Tegumental Glands in the Cirripedia Thoracica.[1]

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With 11 Text-figures.

CONTENTS.

1. INTRODUCTION.

WORKING on Decapod Crustacea, in particular on Homarus vulgaris, Yonge (1932) showed that the exo-skeletal integument consists of two portions, a thin superficial cuticle, which is hard and relatively impermeable, containing adsorbed lipin, and underlying this a thick, relatively soft, freely permeable chitin. These two layers are structurally and chemically distinct and have different origins, the cuticle being formed not by the chitinogenous epithelium but by numerous tegumental glands situated beneath the epidermal epithelium. The presence of similar glands with intracellular ducts was also demonstrated in other groups of the Crustacea.

The fundamental significance of the cuticle as a protective and insulating layer was shown not to be incompatible with

[1] Owing to Dr. H. J. Thomas's absence on active service this paper, part of his thesis for the degree of Doctor of Philosophy, has been prepared for publication by Mr. R. Bassindale.

accessory functions such as the binding of the eggs to the pleopods of the female (Yonge, 1938), the attachment of sand grains to the setae in the statocysts (Lang and Yonge, 1935), and the formation of threads constituting the nest of certain Amphipoda, e.g. Ampelisca (Yonge, 1932).

The present investigation of the tegumental glands of the Cirripedia was undertaken in the light of the above work. The greater part of it was carried out at the University of Bristol, experiments on living material being conducted at the Plymouth laboratory of the Marine Biological Association during the spring of 1938. Thanks are due to Professor C. M. Yonge at whose suggestion and under whose supervision the investigation was conducted, and also to Mr. R. Bassindale, M.Sc., for valuable advice.

2. Material and Methods.

The following species were obtained fixed in formalin and also in either Bouin's fluid or corrosive sublimate or both: Lepas hilli Leach from Tortugas; Lepas anatifera Leach (also in Zenker) Scalpellum scalpellum Leach, Balanus perforatus Brugière Balanus balanoides Leach, and Alcippe lampas Hancock, all from Plymouth. In addition specimens of Lithotrya valentiana Gray in 90 per cent. alcohol from the Great Barrier Reef were available.

Fixation of the tegumental glands was good in specimens treated with 10 per cent. formalin, Bouin, Zenker or corrosive sublimate, the last being probably the best. The specimens of Lithotrya valentiana were of little histological value, but permitted the determination of the distribution of tegumental glands and of the distribution and staining reactions of their secretions.

Microtome sections were cut from 4 to 8 μ thick. Good results for general purposes were obtained by staining in Delafield's haematoxylin with eosin as a counterstain, but for finer details Heidenhain's haematoxylin counterstained with Biebrich scarlet was most suitable. The demarcation between chitin and other secretions was best displayed by Mallory's triple stain.

Hand sections 10 to 20 μ thick were cut from the integument of all regions of large *Lepas anatifera* (preserved in 10 per cent. formalin) and subjected to stains and microchemical tests. The iso-electric points of both layers of the integument were determined following the technique used by Yonge (1932) and in addition small pieces of the integument were subjected to Campbell's modification of Van Wisseligh's test for chitin (Campbell, 1929).

Owing to the thinness of the cuticle on the chitin outside the mantle cavity it was not possible to observe its reaction with reagents acting on hand sections. However, by decalcifying the calcareous plates from large *Lepas anatifera* the thin superficial cuticular covering was obtained. This proved suitable for examination.

3. Nature of the Integument.

In the pedunculate Thoracica, e.g. *Lepas*, *Scalpellum*, *Lithotrya*, the mantle and the enclosed body forms the capitulum and there is a long stalk, the peduncle, which attaches the capitulum to the substratum. In the sessile Thoracica, e.g. *Balanus*, the peduncle is absent and the calcareous plates of the capitulum articulate to form a solid wall.

The chitinous exo-skeleton may be divided into two regions, that covering the outer surface of the capitulum and peduncle, which may include numerous calcareous plates, and that lining the mantle cavity and covering the body proper. The latter or *inner* integument is thin and is shed regularly at the moult. The former or *outer* integument is thick and laminated, due to the fact that, except on the peduncle of *Lithotrya*, it is not shed at the moult, thus recalling similar incomplete moults of the Conchostraca and some Cladocera.

From the examination of microscopic and hand sections and by means of the tests employed by Yonge (1932) and Campbell (1929) it was established (see Tables I and II below) that in all species examined the integument consisted of a thick layer of chitin underlying a much thinner layer of cuticle—both layers being continuous over the whole surface of the body. In mature *Lepas hilli* the chitin varied in thickness from 250 μ on the peduncle to 3 μ in the hind gut, and the cuticle, with an average thickness of some 1·5 μ, varied from 10 μ on the fused 2nd maxillae (labium or lower lip) to 0·4 μ, in the hind gut. Ducts passing through the integument were present in all species examined and similar ducts run through the calcareous valves of the capitulum of some species.

4. Tegumental Glands.

There are three types of tegumental glands: (*a*) those scattered over the outer integument, (*b*) the cement glands, and (*c*) those usually termed 'salivary' glands situated on or near the 2nd maxillae. The last two types are well known. The cement glands are concerned with the attachment of the animal to the substratum and not, as are the other two types, with the secretion of the body cuticle.

