna is folded so that the tip of the second segment also touches the substratum, the passage between the second and third segment is sealed off and permits the suction to be maintained without further muscular exertion. The folded position of the antenna is locked by the socket and prong formation on the rigid chitinous shells of the second and third segments. This locking device is somewhat similar to that of the Elateridae or Click Beetles. After the antenna is locked into the folded position, the cyprid must struggle violently to free itself again.

If the cyprid is satisfied with the settling site, the antenna is permanently locked in the suction position, which will hold the suction cups in place even

after the death of the cyprid. The adhesive power of the suction cups alone is usually quite sufficient to keep the cyprid from being washed away by currents.

The atmospheric pressure and the weight of the water column above the suction cups work against the vapor pressure under it to hold the suction cups in place.

The standard atmospheric pressure $p_a = 1.0332 (10^3) \text{ g cm}^{-2}$

The water pressure at six feet exposure $p_w = 1.88 (10^2) \text{ g cm}^{-2}$

The vapor pressure at 14°C average temperature $p_v = 1.62 (10^1) \text{ g cm}^{-2}$

Total pressure $P = p_a + p_w - p_v = 1.2 (10^3) \text{ g cm}^{-2} \approx 1.2 (10^6) \text{ g cm sec}^{-2}$

The area within the rim of one suction cup $a = (10^{-3})^2 \pi = 3.14 (10^{-6}) \text{ cm}^2$

The total adhesive force of a pair of suction cups at optimum performance

$$F = 2Pa = 7.5 \text{ g cm sec}^{-2}$$

The adhesive force must be greater than the force of the water current exerted on the elliptical profile of the body, if the cyprid is to remain in place.

On the average, the cyprid of *Balanus crenatus* is 330 microns tall and 800 microns long.

The area of the elliptical profile $A = 2.08(10^{-3}) \text{ cm}^2$

The drag force of the current counteracts the adhesive force

$$F = \frac{1}{2} C_D \rho A v^2 = 7.5 \text{ g cm sec}^{-2}$$

C_D = drag coefficient

ρ = density of seawater = 1.025 g cm^{-3}

v = velocity of water current

Due to the small size of the cyprid, it is usually in the boundary layer.

The current velocity inside the boundary layer is

$$v = V \left(\frac{2y}{\delta} - \frac{y^2}{\delta^2} \right)$$

V = main current velocity

y = main distance of cyprid from substratum

= $1.65 (10^{-2}) \text{ cm}$

δ = thickness of boundary layer = $\frac{5.2 \times}{\sqrt{Re}}$

$$R_e = \text{Reynolds number} = V \frac{x \rho}{\mu}$$

x = distance of cyprid from leading edge of the
substratum = arbitrarily 1 inch

μ = absolute viscosity of seawater of 30 ‰ salinity

$$\text{and } 15^\circ\text{C} = 1.21 \times 10^{-2} \text{ g cm}^{-1} \text{ sec}^{-1} \quad (32)$$

$$v = V \left[2 \left(\frac{y}{5.2x} \sqrt{\frac{x\rho}{\mu}} \right) V - \left(\frac{y}{5.2x} \sqrt{\frac{x\rho}{\mu}} \right)^2 V \right]$$

$$\text{if } a = \frac{1}{\left(\frac{y}{5.2x} \sqrt{\frac{x\rho}{\mu}} \right)^2} = 2.97(10^3)$$

$$\text{then } V^4 - 4aV^3 + 2VaV^2 + a^2V^2 = 0$$

Preliminary calculations show that V current velocity will be in the order of magnitude of 10^2 cm sec^{-1} , then

$$R_e = V \frac{x \rho}{\mu} = 4.3(10^4)$$

$$\text{and } C_D(\text{circular disc}) = 1.12 \text{ at } R_e > 10^3 \quad (16) \text{ and } (19)$$

$$v = \sqrt{\frac{2F}{C_D \rho A}} = 7.94(10^1)$$

$$V^4 - 1.188(10^4) V^3 + 4.7(10^5) V^2 + 5.55(10^{10}) = 0$$

$$\text{by approximation by Newton's method } V_2 = V_1 - \frac{f(V_1)}{f'(V_1)}$$

$$V \approx 186.4 \text{ cm/sec} \approx 3.6 \text{ knots}$$

The cyprid can, therefore, withstand currents up to 3.6 knots using only its suction cups. This value is in good agreement with experimental data. (30)
(There is another value of about 230 knots as a solution of the fourth power equation. At this high velocity the laminar current goes through a transition into turbulent current; the drag coefficient (C_D) drops to about $3(10^{-3})$ resulting in a drastically reduced current force.)

The initial attachment, then, is only a mechanical adhesion by means of these hydraulic suction cups. The cement secretion, although usually present as a reinforcement, is not essential for permanent attachment.

CEMENTING MECHANISM

BACKGROUND

The basic anatomy of the cementing organs of both families of adult barnacles was described by Darwin (11,12). Based on studies on the Lepadidae, Darwin believed the cementing apparatus to be a modified part of the reproductive system; but Krohn (24) recognized the cementing apparatus as a separate organ. Pagenstecher (26) indicated that the cement ducts of an adult Lepas lead back into the remains of the cyprid antennae. Gruvel's (22) monograph borrows mostly from Darwin's description of the cement glands and ducts, but contributes a report on the functions of the glands of the Lepadidae. Krüger (25) studied the histology of the cement gland of Scalpella and recognized the periodic function of such glands. Based on Darwin's (11,12) and Gruvel's (22) work, Thomas (33) speculated that the cement glands of sessile cirripedia degenerate after functioning only for one molting period, and that they are probably modified tegumental glands.

EXPERIMENTAL

The mechanical attachment of the cyprid antennae is usually reinforced by a cement secretion. Both the suction cups and the tips of the second segments become imbedded in one dome-shaped cement patch. This patch seems to be secreted in two steps, since two concentric irregular circles can be recognized (Fig. 7). The inner circle is about 30 microns thick and 60 to 80 microns in diameter; the outer circle is only about 5 microns thick and 150 to 200 microns in diameter. Occasionally these two circles show different staining characteristics which could be due to a number of various causes, such as difference in age, oxidation, pH, degree of polymerization, chemical, or physical composition, etc.

Darwin (14), and recently Bernard and Lane (2), indicate that the cement is secreted by a pair of glands located behind the cyprid's compound eyes. Our investigation of these ovoid or kidney-shaped structures shows them to be 60 to 90 microns thick and 150 microns long (Fig. 8). A pair of ducts of about 10 microns in thickness

lead from the glands into the second segments of the antennae, where they are surrounded by clusters of cells, connective tissue and muscle fibers. The orifices of these ducts have not been located. The orifice is not likely to be inside the base membrane of the suction cup, because the secretion of the cement would break the vacuum and the hold before the cement could harden and become effective. Microtome sections through the patch and suction cups show little or no cement under the base membranes; the cement is mainly found piled up around and nearly covering the second and third segments. This observation seems to indicate the existence of an opening outside the suction cups.

The dissection of *Balanus glandula* cyprids, which achieved permanent attachment and secreted cement, shows that the cluster of cells surrounding the cement ducts in the second segment are connected to distinct spots in the second segment, which have staining characteristics similar to those of the cement (Fig. 9). In preparations of adult specimens we find that these same spots are the originating points for the main channels of the cementing apparatus (Fig. 10).

