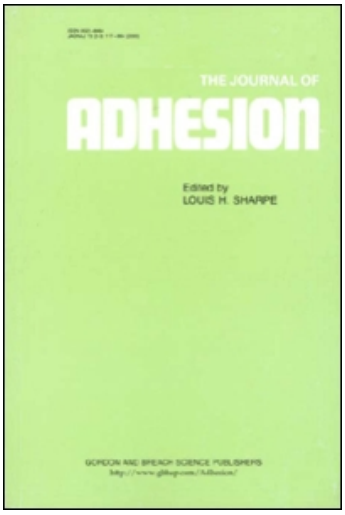

The Journal of Adhesion
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713453635

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To cite this Article Walker, Graham(1981) 'The Adhesion of Barnacles', The Journal of Adhesion, 12: 1, 51 — 58
To link to this Article: DOI: 10.1080/00218468108071188
URL: http://dx.doi.org/10.1080/00218468108071188

J. Adhesion, 1981, Vol. 12, pp. 51–58
0021–8464/81/1201–0051 $06.50/0

Printed in Great Britain

The Adhesion of Barnacles

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(*Received January 19, 1981*)

Adhesives for permanent attachment of barnacles are secreted at two stages in the life history. The settlement stage larva exudes a quantity of cement (cyprid cement) from the paired cement glands onto the substratum and this envelops the attachment organs. The permanently attached larva then metamorphoses to the juvenile and after a short growth interval, during which the juvenile is still dependent on cyprid cement, the newly formed adult cement apparatus first secretes the adult cement. Adult cement can be collected and analysed biochemically; results show it to be $>70\%$ protein. The mechanisms of adhesion are discussed.

INTRODUCTION

Barnacles are crustaceans specialised for a sedentary existence. However there is a dispersal larval form, the nauplius, which is usually released into the plankton where it feeds and moults to the 6th stage before metamorphosing to the cypris larva, the settlement stage larva. The cypris larva, which does not feed, has the single important role of finding a suitable substratum on which to permanently settle. This task is accomplished using an armament of sensory setae, before the larva settles permanently by releasing a cement onto the substratum. This cement will be referred to as cyprid cement. Once permanently attached the larva metamorphoses to the juvenile, which in free-living barnacles may either take the form of an inverted truncated cone with the basis attached to the substratum (sessile barnacles) or have a stalk (peduncle) which elevates the food collecting region some way from the substratum (stalked barnacles). In both these adult forms further cement (adult cement) is intermittently released onto the substratum to form the adhesive.

Presented at the International Conference on "Adhesion and Adhesives" of the Plastics and Rubber Institute held at Durham University, England, September 3–5, 1980.

THE ATTACHMENT OF THE CYPRIS LARVAE

The exploratory behaviour of the cypris larvae on surfaces prior to settlement is well documented[1–3]; it is also now well established that they are able to detect and respond to various chemical and physical features of substrata.[4–8] During this exploratory phase the cyprid moves using the paired antennules in stilt-like fashion. The external morphology and internal organisation of various regions of the antennules of *Balanus balanoides* cyprids have been described.[9–11] Several different setal sense organs were found on and around the adhesive disk of the attachment organ and terminal segment.

The antennules adhere temporarily to the substratum during the exploratory phase. The surface and margin of the disk are covered with cuticular villi[10] and unicellular glands (antennulary glands) open onto the disk surface at many different points. The villi serve to increase the surface area of the disk and promote more efficient adhesion, whilst secretion from the antennulary glands acts as the adhesive.[10] Any proposed mechanism for temporary adhesion must take into account the ability of the cyprid to detach the antennules from the substratum. Recent work[12] has shown that cyprids can have a tenacity up to 4 Kg/cm^2 when adhering temporarily. It was also demonstrated that tenacity increases progressively throughout the settlement season. Early in the season, when the "urge" to settle is not as great, tenacity can be below atmospheric pressure (1.033 Kg/cm^2).