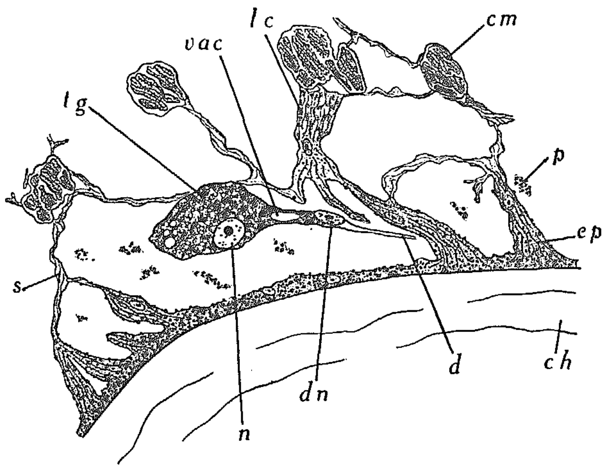
(*a*) The Glands of the Peduncle and outer Surface of the Capitulum in four Pedunculate Species.

The organs in the body of the Cirripedia are held in a spongy lacunar tissue which gives way at the surface to a regular epithelium consisting of a single layer of striated conical cells occasionally interrupted by cubical cells. Immediately below this epithelium lie the individual cells of the tegumental glands, and although these glands have not been previously described the ducts passing through the chitin of the peduncle were mentioned by Darwin (1851) and others (see discussion).

(i) Lepas hilli.

In Lepas hilli there are numerous gland-cells beneath the epithelium underlying the integument of the peduncle (Text-fig. 1, *tg*). Each cell is large (40 μ long) and roughly oval in section, although somewhat irregular due to the connective tissue strands which hold it in position. The actively secreting cell has a large central nucleus. After fixation in Bouin's fluid or 10 per cent. formalin this shows a few large granules and a prominent nucleolus. The cytoplasm contains numerous rounded vacuoles (*vac*) which in the region opposite the origin of the duct may attain considerable dimensions. Numerous small granules, presumably secretion, are also present; these are generally rounded and have affinity for both cytoplasmic and nuclear stains, particularly for the former. The cytoplasm of the inactive gland-cell is practically homogeneous, without vacuoles or granules, being lightly coloured by nuclear stains and unaffected by cytoplasmic stains.

At one end of its long axis the gland-cell merges into a duct

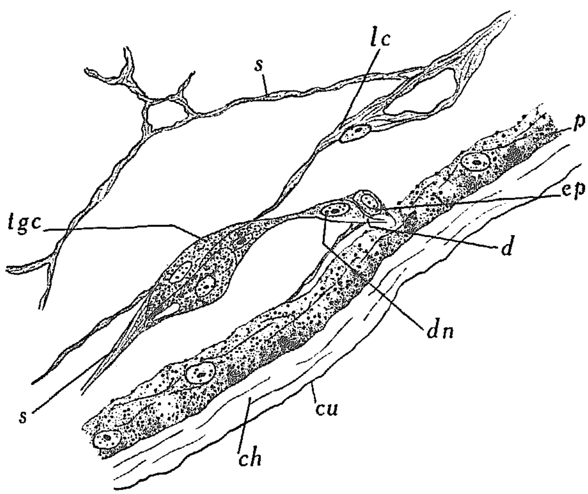


TEXT-FIG. 1.

Lepas hilli. Longitudinal section of stalk showing unicellular tegumental gland. × 700. *ch*, chitin; *cm*, circular muscle; *d*, intracellular duct; *dn*, duct nucleus; *ep*, epithelium; *lc*, lacunar tissue; *n*, nucleus; *p*, pigment; *s*, branching connective tissue strands; *tg*, tegumental gland; *vac*, vacuole.

of which the lumen may project considerably into the cytoplasm of the gland-cell, or in some cases a large vacuole may be present (Text-fig. 1, *vac*). This short funnel-shaped portion of the duct formed by the gland-cell is wide and somewhat irregular; it leads immediately to a separately formed intracellular duct (*d*) which is long, narrow, and of uniform bore. At the junction of the duct from the gland-cell and the intracellular duct is situated a large duct nucleus (*dn*). This is typically elongated, flattened, and closely applied to the surface of the duct. It possesses a number of deeply staining granules and has a fairly prominent nucleolus. The duct nucleus is surrounded by a narrow layer of clear cytoplasm similar to that forming the walls of the duct. This is unbranched and runs for some way through the epithelium, parallel to the integument, before turning outwards as a much coiled tube leading through the epithelium and integument, and opening by a pore to the exterior.

In addition to these large unicellular glands associated with



TEXT-FIG. 2.

Lepas hilli. Longitudinal section of the capitulum showing compound tegumental gland from the chitinous region below the adductor muscle. × 700. *cu*, cuticle; *tgc*, compound tegumental gland. Other lettering as before.

the stalk region there are compound tegumental glands underlying the epithelium on the outside of the capitulum. These consist of groups of five or six small gland-cells (15 μ long) opening into a common intracellular duct leading to the exterior (Text-fig. 2, *tgc*). Histologically, these cells are similar to the unicellular glands described above. The nucleus, however, is longer in proportion to the size of the cell and a prominent nucleolus is not so characteristic a feature.

Depending on the position, the glands may be compact or diffuse. In regions where the epithelium consists largely of cubical cells (e.g. below the adductor muscle) the gland is compact (Text-fig. 2). In this case collecting ducts are short and fine, the condition being similar in many ways to that found in Decapoda. If, however, a copious lacunar tissue is present, the tegumental gland is diffuse, with long, wide collecting ducts (much as in Scalpellum, Text-fig. 6), a condition recalling that in the cement glands.

As in the case of the unicellular glands, each cell narrows off at one end, giving rise to a short, wide, irregular duct, formed by the gland itself. These collecting ducts join together and are continuous at their outer end with the separately formed main intracellular duct, which bears at its upper end a typical duct nucleus. The duct itself is of uniform bore, fine and unbranched, being similar to that of the unicellular glands, though usually of greater length.