We know from the literature that the settled and attached cyprid moults the cyprid carapace and the exoskeleton of the body--with the exception of the embedded parts of the antennae--and soon takes up its adult shape. In the Balanidae, the main channel begins to grow perpendicular to the rostral--carinal axis toward the perimeter of the baseplate. In each growing period, new nodules form on the main channel and become the originating points of the separate duct networks. Darwin (12) and Gruvel (22) believed that these nodules are the cement glands, and that the Balanidae grow a new pair of such glands in each growing period because the former ones degenerate. (They recognized, however, that the Lepadidae have only two permanent cement glands.) Each nodule is larger than the former one and each duct network is more complicated than its predecessor. These networks grow on top of each other and end in numerous orifices at the corresponding baseplate perimeter. The end of the duct widens and forms a funnel to spread the cement around the perimeter under the growing baseplate in concentric circles.

The duct system develops in the epithelium of the mantle which is in contact with the calcareous baseplate. First, a number of cells concentrate around the area where the new ducts and nodules are going to develop (Fig. 11 and Fig. 12); then, the cells elongate and pack very close together (Fig. 13) so that the outline of the duct system appears clearly (Fig. 14). In further development, most of the cells disappear, leaving only a few clinging to the outside of the completed wall (Fig. 15). The developed ducts are still in intimate contact with the epithelium layer and the ducts will break if the epithelium layer is broken. Finally, the epithelium layer recedes completely from the formed ducts and the ducts are no longer affected by any damage to the epithelium layer (Fig. 16). Microtome sections show that the completed ducts are part of a thin acellular membrane that spreads across the baseplate and piles on top of the older such layers. This membrane corresponds to the other parts of the exoskeleton of the barnacle body and mantle, which are moulted away before each growing period. Similarly, the completed new duct network and the connecting membrane is moulted away in the barnacle. Since this membrane is in contact with the calcareous shell and not in open space, it cannot be expelled and, therefore, remains to become an integral part of the shell, permanently embedded in calcareous material. Despite the results of Costlow and Bookhout (3,4,5), who were unable to show any empirical correlation between moulting and baseplate growth, these have to coincide since each new duct network ends in orifices around the new baseplate perimeter and each duct network is physiologically moulted away with the rest of the exoskeleton.

The nodules develop through identical steps and at the same time as the ducts (Fig. 13). Morphologically, the nodules present the same appearance as the ducts (Fig. 17). No cell components, indicative of secretory functions, were ever found in the nodules. Therefore, contrary to previous beliefs, the nodules can not be cement glands but probably only collecting and distributing chambers for the cement.

Microscope preparations of *Balanus* indicated, that the main channel has a more important function than simply serving as a connection between the successive

nodules. Beyond the newest nodule, the main channel continues toward the perimeter of the baseplate. This main channel extension is about 30 microns in diameter and its walls consist of tightly packed cells. The 10 to 12 micron lumen is filled with a substance with staining characteristics similar to those of the cement. Near the perimeter, the main channel extension rises through the mantle tissue from the vicinity of the baseplate to just below the epithelium of the mantle cavity (Fig. 18). Here, the channel expands into a larger cell cluster (Fig. 19) which is the originating point for numerous side-channels that lead into different directions. It is noteworthy that the staining characteristics of the contents of the main and the side channels are different. Also, the cement-staining reactions seem to diffuse into the cells of the cluster at the meeting place of the main and side channels.

At the end of each side channel, there is a large round organ more than 100 microns in diameter containing vacuolae and substances having various staining characteristics (Fig. 20). These organs appear to be very similar to those we find in the Lepadidae and which were reported to be giant moncellular cement glands (22, 25) (Fig. 21). In both the Balanidae and the Lepadidae, there are usually several such glands on each side of the mantle and they form clusters.

DISCUSSION

The cement gland appears first in the planktonic cyprid larva stage of the barnacles. During the settling procedure and metamorphosis to the adult form most of the cyprid organs undergo histolysis, tissue changes, reorganization, and migration. Bernard and Lane (2) show that, in *Balanus amphitrite niveus*, the cement glands: "the post optic organs undergo considerable functional modification" during and after settling, but "they retain the same size and position." They indicate, however, that these glands disappear in the next stage of development, which they call the "decorticated settler" stage. This stage is supposed to take place between the settled cyprid and the young adult stage. It is characterized

by a completely amorphous, "soft, translucent, gelatinous, cellular mass," with "no sign of appendages or of crustacean segmentation."

In *Balanus crenatus* and *B. glandula* we failed to recognize this decorticated settler stage. These species seem to moult the cyprid carapace and the body exoskeleton to reveal a fairly articulate adult form. Under the shedding exoskeleton of the cyprid thoracic appendages, the adult cirri appear, which curve into the opposite direction (Fig. 22). The internal organs also seem to be organized at this point.

We also showed that the main channel of the adult cementing apparatus originates from the same point in the second antennal segment of the cyprid to which the cyprid cementing apparatus seems to be connected (Fig. 9 and Fig. 10). These observations suggest the concept that the cyprid and the adult cementing organs are closely related and that they do not undergo much change during the metamorphosis. The morphological similarity of the cyprid and adult cement glands seems to support this concept.

The cyprid cement glands are probably retained by the adult (Fig. 23). During the settlement and metamorphosis to the adult stage, these glands migrate from their position behind the compound eyes of the cyprid to the perimeter of the new baseplate of the adult perpendicular to the axis of symmetry of the body. As the adult barnacle grows, the cement glands remain in the living tissue of the mantle in the same relative position. The larger the baseplate grows, the more cement is needed for adhesion; therefore, periodically, new cement glands develop and join the existing ones, forming a cluster of glands on each side of the mantle. The individual glands of the same cluster are connected to each other by channels. These channels join in a cluster of cells, from which the main channel on each side conducts the secretion toward the baseplate. The main channel connects the glands and the remains of the cyprid antennae at the point of the initial attachment.

We recognize now, that the whole cementing apparatus--the cement glands; the side channels joining in a cluster of cells; and the main channels which conduct the cement toward the point of initial attachment--is anatomically identical in the two families of the sub-class Cirripedia: the Balanidae and the Lepadidae. The cement glands of both families are permanent, and they function periodically in relation to the moulting or growing periods.

In the Balanidae, in each growing period a new nodule is formed on each main channel and becomes the starting point for each new duct network. The duct network ends at the perimeter of the baseplate in numerous orifices and spreads the cement around the edges. The nodules and the ducts are developed by the epithelium as part of a continuous membrane on the inner side of the baseplate. This membrane is actually part of the exoskeleton and would be moulted away with the rest, but the baseplate prevents this from occurring. This membrane with the ducts and nodules piles on top of the other membranes from earlier growth periods and becomes an integral part of the baseplate. In the Balanus species with calcareous baseplates, this layer becomes permanently embedded in the calcareous material. Once the duct network is moulted, it is no longer living tissue despite the fact that it remains in close contact with the rest of the system by means of the main channel. The moulted ducts, therefore, can not grow and reach the enlarged perimeter of the next growing period. Consequently, a new duct network must be produced to reach the newly developed areas under the enlarged baseplate.

We demonstrated that the contents of the side channels originating from the cement glands abruptly change their staining characteristics at their joining point with the main channel. This indicates a change in chemical composition of the cement at this point. Our knowledge of the chemical composition and the hardening mechanism is still limited. We can safely assume, however, that the hardening process is probably due to some sort of polymerization or cross-linking.