When a site is finally selected, cyprid cement is released from a pair of cement glands within the body (Figure 1); a single duct passes from each gland to open out at several points on the attachment disk surface. Cement is thus extruded onto the substratum, embedding the attachment organs and 4th segments to form the permanent attachment. The suggestion by Harris[13] that the permanent adhesive of barnacles might be quinone-tanned protein was first tested by Knight-Jones and Crisp,[14] who found positive histochemical staining for phenols in the exuded cyprid cement of *B. balanoides*. Hillman and Nace[15] using histochemical techniques detected protein in the cyprid cement of *B. eburneus*, but were unable to show the presence of phenolic amino acids. Saroyan *et al.*[16] not only demonstrated the proteinaceous nature of cyprid cement (*B. crenatus*) but also the presence of phenolic amino acids. Walker[17] made a detailed light and electron microscope study of the whole cyprid cement apparatus of *B. balanoides*; two cell types, α and β, were found in the cement glands. α cells contained secretion positive for proteins, phenolic compounds and a polyphenol oxidase (tyrosinase); β cells appeared to contain proteinaceous secretion only. Newly exuded cyprid cement gave the same histochemical reactions as α cell secretion. These results indicate that, as for the cockroach shell glands, a two component system is operating allowing polymerisation only after component mixing. The occurrence of a

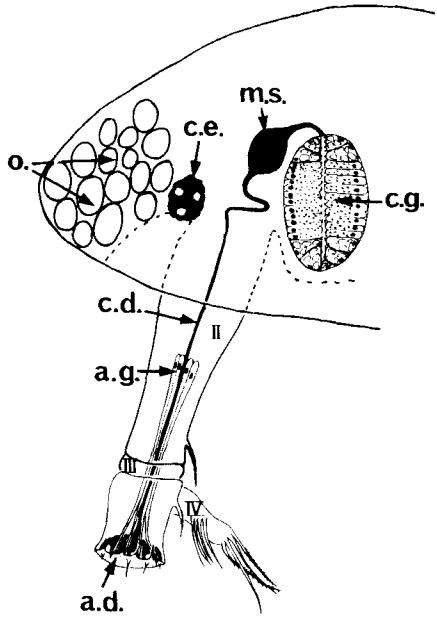

FIGURE 1 Diagrammatic sagittal section through the anterior region of a cyprid showing the cement apparatus. a.d., adhesive disk; a.g., antennulary glands; c.d., cement duct; c.e., compound eye; c.g., cement gland; m.s., muscular sac; o., oil droplets; II, III; IV, segments of the antennule.

mixture of proteins, phenols and polyphenol oxidase indicates that some quinone tanning (polymerisation) is operating:[18,19]

$$\text{protein} + \text{diphenol} \xrightarrow{\text{polyphenol oxidase}} \text{protein} + \text{quinone} = \text{tanned protein}.$$

When the cyprid cement is exuded onto the substratum it totally embeds the attachment organs which are usually sufficiently close together to result in a single patch of cement.

It has been shown at the ultrastructural level that the exuded cement is not an homogeneous mass.[17] The major portion is composed of material forming a loose reticulum, whilst at the outer surface there is a zone of more electron dense material. It is thought that this outer zone results from either (a) the chemical interaction between the cement and seawater or (b) restriction of the tanning process, perhaps because the process needs environmental oxygen.

An alternate hypothesis for the attachment of the cyprid (both temporary and permanent) has been propounded by Saroyan *et al.*[16,20] They believed that the release of cyprid cement was not essential for permanent attachment; even when released they predict that it merely reinforces the purely mechanical hold of the attachment organs acting as suction cups. Studies have now

shown the suction cup hypothesis to be ill-conceived. On morphological grounds alone it has been shown[9,10] that an attachment disk has no effective seal around its edge to prevent the entry of water into the presumed reduced pressure region. Secondly, cyprid cement is now known to pass out through numerous exit points on the disk surface[10,17]; such exudation would terminate suction. As pointed out earlier the tenacity of cyprids during temporary attachment[12] can be 2–3 times atmospheric pressure. The evidence is overwhelming for the total dismissal of the suction cup concept in cyprid attachment.

Present knowledge of the permanent attachment of cyprids can best be summarised in a diagram (Figure 2). Liquid cement is released *en masse* onto a substratum but it does not spread very far, although it presumably fills in any irregularities on the substratum surface. It does, however, successfully flow around the attachment organs and 4th segments. Polymerisation then occurs which at first is restricted to the outer region of the cement, so effectively forming a skin, which may help render the cement immune to biodegradation. The type of bonding between the cement and the substratum is not known. However, permanent attachment relies to a great extent on the morphology of the terminal parts of the antennules; the bell shape of the attachment organs together with the protruding 4th segments ensures a most successful mechanical lock within the cement (Figure 2). As cyprid cement must maintain the attachment of the juvenile barnacle for 40+ days,[21] polymerisation may continue to eventually affect the whole of the cement.