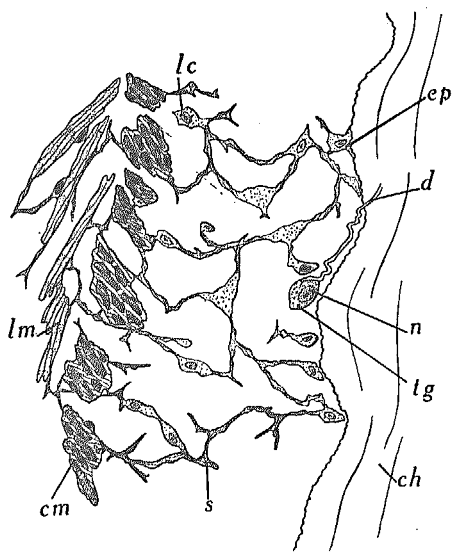
In certain cases, the duct originating from a compound tegumental gland bears in the typical position of the duct nucleus a cell of glandular appearance (Text-fig. 2, *dn*). The nucleus of this cell is surrounded by a granular cytoplasm which, in comparison with the cytoplasm of the typical tegumental gland, is markedly reduced and possessed of considerably fewer granules. The intracellular duct passes through the protoplasm. It is quite short and the condition suggests a developmental stage. Presumably the compound gland originates from a small secretory cell with a short duct leading directly to the exterior and bearing no duct nucleus, similar to the gland-cells of the stalk of Lepas anatifera (Text-fig. 3, *tg*). As further secretory cells become differentiated, the initial cell gradually loses its glandular function and, with loss of its granules and the reduction of its cytoplasm, becomes specialized for the formation of a duct of increasing length. In this way the nucleus of the initial gland-cell becomes a duct nucleus, associated with the long intracellular duct leading from the compound tegumental glands.

(ii) Lepas anatifera.

The tegumental glands underlying the integument of the stalk of Lepas anatifera are of the simplest type examined.

Numerous small unicellular glands, about 10 μ in diameter, occur well within the epithelial layer (Text-fig. 3, *tg*). The gland-cell is somewhat larger than the ordinary epithelial cells, and leads by a short and little coiled duct directly to the exterior. There is no duct nucleus.

Underlying the integument of the outside of the capitulum

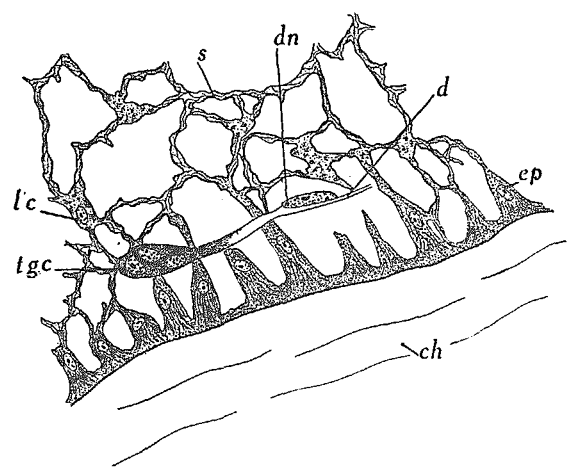


TEXT-FIG. 3.

Lepas anatifera. Longitudinal section of stalk showing unicellular tegumental gland. $\times$ 700. *lm*, longitudinal muscle. Other lettering as before.

are compound tegumental glands, the individual cells of which are about 15 μ in length (Text-fig. 4 *tgc*). They are situated inside the epithelial layer and histologically are similar to those of the capitulum of Lepas hilli. Leading from the gland is a long, coiled duct (*d*) bearing a typical duct nucleus (*dn*). The majority of these ducts pass through the integument in a typical manner. They are of uniform bore and follow a much coiled course to the exterior, where they open by a fine pore. In the region of the integument between the calcareous plates of the capitulum certain of the ducts, which in this region are of particularly fine bore, bear a rounded swelling before opening to the exterior. These are described and figured by Gruvel (1905) under the name of 'organes vésiculeux'.

In one specimen the integument in the upper part of the stalk showed signs of damage (Text-fig. 5, *chd*). Large amounts of



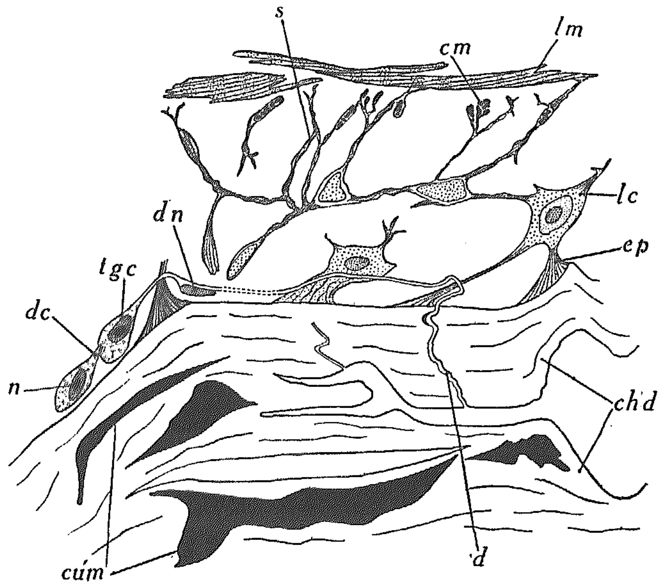
TEXT-FIG. 4.