Such a reaction mechanism can be initiated spontaneously or by catalysts. Studies are being continued to demonstrate whether different glands of the cluster produce different chemicals which are mixed at the joining point to start the reaction; or the changes in the secretion are due to pH changes at the joining point; or some small quantities of catalysts or cross-linking compounds are secreted by the cells around the joining point, to be mixed with the bulk of the secretion passing by. The fact that the cytoplasm of the surrounding cells stains similarly to the hardened cement indicates that the cell cluster possibly secretes small quantities of a chemical of low molecular weight that may be a catalyst or cross-linking compound.

In conclusion, the completed adult cementing apparatus in the Balanidae is anatomically and probably functionally identical with that of the Lepadidae. The cyprid cement gland is retained by the adult and is the basis for the adult cementing apparatus.

SECONDARY ATTACHMENT

BACKGROUND

The walls and the baseplate of the barnacle shell are tightly connected by numerous muscle tissues around the baseplate perimeter at the inside joints of the leading edges. As a result of muscular action, the edges of the walls and the baseplate are pressed tightly on the substratum to leave only a very slight gap. The baseplate may grow into the recessions and over the protuberances of a solid substratum, faithfully duplicating its surface structure (21). The forces produced by the growing barnacle enable it to plow away loose deposits, fouling organisms, and detritus and to dig beneath soft materials, such as clay or certain coatings (1) in order to reach an underlying solid surface. In these ways the barnacle obtains a maximum contact area for adhesion.

The baseplate is generally cemented so firmly to the substratum by an adhesive substance that the shell will usually break when an attempt is made to detach the barnacle. This adhesive substance is secreted at the perimeter of the baseplate and spreads under it to fill any gap between baseplate and substratum. Due to the pressures exerted by the barnacle, normally the gap to be filled and hence, the thickness of the cement layer, is less than 5 microns.

In crowded communities, however, the barnacles may develop abnormally. For example, in such communities, *Balanus balanoides* grows into elongated shapes, and Darwin observed that sometimes only the walls of such specimens reach the substratum, while the non-calcareous basal membrane remains suspended and deeply concave (15). Darwin noticed that "thickish roots" were found hanging from the basal membrane, in the resulting gap. He believed these roots to be cement.

This development form apparently escaped the interest of later investigators since very little reference can be found on this subject in the literature. Crisp describes specimens which survived complete upward displacement by neighboring barnacles but does not mention any adhesive secretion (6). Crisp also found that *Balanus balanoides* have some limited mobility under lateral pressure by neighboring barnacles and can be moved along the surface of a smooth substratum several centimeters away from their original point of attachment. Crisp speculated that the advancing edges form new adhesions as the barnacle gradually undergoes lateral displacement.

EXPERIMENTAL

During the collection of large specimens of *Balanus nubilus*, one of the barnacles suffered a sizable crack in its baseplate. After 24 hours, an abundant white, opaque, rubbery exudate, or secondary secretion was found, filling and sealing this injury (Fig. 24). On numerous occasions it has been observed that barnacles sustain injury such as cracks or breaks in their baseplate as a result of the forces produced either by themselves or their neighbors. If vital organs are not seriously damaged or if the injury is not too extensive, the barnacle may survive such an accident by repairing the injuries with these secretions.

Secondary secretions were found not only in injured barnacles, but also in specimens of *Balanus crenatus* and *B. glandula* that were partially or completely separated from the substratum. Some of these separations appear to have been caused by excessive force exerted by the barnacle itself in an effort

to press the growing edges of the baseplate close to the substratum. The pressures created at the perimeter may result in lifting and detaching the central portion of the baseplate. Such baseplates become concave, and the ensuing gap is usually filled with the secondary secretion (Fig. 25).

Microtome sections of these thick layers of secretions often have a cavernous and vertically striated appearance (Fig. 26). This effect is probably caused by the continuous recession of the baseplate from the substratum. The gap created between baseplate and substratum is filled with the fluid secretion, but before hardening can take place, the baseplate continues to recede. The already viscous secretion may then pull threads of materials between the two surfaces, thus creating a loose structure. These new gaps are then filled with fresh secretions during the next period; and so the process is continued until the recession either ceases or continues at so rapid a rate that the secretory system is no longer able to supply enough material to fill the gap.

Another type of separation can occur in gregarious communities where the sidewalls of neighboring barnacles may be fused together. Since the walls grow up from their bases, a faster growing specimen may lift up and detach a slower growing one from the substratum. The space between the elevated barnacle and the substratum is usually then filled by the opaque secretion. If the gap is too large to be filled, any secretion present may be seen hanging suspended from the baseplate, indicating that an effort was made by the barnacle to reach the lost substratum and to reattach.

In the laboratory, barnacles were detached intact from smooth test panels (Fig. 27) and subsequently reattached to other smooth surfaces, such as glass microscope slides (Fig. 28). These specimens could be then kept alive indefinitely with proper care. A white, opaque substance is secreted, which spreads between baseplate and substratum if they are in close proximity. The closer the

contact of the two surfaces, and hence the thinner the secretion layer, the firmer the reattachment appears to be. The reattachment can be so strong that the shell walls and body will break away before the baseplate can be detached. If the intervening space between baseplate and substratum is too large to be filled, thick droplets of secretion appear and hang from the baseplate (Fig. 29).

Assuming that the secretion is cement, it could be expected that the secretion would originate from the perimeter of the baseplate, where the newly developed cement duct orifices are located. In general, secretion does appear at the perimeter, from which it then spreads under the separated or injured area. Occasionally, however, an extensive separation or injury occurs isolated within the perimeter and the cement secreted at the perimeter cannot reach the effected areas in sufficient quantities. Indications are that the barnacle is able to grow new irregular ducts into such a damaged area (Fig. 30). These emergency or secondary ducts are larger in diameter than the normal ducts and have few or no bifurcations. Such a duct extends directly from the newest formed nodule to the damaged area where it ends in an orifice. Normally only the first nodule formed after metamorphosis would have such a duct leading from it, as with each succeeding growth period, the duct system becomes increasingly complex in the number of branchings before the final orifices are reached.

The secondary ducts usually can be found only in those detached or injured areas where no old primary ducts and orifices can be found, as for example, near the cyprid attachment where the first available duct ends are outside the perimeter of the innermost circle. There is probably a unique mechanism that enables the barnacle to recognize the need for new ducts and to initiate the growth of these unusual ducts. Likewise, the dissolution of the calcareous matter to permit the growth of new ducts into old sites must somehow occur; similar processes are known in nature.

In the majority of cases, however, when detachment involves baseplate areas inside the baseplate perimeter, the reattaching secretion seems to originate

from old primary duct ends. This reuse can be seen in whole mounts of the baseplates where several layers of additional secretion lie around the old duct ends (Fig. 31). These secretions appear in discrete layers, indicating that there was enough time for one layer to harden before the next one was laid down. Since, in the course of normal development, the edge of the growing baseplate is pressed tightly on the substratum and the cement is spread in a very thin layer, those thicker layers of secretion must have appeared at a time subsequent to the growth period during which the primary secretion took place. Therefore, we consider these thicker layers to be secondary secretions or secondary cement.

DISCUSSION

It was shown that in most cases, an injured or detached barnacle uses the old duct system for repair or reattachment. This reuse is possible only if the duct system connected to these areas is still functional and the passages are still open. It would seem that since the ducts were filled with primary cement, when the cement hardens, the ducts would be plugged with solidified material and thereby rendered useless for subsequent secretion. Microscopic preparations, however, show most of the old, previously used ducts to be empty (Fig. 26).

It is believed that initially the cement is a fluid of low viscosity and solidifies within a short time after secretion from the duct system. The presence of an occasional duct filled with hardened cement suggests that the hardening process is not restricted to the hydrospace outside the duct system and that the cement is able to harden in the ducts, but is somehow removed before it can set. This removal may be due to a flushing process.