THE ATTACHMENT OF THE ADULT BARNACLE

Controversy exists as to whether the adult cement apparatus develops from the paired cyprid cement glands. Walker[21] showed clearly that after settlement the α cells of the cement glands of *B. balanoides* cyprids dedifferentiated, then redifferentiated into adult cement cells. Further adult cells developed from cells of the collecting canals. The direct development of the adult system from the larval cementing organs is also the view of Saroyan *et al.*[16]. Lacombe and Liguori[22] and Cheung and Nigrelli[23] on the other hand believe that the adult cement cells arise only from the cells of the larval collecting ducts. This argument takes place from entrenched positions. Cheung and Nigrelli[24] believe the cyprid cement and adult cement to be histochemically different, whereas the other view[16,21] is that the cements are chemically similar.

The adult cement apparatus of *B. balanoides* first releases cement onto the substratum some 40+ days after settlement[21] and as growth proceeds the apparatus (cement cells and duct system) develops, keeping pace with the

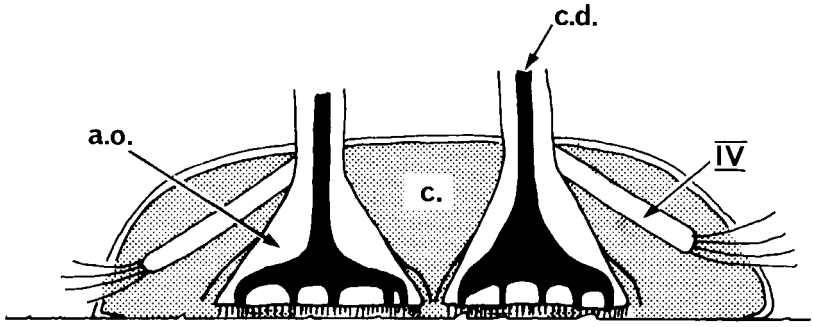

FIGURE 2 Diagram showing the exuded cement and the embedded terminal parts of the two antennules. a.o., attachment organ; c., cement; c.d., cement duct; IV, 4th segment.

enlarging basis. As cement cells are modified cuticle-forming cells[25] a link between cement secretion release and some phase of the moult cycle seems most likely, but so far this has not been demonstrated.

Cytology and histochemistry

Bocquet-Védrine[26] was the first worker in recent times to re-explore the cytology of adult cement cells (*Elminius modestus*); this was followed by the detailed studies of Lacombe[27–29] on *Balanus tintinnabulum* and the comparative study[27] on the cells of *B. tintinnabulum* and *Lepas anatifera*. Such studies, and others,[30, 31] show that cement cells of barnacles (*L. anatifera* is an exception) have two distinct cytoplasmic regions—regions of secretion synthesis and regions of secretion accumulation. The secretion in the accumulation regions stains positively for proteins and phenolic amino acids.[30, 31]

Biochemical analysis

It is not possible to collect sufficient, uncontaminated cyprid cement for analysis, but adult cement can be obtained in sufficient quantity. It is well known that growing sessile barnacles can gradually dislodge one another when they come into contact. Much cement is produced onto the basis of such raised up individuals and is easily collected for analysis.[32, 33] Scraping over the basis to collect cement leads to contamination and meaningless analysis (see Barnes and Blackstock[34]).

Newman *et al.*[35] refer to unpublished observations of carefully detached *B. amphitrite* reattaching to glass slides. Such a reattachment phenomenon led to the development of the "milking in hydrospace" technique[16] for