Lepas anatifera. Longitudinal section of capitulum showing compound tegumental gland. × 700. Lettering as before.

cuticular material were observed in the tissues within the chitin of the damaged area (*cum*). Underlying such regions are large, diffuse, compound tegumental glands. The component cells of these are of greater size (17 μ in diameter) than the normal unicellular gland of the stalk region, and are characterized by a dense nucleus with a very pronounced nucleolus. The gland-cells are flask-shaped and lie within the epithelium immediately below the chitin. At one end of its longitudinal axis the cell gives rise to a long, somewhat irregular and tapering collecting duct (*dc*). A number of these ducts join together conveying the secretion from the plate-like area of gland-cells to the main intracellular duct which is exceptionally long, and bears at its upper end a typical duct nucleus (*dn*).

(iii) Scalpellum scalpellum.

In this species the whole of the outer integument, including that of the stalk, possesses calcified areas. Associated with this integument are compound tegumental glands of a type similar to those underlying the calcified capitulum of Lepas hilli

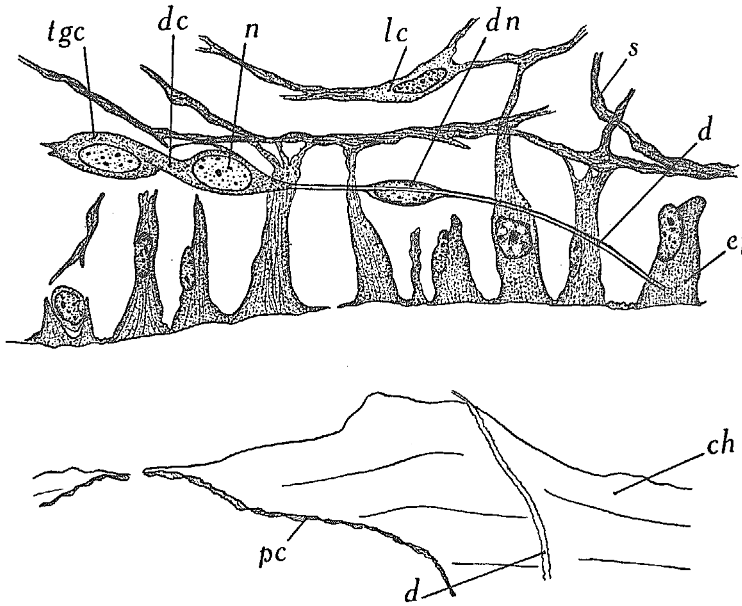


TEXT-FIG. 5.

Lepas anatifera. Longitudinal section of the stalk showing compound tegumental glands in the region of damaged integument. × 700. *chd*, damaged chitin; *cum*, mass of cuticular substance; *dc*, collecting duct of gland-cell. Other lettering as before.

and Lepas anatifera. The glands are diffuse and the component cells large (30μ in diameter). The nucleus (Text-fig. 6 *n*) is exceptionally large and after fixation in Bouin's fluid a nucleolus is not apparent. The gland-cells are joined together by wide irregular collecting ducts which pass to a long, somewhat narrow intracellular duct. This bears a typical duct nucleus (*dn*), is slightly coiled, and leads through the epithelium and integument.

Passing through the integument the ducts follow the typical much coiled course. In the region of the capitulum they traverse the calcareous plates. In the stalk region on the other hand the ducts run around the scales, but their openings are uniformly distributed at the surface. The external opening is a



TEXT-FIG. 6.

Scalpellum scalpellum. Longitudinal section of stalk region showing compound tegumental glands. × 700. *dc*, collecting duct of gland-cell; *pc*, cuticle lining cavity of calcareous scale. Other lettering as before.

pore of the same diameter as the duct and in general lies flush with the surface of the cuticle. Sometimes, however, it is mounted at the end of a short, stout, chitinous papilla.

(iv) Lithotrya valentiana.

Ducts from the tegumental glands are readily detected in the integument on account of the characteristic staining of the contained secretion, which differs markedly from the chitin.

Thus, although the material available was not suitable for histological study, the distribution of tegumental glands was readily determined.

Ducts with the typical characteristics are common in the whole of the chitin of the stalk, and of the chitinous parts on the outside of the capitulum. As in the case of the other genera examined no ducts were found in the chitin lining the mantle cavity or covering the body proper.

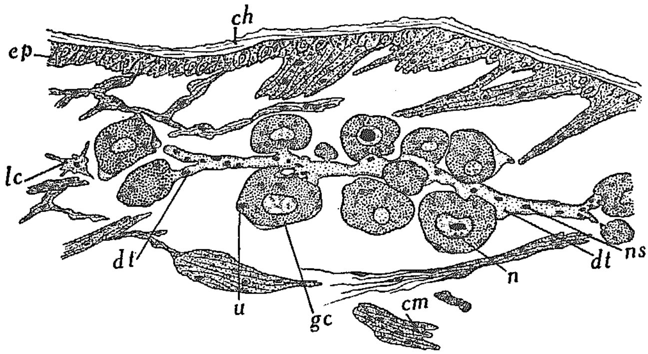
Beneath the epithelium large glandular masses were observed apparently very similar to the unicellular glands of the stalk of Lepas hilli.

(*b*) Cement Glands.

In addition to the tegumental glands above described there are, in the peduncle of pedunculate thoracic cirripedes, well developed glands which secrete a substance which attaches the animal to the substratum. Similar glands are found in the basis of operculate thoracic cirripedes. These cement glands are well known, and their gross anatomy has been described by both Darwin (1851) and Gruvel (1905). The glands normally consist of paired masses of cells whose secretion is collected by two main ducts which may or may not unite before opening at the base of the peduncle in the Pedunculata, or in the centre of the basis in the Operculata. Slight variations occur in different species, the most notable being in Lithotrya where, from a swelling in the duct, fine canals discharge cement through a calcareous attachment plate at the base of the peduncle.