The flushing of the ducts after cement secretion and the recementing capabilities of the barnacle, as we now theorize, can be explained only if the cement is produced somewhere outside the duct and nodule system, and not in the nodules as previous investigators believed (11,15,22). We showed that the true glands are located in the living mantle tissue at the end of the main

channel. The freshly produced liquid cement flows through the main channel from its origin, the peripherally located glands, toward the centrally located initial attachment. At the site of the outermost and newest nodule or distributing chamber, the cement pours out into the adjoining duct network to be secreted through the duct ends at the perimeter. The cementing period ends when the flushing fluid, which is probably water-soluble, displaces the still liquid cement from the ducts (Fig. 34B). In the course of normal development, this flushing substance forces the cement out beyond the duct orifices and away from the edges of the baseplate. The cement hardens in this position under the cuticle of the joint connecting baseplate and walls. In this manner, a ten to forty micron wide circular channel is formed between the edge of the baseplate and the hardened cement, leaving the flushing fluid contained within this seal (Fig. 32). This flushing fluid may be modified cement, consisting of its monomers or of derivatives that may lack catalysts or contain polymerization inhibitors.

Although the nodules, as well as the rest of the duct network, are embedded in calcareous material in those species which possess calcareous baseplates, the nodules are not functionless organs. The nodules, in conjunction with the main channel, represent a unique system that is still able to function efficiently to prevent the flushing fluid from backing up and mixing with the new cement and to direct the cement to where it is needed.

The main channel goes through the nodules as what appears to be a continuous tube rather than as a simple connection (Fig. 33). On the basis of staining characteristics, the main channel appears to be composed of tissue different from that constituting the nodules and ducts. The main channel develops previous to the nodules; its walls are smooth and not scaly like the walls of nodules and ducts. The nodules and ducts are probably composed of chitinous material and are resistant to deterioration long after the death of the animal, while the portion of

the main channel inside the nodule soon deteriorates. This portion of the main channel is probably semipermeable, permitting transflux into the nodule only. As long as the flushing fluid fills the ducts and nodules, the system remains in balance and no liquid passes through the semipermeable walls. In the course of normal development, therefore, the new cement does not go beyond the outermost and newest nodule because the rest of the main channel and duct network is filled with the flushing fluid and no room is available for the cement (Fig. 34A). The new cement simply pours into the new nodule and duct network to be secreted at the perimeter.

However, if the baseplate separates from the substratum, the cement seal of some duct-ends breaks and the flushing fluid drains out of the corresponding ducts and nodules. In the same fashion, a fracture in the baseplate would sever some ducts and the flushing fluid would also leak out at the injury site, followed by the cement (Fig. 34C). Since the duct networks of different growing periods are completely isolated from each other except at the nodules, which are connected only by the main channel, the flushing fluid drains only from duct ends which are effected by the injury and only from those ducts which represent the shortest route from injury to the corresponding nodule. The pressure inside this nodule decreases due to fluid loss and the liquid from the main channel passes through the semipermeable walls into the nodule cavity where it is also lost to the injured site. This loss of fluid is followed by the fresh cement out to the injury. Other ducts, unconnected with the injured area, remain filled with the flushing fluid and, hence, the cement bypasses those nodules which serve the unaffected duct network.

Thus, the nodules serve as distributing chambers and that portion of the main channel within the nodules controls and regulates the flow of cement and flushing fluid, as would a valve. The nodules of different age are situated near each other and are connected by the main channel. This arrangement puts

all the nodules, the corresponding duct network, and especially the duct ends, regardless of the network to which they belong, almost equidistant from the cement glands at the end of the main channel. Thus, it is practically as easy to secrete cement through older ducts as through the newest, peripheral duct system. After emergency use, the system is again flushed out and ready for further reuse. In repeated use, however, the flushing process is not always complete because usually larger and irregular amounts of cement are secreted at the emergency locations, thus plugging the orifices. Hence, those reused ducts and nodules could contain hardened cement.

Since this secondary cement is secreted at the same time as the primary cement and is a product of the same cementing apparatus, it can be inferred that the two substances are chemically identical.

CONCLUSIONS

1. The initial attachment of the barnacle is only a mechanical adhesion by means of the hydraulic suction cups at the tip of the cyprid antennae. The initial attachment is usually reinforced by an adhesive cement, but the cement is not essential for achieving permanent attachment.

2. Both families of Cirripedia, the Balanidae and the Lepadidae, have an anatomically identical cementing apparatus: (1) periodically functioning permanent glands on both sides in the mantle; (2) cement glands consisting of smaller units, connected at one point at which the chemical properties of the secretion are altered; and (3) main channels which conduct the secretion toward the initial attachment point.

3. The cyprid cement glands are retained in the adult, where they are found in the living mantle tissue perpendicular to the axis of symmetry of the barnacle. These glands become the basis of the adult cementing apparatus.

4. The development steps of the duct network in the Balanidae indicate that the growth periods of the baseplate correspond with the moulting periods.

5. A flushing mechanism removes the cement from the cementing apparatus before hardening can take place. Therefore, the duct network is kept open and ready for emergency reuse. The nodules, formerly thought to be the cement glands, function as regulating valves for both the cement secretion and the flushing process.

6. We demonstrated that the primary cement secreted by the adult barnacle during normal development and the emergency secondary cement are products of the same cementing apparatus and are secreted simultaneously. It is expected, therefore, that the primary cement and the secondary cement are chemically identical substances. As it was shown that the cyprid cement is retained and becomes the basis of the adult cementing apparatus, the cyprid cement should also be a similar chemical substance.

Recommendations

In view of the encouraging results of the preliminary phase of the project, results which, in themselves, represent a basic contribution to the Biological Sciences on barnacle attachment, the following recommendations are made:

1. Continue studies to
 - a. Determine the chemical and physical nature of barnacle cement.
 - b. Determine the chemical mechanism of cement hardening.
 - c. Synthesize a "barnacle cement type" adhesive for underwater and wet surface conditions.
 - d. Produce improved antifouling coatings with antiadhesion properties.
2. Authorize funds for further study under the NAVSHIP RDT&E Program.

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LIST OF FIGURES

Figure 1. Section of cyprid antenna: (A) Third segment, (B) Second segment (10 μ section, Mallory's Trichrome, 625x)

Figure 2. Balanus crenatus cyprid antenna: (A) rigid shell, (B) flexible base (wholemount, Mallory's Trichrome, 625x)

Figure 3. Balanus glandula cyprid attachment in adult baseplate (A) third segment and (B) folded portion of second segment. No cement. (wholemount, TriPARS, 425x)

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Figure 5. Section through initial attachment of Balanus crenatus adult: (A) folded portion of second segment, (B) bell-shaped third segment, (C) cyprid cement patch. (10 μ section, Mallory's Trichrome, 625x)

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Figure 11. Slight concentration of cells around area where new duct will be formed. (wholemount, Mallory's Trichrome, 475x)

Figure 12. Increased concentration of cells about new duct site (wholemount, Mallory's Trichrome, 475x)

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Figure 14. Cells pack very closely, clearly showing outline of new duct. (wholemount, Mallory's Trichrome, 475x)

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Figure 16. Completed duct has now become separate from the epithelium. (wholemound, Mallory's Trichrome, 475x)

Figure 17. (A) Nodules with (B) main channels passing through them, morphologically are very similar to the (C) ducts which branch from them. (wholemound, Gallego-Garcia, 375x)

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Figure 19. (A) Cell cluster found at end of main channel extension, (B) cement gland (12 μ section, Mallory's Trichrome, 250x)

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Figure 22. Adult cirri emerging from moulted cyprid exoskeleton in living *Balanus crenatus* specimen. (100x)

Figure 23. Metamorphosis of cyprid to adult barnacle showing the migration of the cyprid cement glands to the position in which they are retained and function in the adult.