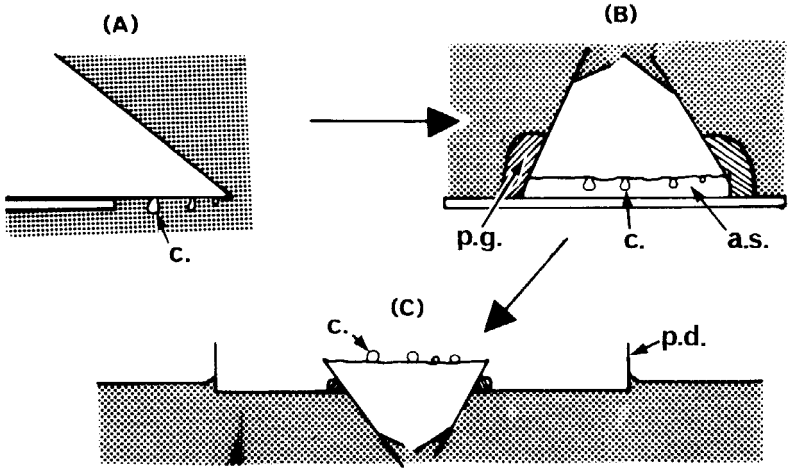

FIGURE 3 Diagrams illustrating the three (A, B, C) methods of obtaining adult cement. a.s., air space; c., cement droplets; p.d., petri dish; p.g., plasticine girdle.

collecting *B. crenatus* cement (Figure 3A); detached barnacles were allowed to reattach to glass slides over part of their basis area; the remaining cement was secreted onto overhanging parts of the basis and could be collected. This cement is called secondary cement.[16] Walker,[33] mindful of obtaining cement which was uncontaminated, placed plasticine girdles around detached *Balanus hameri* so that the exuded cement went into an enclosed air space (Figure 3B). Cheung *et al.*[36] further modified this technique; they floated petri dishes with harnessed inverted *B. eburneus* (Figure 3C) so that the uncontaminated cement was released directly into air.

Stalked barnacles produce large amounts of cement which is easily collected from the base of the stalk for analysis.[34,37] Most analyses of barnacle cement estimate the total lipid, carbohydrate and protein contents and so far there is agreement that protein is the major component (>70% w/w), although some controversy exists over the proportion of lipid present.

Polymerisation of adult cement

Cheung *et al.*, using the collection technique in Figure 3C showed that although cement first appears on the basis as a clear non-viscous fluid it rapidly changes (polymerises) to the solid state (secondary cement).[36] Temperature changes ($-10 \rightarrow +45°C$), enzyme inhibitors and certain chemicals do not affect this polymerisation process and so it was concluded that the process was time dependent and requires no exogenous catalyst.[36] It is believed that the newly released liquid cement is able to spread between

the basis and substratum before polymerising. However, the cement does *not* form a continuous layer under the basis. In stalked barnacles the cement amasses around the stalk base; new cement is intermittently released into two ducts each of which opens out through an antennule. How new cement continues to be passed out along this route if the already exuded cement is polymerised remains problematic.

Quinone tanning has been proposed as the polymerisation process which takes place in adult barnacle cement (and cyprid cement), purely by analogy with the process[38] within insect cuticle. Very recently, however, doubt has been cast on this theory of covalently cross-linking proteins with quinone derivatives. Vincent and Hillerton[39] have revived an earlier concept[40] that controlled dehydration is the key process in stiffening cuticle, the quinone acting by selectively occupying strongly hydrated groups and effectively squeezing the water out. Such chemical dehydration would also work under water.

Mechanism of adhesion

The mode of growth of sessile barnacles makes it impossible for the shell (side-wall plates) to be firmly bound to the substratum. The basis alone is cemented down whilst the shell plates are held down by specialised muscles (fixation fibres) which can relax to allow accretion at the basal margin of the shell. The only study so far carried out which is concerned solely with measuring the adhesive strength of barnacles was undertaken by Despain *et al.*[41]; using the "blister test" they determined the adhesive energy (γ_a). Their figures for the adhesive energy of adult cement on different surfaces ranged from 1.75 → 122 ergs/cm^2. Carderelli *et al.*[42] mention tenacity (force per unit area of basis) figures of between 2–4 Kg/cm^2 for a sessile barnacle attached to metal, but do not say how such tenacity was measured.

Crisp[43] predicted that adhesion of adult sessile barnacles was of the Stefan type, the cement being envisaged as a high viscosity fluid rather than a chemically bound rigid solid. Observations that barnacles with a membranous basis are capable of lateral movement under sustained pressure[44] lends support to the Stefan type of adhesion. However, such adhesion cannot explain cyprid attachment or that of stalked barnacles where successful mechanical marriage between antennules, cement and substratum seem all important.

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