In Lepas anatifera, which is typical of the Pedunculata, each gland-cell is roughly spherical and between 30 and 40 μ in diameter (Text-fig. 7). The cytoplasm of an actively secreting cell is finely granular and without vacuoles. The nucleus has a large nucleolus, and fine granules are sometimes present in addition. Certain variations in the appearance of the cells indicate that they undergo a secretory cycle identical with that described by Krüger (1923) for the cement glands of Scalpellum scalpellum and Scalpellum stromii.

The branched ducts, which project slightly into the gland-cells, are of uniform bore, and consist of a syncytium with evenly distributed granular nuclei. The main duct is similar except that the nuclei are less numerous and the cells consequently more flattened. Small flattened cells, with nuclei and cytoplasm identical with those of the collecting ducts, are frequently seen adpressed to the gland-cells (Text-fig. 7, *u*). These are presumably products of the proliferation of cells which



TEXT-FIG. 7.

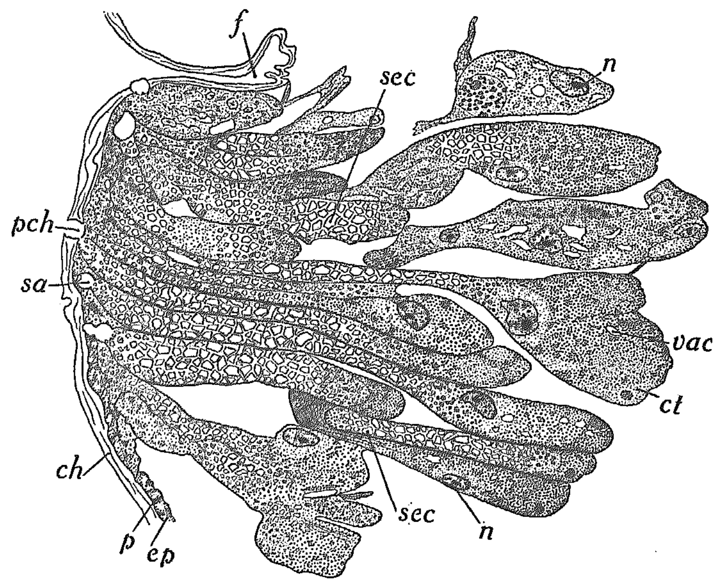
Lepas anatifera. Longitudinal section of stalk showing part of the cement gland. × 300. *dt*, tributary duct from the gland-cell; *gc*, cement gland cell; *ns*, nuclei of duct syncytium; *u*, undifferentiated cells. Other lettering as before.

has given rise to the gland mass and the ducts. Probably they are continuous with the cells of the collecting duct, and with them form an envelope around the gland-cells.

(*c*) 'Salivary' Glands.

Large masses of unicellular glands open on the fused second maxillae or labium of Cirripedia Thoracica, and similar glands open near the base of the first, second, or third thoracic appendages. These have been termed 'salivary' glands although attention has been drawn to their unsuitable position, particularly of those at the base of the appendages. They have been figured by Gruvel (1905), and secretory material is a very prominent feature in the very large cells. The glands of Lepas hilli (Text-fig. 8) are typical and hand sections of the integument overlying them shows that the chitin is covered by a special thick cuticle which is under tension and often tears away in paraffin sections (as in the Decapoda; Yonge 1932).

Gruvel (1905) ascribed to the 'salivary' glands the production of a 'bol alimentaire' which cements together food particles scraped off the cirri and which is swallowed with the contained

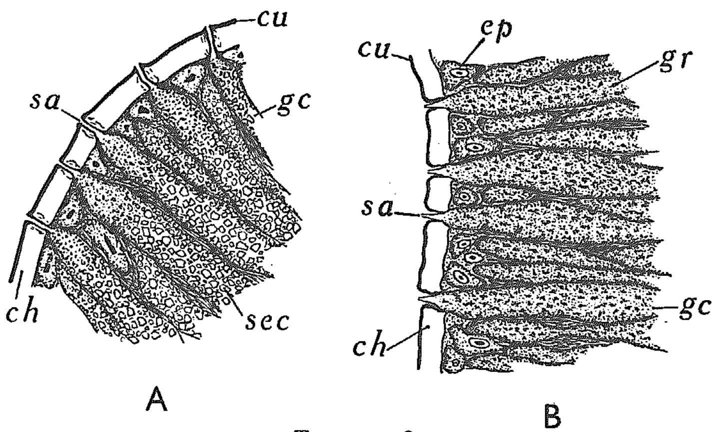


TEXT-FIG. 8.

Lepas hilli. Longitudinal section of the capitulum showing the 'salivary' gland. × 300. *ct*, cytoplasm; *f*, fold of chitin; *pch*, chitin pit; *sa*, secretory pore of cell; *sec*, large secretory agglomerations. Other lettering as before.

food particles. Broch (1919) pointed out their unsuitable position, since even the labial gland has many openings on the lateral, outer face of the second maxillae, and the other glands are more remote from the mouth. He suggested that the glands serve to poison the prey.

Since living pedunculate material is rare, these glands were studied in the large operculate barnacle Balanus perforatus. Here, the second pair of glands at the cirrus base (and underlying the suboesophageal ganglion) are identical in appearance with the glands of Lepas hilli in that the cells open flush with the surface by ducts of uniform bore (Text-fig. 9 A) and the secretion is in the form of coarse agglomerations. The labial glands of Balanus perforatus, however, differ in that the secretion is more often finely granular (after the same fixative), although coarse granules may sometimes be present



TEXT-FIG. 9.