Figure 24. Large specimen of *Balanus nubilus* suffered a crack in its baseplate. Within 24 hours, the secretion seen here hardened and repaired the crack.

Figure 25. Abnormal concave baseplate on *Balanus crenatus* specimen detached from a solid substratum in the laboratory shows abundant secretions in an attempt by the barnacle to fill in the central gap.

Figure 26. Sagittal section through baseplate area of *Balanus crenatus* that was attached to paraffin shows (A) thick irregular layers of cement secretion, (B) empty ducts, (C) duct containing cement and (D) substratum. (10 μ section, Mallory's Trichrome, 250x)

Figure 27. Baseplate of *Balanus crenatus* specimen after detachment from test panel in the laboratory.

Figure 28. Baseplate of *Balanus crenatus* shown in Figure 27 after it reattached to a glass microscope slide.

Figure 29. Reattached *Balanus crenatus* specimen. Side A was reattached firmly to a glass microscope slide, while B was left suspended. Drops of secretion hang from the baseplate on the side where the barnacle was unable to reattach and adhere to the substratum.

Figure 30. Growth of new, irregularly-shaped ducts originating from (A) nodules into the (B) damaged initial attachment area. (wholemound, TriPARS, 100x)

Figure 31. Thick, circular layers of secondary cement at the orifices of old ducts. (wholemound, TriPARS, 255x)

Figure 32. Perimeter of an inner baseplate circle with (A) empty duct and (B) funnel-shaped orifice leading to the perimeter. The flushing process, following the cement secretion, has washed both the duct area and (C) a circular ring around the edge of the baseplate free of (D) cement before hardening took place. (wholemound, Mallory's Trichrome, 375x)

Figure 33. Section through baseplate of an adult *Balanus crenatus* showing (A) the main channel and (B) the nodules through which it passes. (12 μ section, Mallory's Trichrome, 325x)

Figure 34. (A) Secretion of cement from cement gland to newest nodule and duct network during normal development, (B) flushing of duct network following cement secretion and (C) secretion of cement when a separation from substratum has occurred in the region of an old duct

PLATE 1



FIG. 1



FIG. 3



FIG. 4



FIG. 2



FIG. 5

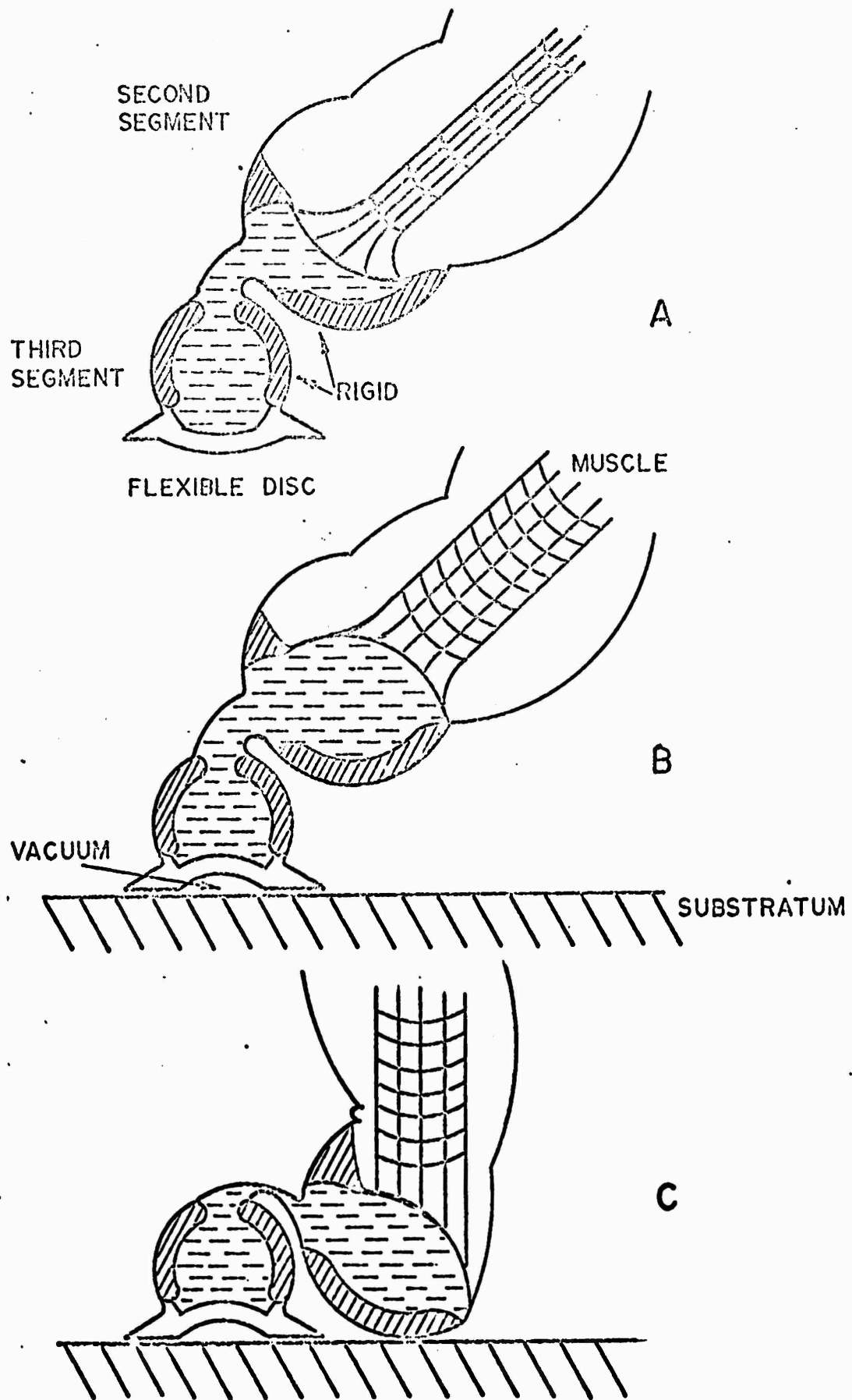


FIG. 6.

PLATE 2



FIG. 7



FIG. 9



FIG. 8



FIG. 10

PLATE 3

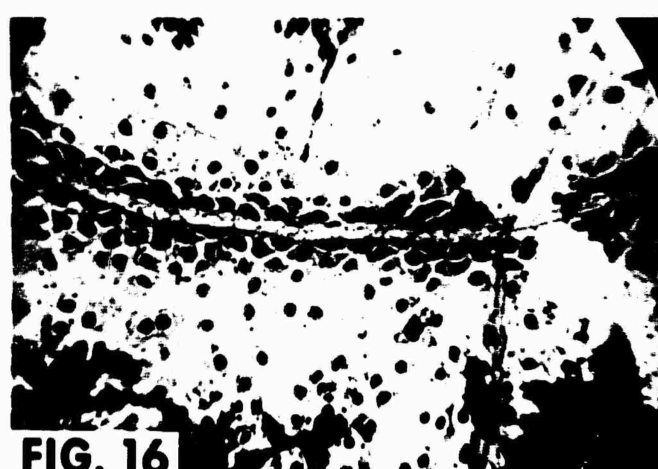
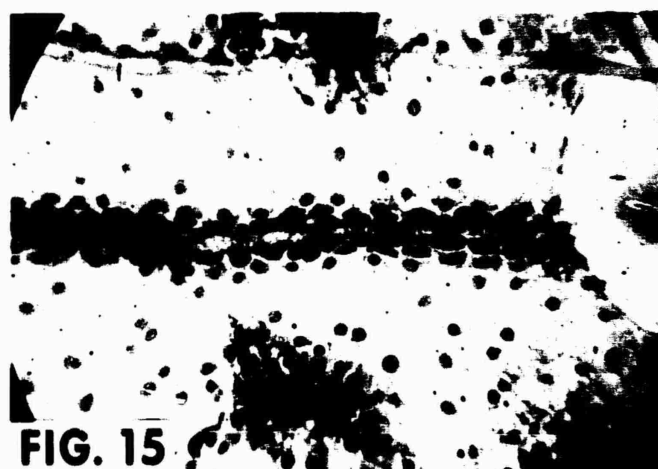
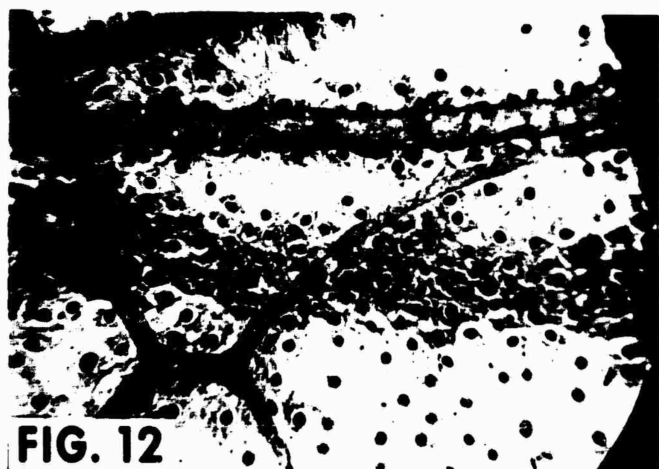
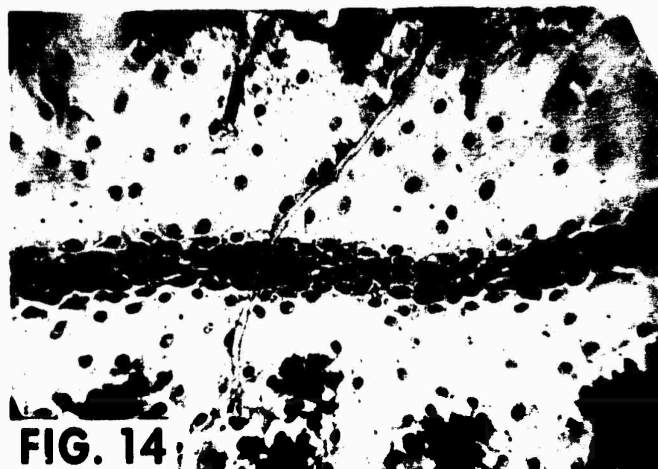
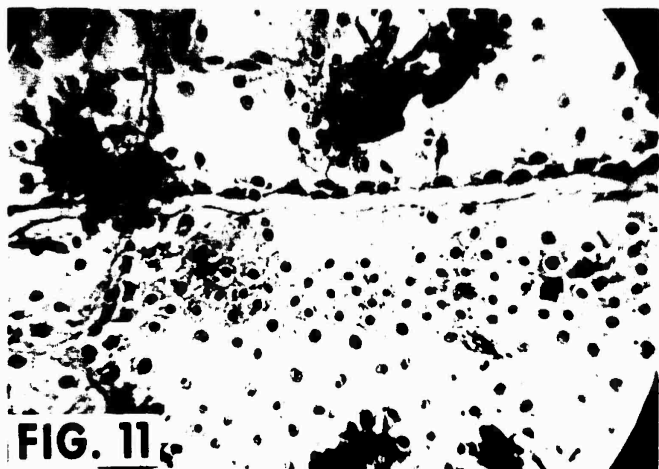