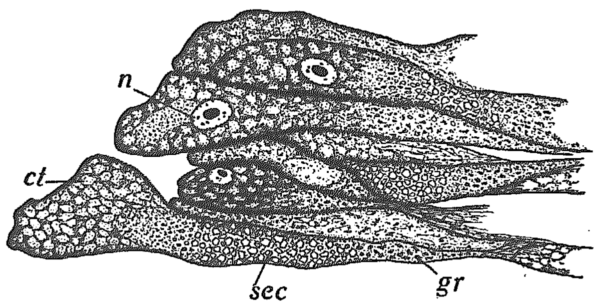
Balanus perforatus. Semi-diagrammatic representation showing the difference between (A) the glands of the suboesophageal ganglion and (B) the glands of the second maxillae. × approx. 300. *gc*, 'salivary' gland-cell; *gr*, secretion forming fine granules; *sa*, secretory pore of gland-cell. Other lettering as before.

in the inner half of the cell (Text-fig. 10). In addition the secretory pore is in the form of a conical nozzle situated in a pit in the chitin (Text-fig. 9 B).

Balanus perforatus is easy to keep alive in circulating sea-water and individuals of similar size were chosen for experiment. Moults occurred about every fifth day, and specimens were fixed at varying periods after the moult. In addition individuals were placed for varying periods in thick suspension of carmine particles or blood corpuscles and then fixed.

In a carmine suspension the barnacle was observed to make regular sweeps with its feeding cirri, and then to discard from the mantle cavity a carmine-coloured mass which consisted of carmine particles bound in a tangle of threads of a hyaline non-sticky substance. This substance was found to be unaffected by digestive juices from the alimentary canal, nor was it ever discovered in the alimentary canal. It seems, therefore, that the 'bol alimentaire' of Gruvel is formed but not swallowed, and that its formation is associated with the rejection of unwanted particles.

Specimens subjected to a thick suspension of carmine particles



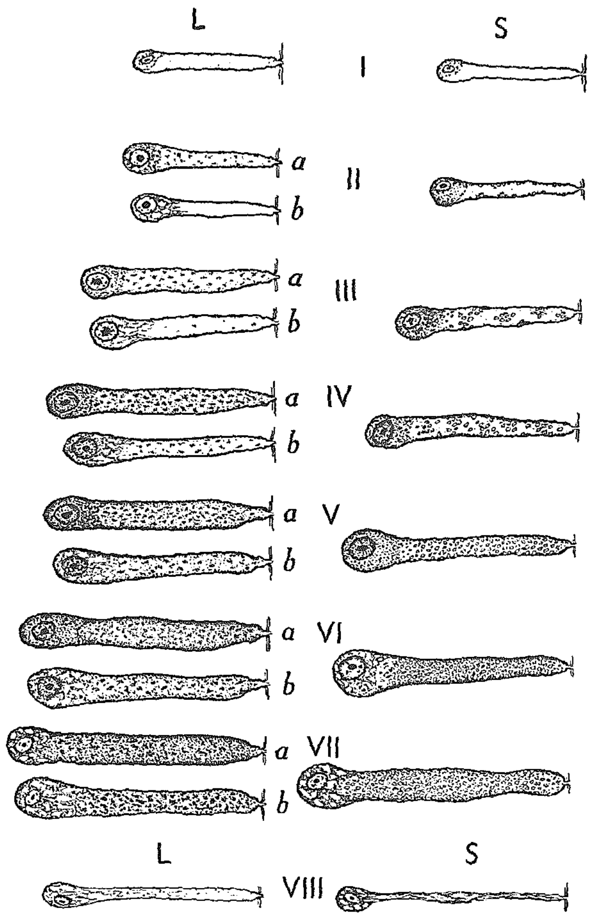
TEXT-FIG. 10.

Balanus perforatus. Longitudinal section showing salivary gland-cells. × 300. *gr*, secretion in form of fine granules. Other lettering as before.

were capable of rejecting two masses of aggregated particles, but after this the cirri became clogged and collecting soon ceased. Furthermore, such animals could not perform the function of rejection even two days after such treatment. It seemed that the source of the entangling material was exhausted, although sections often showed the presence of considerable amounts of secretory material within the gland-cells of the 'salivary' glands of the maxillae. Animals fed on blood corpuscles showed no signs of secretion from either set of glands.

In order to make a chemical examination of the entangling material finely ground silver sand was allowed to fall across the cirri of a feeding barnacle, and the rejected mass of entangled sand particles was subjected to the tests described later.

The glands were obviously not concerned exclusively with the rejection of material, and their condition was examined in sections of animals fixed at varying periods after the moult. The activity of both labial and suboesophageal glands showed a marked correlation with moulting. The secretory cycle is



TEXT-FIG. 11.

Balanus perforatus. Diagrammatic representation of the secretory stages of the 'salivary' glands. L. The glands of the second maxillae, *a*, before secretion of rejectory material; *b*, after secretion of rejectory material. S. The glands in the region of the suboesophageal ganglion. × approx. 400.

illustrated in Text-fig. 11 for both suboesophageal (S) and labial (L) glands. The series *a* and *b* of the labial glands indicate the difference between animals fed normally (*a*) and animals subjected to carmine suspensions and so exercising the rejection mechanism (*b*).