PLATE 4



FIG. 17

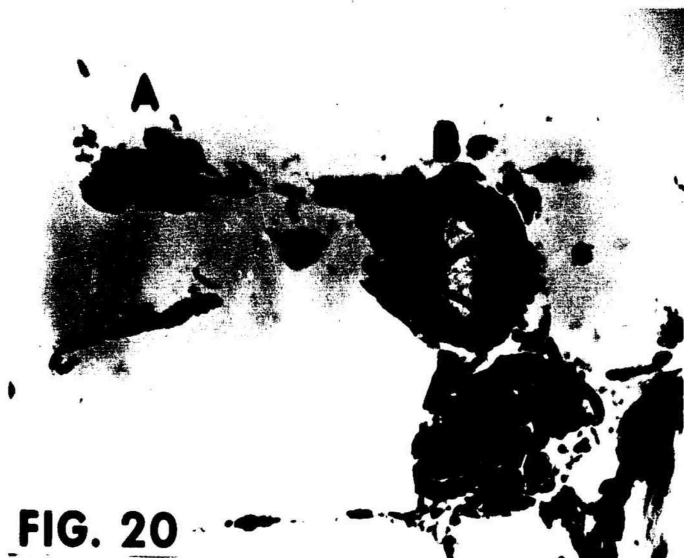


FIG. 20

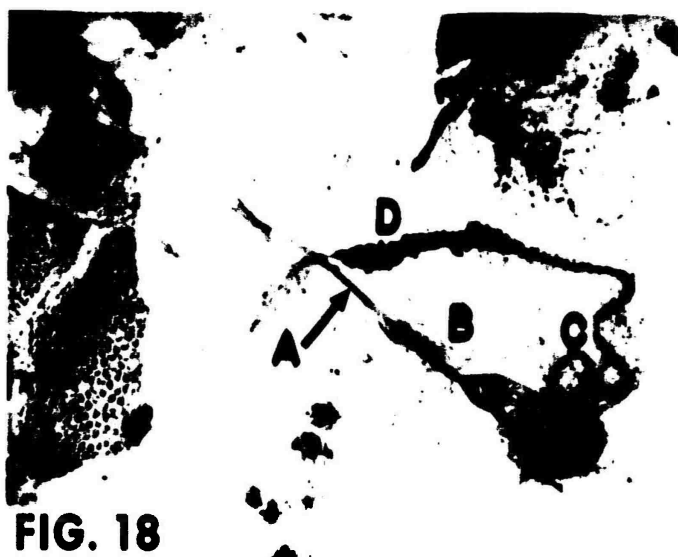


FIG. 18



FIG. 21

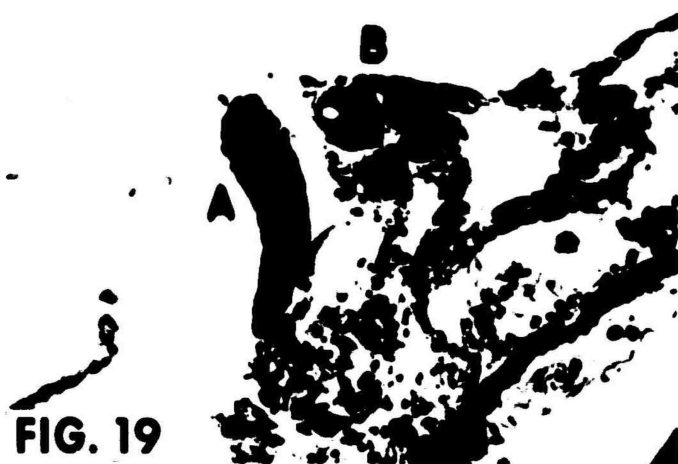


FIG. 19



FIG. 22

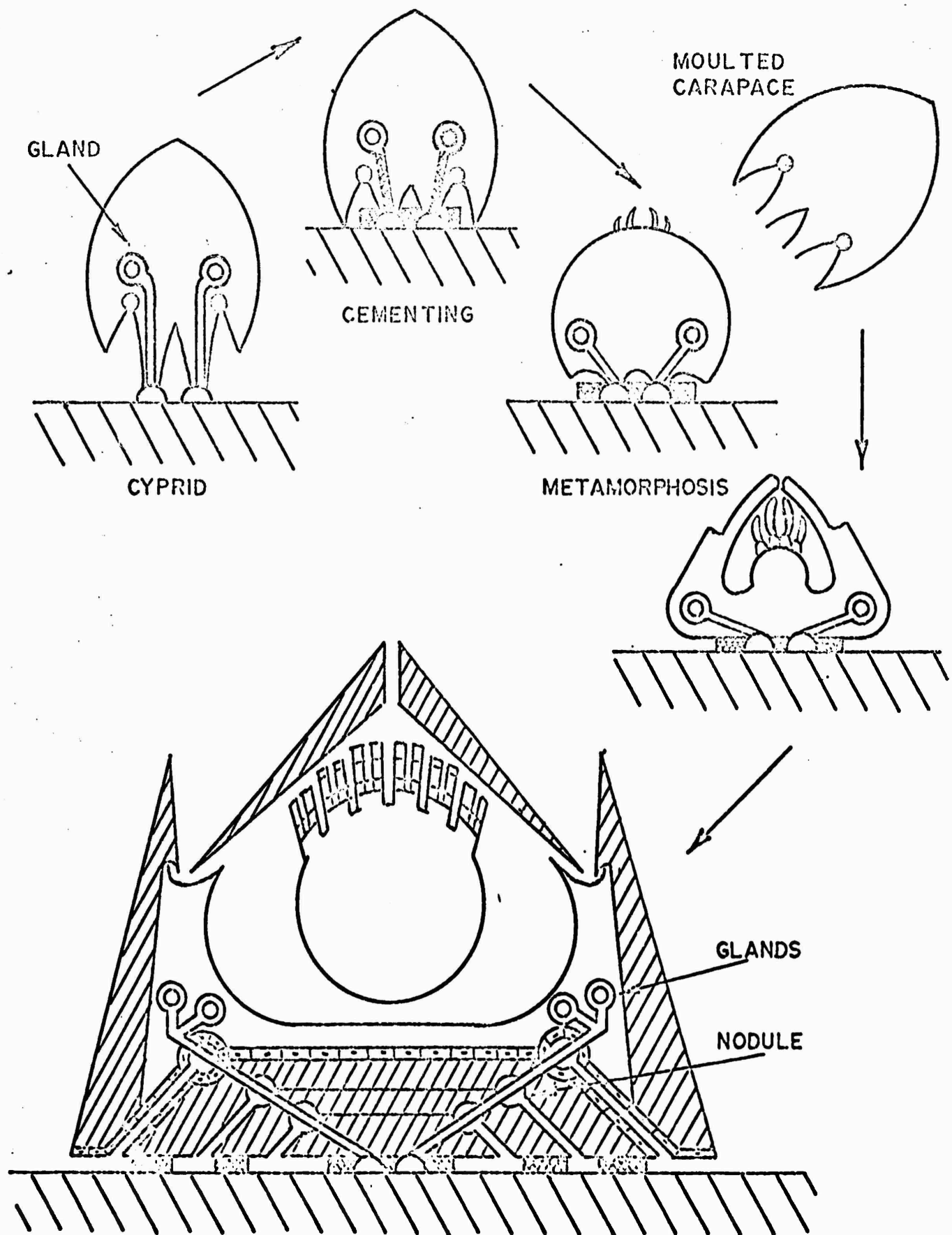


FIG. 23.

PLATE 5



FIG. 24



FIG. 25



FIG. 26

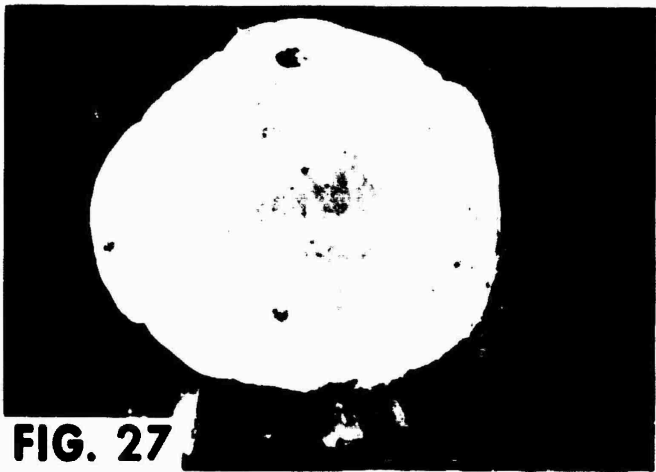


FIG. 27

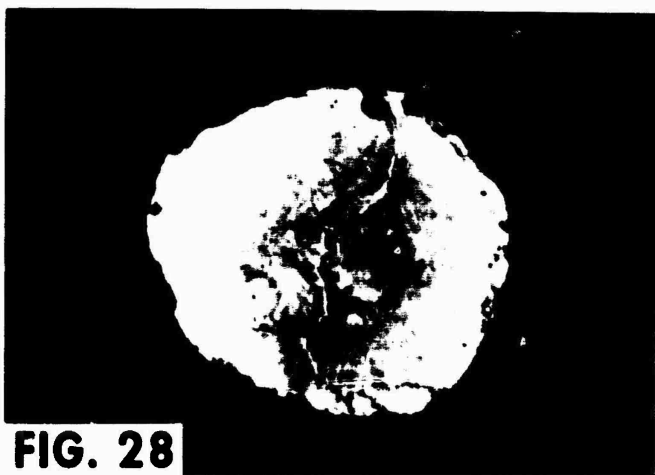


FIG. 28

PLATE 6



FIG. 29



FIG. 31

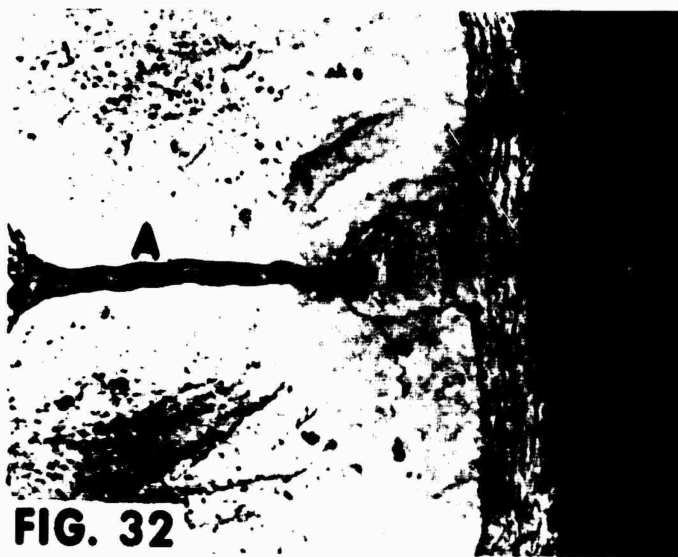


FIG. 32



FIG. 30

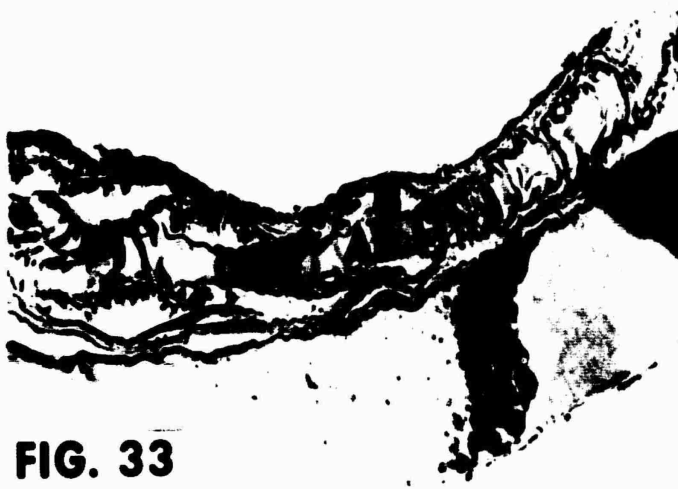


FIG. 33

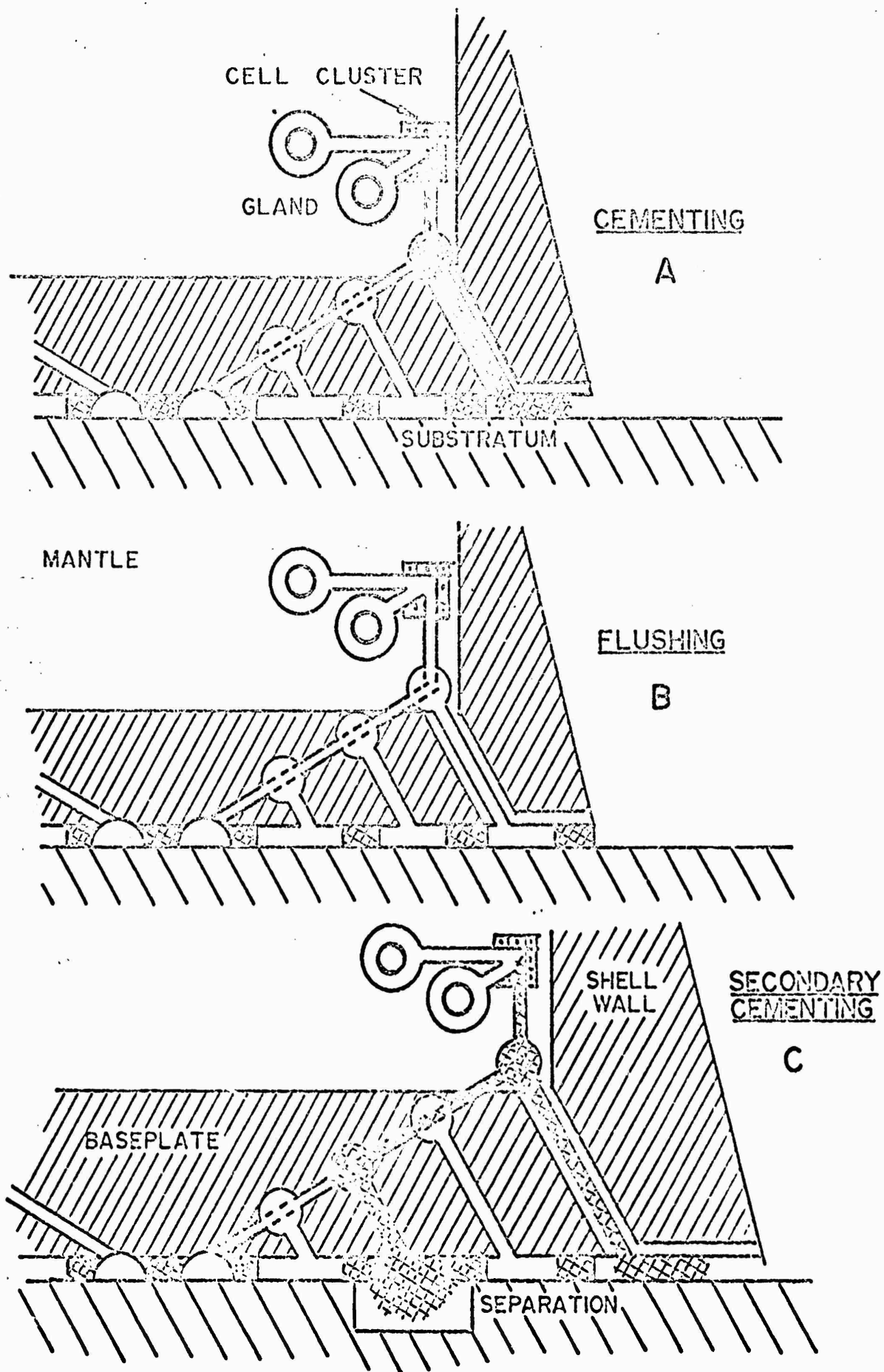


FIG. 34.

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13. ABSTRACT The initial attachment of the barnacle is shown to be a purely mechanical hold by the suction cups of the cyprid antennae. An adhesive cement may be secreted for reinforcement but is not essential for permanent attachment. The Balanidae have permanent, periodically functioning glands which are located in the living mantle tissue. These glands develop directly from the cyprid cement glands. The cement glands and the rest of the cementing apparatus of the Balanidae are basically identical with those of the Lepadidae. The cementing apparatus is flushed after each cement secretion. In this way, old ducts are kept open for emergency repair or reattachment. This emergency secretion is expected to be chemically identical to the cyprid and the normally secreted adult cement.			

Mare Island Paint Laboratory, San Francisco Bay Naval Shipyard, Vallejo, California 94592 ATTACHMENT MECHANISM OF BARNACLES, The Study of the Adhesion of Calcareous Types of Attaching Marine Organisms--First Progress Report by J.R. Saroyan, E. Lindner & C.A. Dooley Paint Lab. Rep. 68-1, Feb 1968, 48 pp., incl illus., tables & appendixes (Project SF020-99-02 Task 11906) Unclassified Report The initial attachment of the barnacle is shown to be a purely mechanical hold by the suction cups of the cyprid antennae. An adhesive cement may be secreted for reinforcement but is not essential for permanent attachment. The Balanidae have (continued on back)	UNCLASSIFIED Barnacle Attachment Adhesive cement Balanus Cirripedia Lepadidae Fouling Antifouling Biology DISTRIBUTION: Obtainable from DDC	Mare Island Paint Laboratory, San Francisco Bay Naval Shipyard, Vallejo, California 94592 ATTACHMENT MECHANISM OF BARNACLES, The Study of the Adhesion of Calcareous Types of Attaching Marine Organisms--First Progress Report by J.R. Saroyan, E. Lindner & C.A. Dooley Paint Lab. Rep. 68-1, Feb 1968, 48 pp., incl illus., tables & appendixes (Project SF020-99-02 Task 11906) Unclassified Report The initial attachment of the barnacle is shown to be a purely mechanical hold by the suction cups of the cyprid antennae. An adhesive cement may be secreted for reinforcement but is not essential for permanent attachment. The Balanidae have (continued on back)	UNCLASSIFIED Barnacle Attachment Adhesive cement Balanus Cirripedia Lepadidae Fouling Antifouling Biology DISTRIBUTION: Obtainable from DDC
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