At the moult, or just after it, cells of both glands are devoid of secretions (Text-fig. 11, viii). Subsequently in the sub-oesophageal glands (Text-fig. 11 s, i–vii), and in the labial glands of animals feeding normally (Text-fig. 11 L, i–vii, *a*), the cells gradually accumulated secretory material, becoming large and heavily charged immediately prior to the moult. After the moult the cells are shrunken and possess a small nucleus with a few granules. The cytoplasm is homogeneous and vacuolated. Soon the cells enlarge, but appear empty except for the granular cytoplasm surrounding the now nucleolate nucleus at the base. The cell continues to grow, and the cytoplasm contains more granules and increases in amount. Prior to the moult the granules of secretion accumulate in the distal two-thirds of the cell and appear as large aggregations after fixation. About this time the cytoplasm at the base becomes vacuolated, and it may be the enlargement of these vacuoles which discharges the secretion. The secretory cycle of the individual cell is identical with that of the cement gland cells of Lepas (see above) and of Scalpellum (Krüger, 1923).

In an animal fed on carmine particles the glands on the labium show slight modifications from the above cycle. Limited numbers of secretory granules appear near the cell opening, and the cytoplasm develops vacuoles at an early stage. At this period all the available secretion may be discharged during particle rejection (Text-fig. 11 L, ii, *b* and iii, *b*). Later, however, not all the secretion is discharged, and there is a reserve accumulation (Text-fig. 11 L, iv–vii, *b*) which is not discharged during rejection but is preserved for the moult.

5. Secretions of the Glands.

The integument in Decapod and other Crustacea consists of a thick layer of chitin secreted by the epithedermal cells, and of a thin layer of cuticle secreted by special tegumental glands. In the above account the cirripede integument is shown to consist of similar layers and three types of glands which may correspond to tegumental glands are present.

Tests were applied to identify the chemical nature of the cirripede integument, and Tables I and II summarize the results.

TABLE I.

Reactions of the Chitin and Cuticle of the External Surface, Cuticle of the Mantle Cavity and Cement of Lepas anatifera, and of the Rejectory Secretion of the 'Salivary' Glands of Balanus perforatus.

	Chitin.	*Cuticle of external surface.*	*Cuticle of mantle cavity.*	*Cement.*	*Rejectory secretion of 'salivary' glands.*
1. Iso-electric point.	5·0	3·4	3·5	3·5	3·4
2. Conc. HCl.	Partial soln.	Unchanged	Unchanged	Unchanged	Unchanged
3. Warm saturated KOH solution.	Insoluble	Dissolves	Dissolves	Dissolves	Dissolves
4. Sudan III.	Unstained	Pink	Pink	Pink	Pink
5. Osmic acid	Light brown	Black	Black	Black	Black
6. Millon's reagent	Faintly pink	Deep brick red	Deep brick red	Deep brick red	Deep brick red

On addition of hydrochloric acid there was an immediate contraction and a marked solution of the chitin. Complete solution did not occur even on warming. Pure chitin with warm concentrated acid is converted into the soluble glucosamine hydrochloride. The incomplete solution may be due to the effect of the formalin in which the Lepas was fixed and which is known to affect amino groups.

Campbell's test for chitin (Campbell, 1929) gave positive results for all regions of thei ntegument (not for the cement), thus confirming the evidence recorded in Table I, especially the iso-electric point (5·0) which agrees with that found by Yonge (1932) for the chitin of Homarus. The properties of the cuticle of the external surface and of the mantle cavity, recorded in Tables I and II, also agree with those of the cuticle in Homarus (including the iso-electric point at 3·4–5), also with those of the cement which binds the eggs to the pleopods

TABLE II.

Staining Reactions of the Chitin and of the Secretions of the Tegumental, 'Salivary', and Cement glands of Lepas hilli, and of the secretion of the glands of the Second Maxillae of Balanus perforatus.

		Secretions of			
	Chitin.	*Tegu-mental glands.*	*'Salivary' glands.*	*Cement glands.*	*Glands of the 2nd Maxillae of* Balanus.
1. Delafield's haematoxylin with eosin.					
	Faint blue				
(*a*) In cells	..	Red	Reddish purple	Purple	Purple
(*b*) In ducts	..	Reddish purple	..	Reddish purple	..
(*c*) After liberation	..	Reddish purple	Reddish purple	Reddish purple	Reddish purple
2. Heidenhain's haematoxylin with Biebrich scarlet.					
	Pale brown				
(*a*) In cells	..	Dark red	Dark red	Dark red	Dark red
(*b*) In ducts	..	Dark red	..	Dark red	..
(*c*) After liberation	..	Dark red	Dark red	Dark red	Dark red
3. Mallory's triple stain.					
	Blue				
(*a*) In cells	..	Red	Red	Reddish orange	Red
(*b*) In ducts	..	Reddish orange	..	Reddish orange	..
(*c*) After liberation	..	Reddish orange	Reddish orange	Reddish orange	Reddish orange

in the Decapoda (Yonge, 1938), and the secretion which attaches sand grains to the sensory setae in the statocysts (Lang and Yonge, 1935). In addition the properties of both the cement in the Cirripedia, and of the labial secretion which aids in rejection in the Operculata, are identical with those of the cuticle indicating a fundamental similarity between all the glands concerned. The presence of adsorbed lipin, indicated by positive reactions with Sudan III and osmic acid, is of special interest.

The presence of this in the cuticle of the Decapoda was experimentally demonstrated by Yonge (1936) through its effects on permeability.

The integument of the Cirripedia, therefore, consists of a thick layer of chitin and a thin superficial layer of cuticle, and the cement, labial, and suboesophageal glands are all modified tegumental glands. The apparent absence of cuticle on that part of the integument covered by cement is a further indication that the cement glands represent the modified tegumental glands of this region, while the manner in which the cement spreads round the base of the peduncle indicates the possession of low surface tension, as postulated by Yonge (1932, 1938) for the cuticle and the egg-binding cement of the Decapoda. Moreover, the compound glands of the capitulum of Lepas hilli are very similar to the uterine glands of Chirocephalus which secrete the cuticular outer egg membrane (Mawson and Yonge, 1938).

6. Discussion.

Three types of gland have been described, (1) the newly described glands of the outer surface of the barnacle, (2) the cement glands, (3) the so-called 'salivary' glands. All three secrete material having identical properties and similar to the cuticle of the Decapoda. The presence of three different sets of glands in the cirripedes seems to be associated with their sedentary habit. It has been shown in the Decapoda that glands identical with those which secrete cuticle at the moult produce the same substance for cementing purposes, e.g. for attaching eggs to pleopods or sand grains to statocyst setae (Yonge, 1938; Lang and Yonge, 1935). It may be imagined, therefore, that when the cirripedes became sessile the attachment cement was supplied by the available tegumental glands. These have now become specialized as the cement glands and produce a copious secretion.

After the assumption of a sessile habit the acquisition of a very thick protecting layer would be an advantage.[1] This has

[1] The inability of the animals to obtain refuge, like the Decapoda, during the helpless period before the new integument calcifies, would alone render moulting of the outer integument impracticable. C. M. Y.

been developed in the cirripedes by retaining the old integument at the moult in the parts exposed to the environment and by the secretion of calcareous material to form protecting plates. Such a thickened integument would be unsuitable for the body proper and for the lining of the mantle (which is probably used as a respiratory surface), and moulting proceeds normally in these regions. On the outer surface where abrasions must be repaired and where cracks in the chitin occur due to the enlargement of the peduncle, tegumental glands secreting continuously must be present; but for the region within the mantle cavity glands secreting only at ecdysis would suffice. These conditions are fulfilled by the large number of small glands on the outer surface as described in Lepas hilli and other species, and by the so-called 'salivary' glands within the mantle.

Histological evidence indicates that, in the Pedunculata, the cells of the tegumental glands of the outer surface and of the labial and suboesophageal glands regenerate after secretion. Krüger (1923) came to similar conclusions as a result of his study of the secretory cycle in the cement glands of Scalpellum. But whereas the component cells of the tegumental glands of the outer surface and of the cement glands are not all in the same phase so that the glands secrete continuously, in the labial and suboesophageal glands the cells are in step and secrete only at the moult. In the Operculata the cells of the labial and suboesophageal glands regenerate after secretion, but those of the cement glands degenerate, new gland-cells developing from the walls of the duct (Gruvel, 1905). But here all the cells of the cement glands are in the same phase, secretion occurring only at the moult. This is certainly correlated with the discontinuous growth of the Operculata (Darwin, 1853), cement being required only at ecdysis.

Although the small and unevenly distributed tegumental glands of the outer surface of the Pedunculata are here recognized for the first time, the ducts have been seen previously. Koehler (1889) described 'canals' through the integument of the stalk of Pollicipes cornucopia some of which run to the calcareous scales. At the base of the scale, these ducts bear a swelling from which fine 'canals' run through the scale to the surface. Gruvel (1905) has figured these 'organes de Koehler' as well as the 'organes vesiculeux' of Lepas anatifera, and the canals running through and perpendicular to, the chitinous and calcareous parts of the integument of Cirripedia in general. Gruvel attributes a nervous function to these structures and figures the swellings on the 'canals' in the 'organes de Koehler' and 'organes vesiculeux' as cellular structures. Careful examination of the latter showed that in reality they are non-cellular, being nothing more than swellings on the 'canals' which continue through the integument to the surface.

'Organes de Koehler' are not present in the calcareous scales of the peduncle of Scalpellum scalpellum, since these differ from those of Pollicipes in being completely encased in the chitin, so that the ducts are able to run within the chitin round the scale and open with a uniform distribution on the surface of the integument.

Careful study of the 'canals' in the available species shows that they are in association with ducts from typical secretory cells. Gruvel has stated that they contain prolongations of nerves, but their contents are invariably homogeneous with staining reactions identical with those of the cuticle. Occasionally the 'canals' are completely empty. They represent in fact the continuation of ducts from the tegumental glands (as in Homarus (Yonge, 1932)) and serve to carry the secretion from the gland, through the integument, to the exterior where, on account of its low surface tension (as postulated by Yonge (1932, 1938) for the decapod Crustacea), it spreads out to form a thin continuous layer over the chitin.

In the case of the 'salivary' glands it was shown that the glands on the maxilla in an operculate barnacle are partly concerned with the rejection of excess particles in the sea-water, since, (*a*) they secrete cuticular material, (*b*) they become partly exhausted in an animal kept in a thick carmine suspension, and, (*c*) there are no other glands available to produce the entangling threads which are of cuticular material. Nevertheless their main rhythm coincides with the production of cuticle at the moult, and the cuticle is thickest in the vicinity of the glands and becomes thinner with increasing distance from them. They must, therefore, be regarded as tegumental glands, and the misleading name 'salivary' should be discarded. In addition the name maxillary gland applies to the excretory organ of the whole of the Crustacea, so that for the tegumental glands on the fused second maxillae a new name must be used. Labial tegumental glands is probably the most suitable. For the glands behind the labium, or in the bases of the first three pairs of cirri, a general term 'suboesophageal tegumental gland' would serve to indicate their position close to the suboesophageal ganglion.

During the examination of the labial and suboesophageal tegumental glands of Balanus perforatus certain differences were noticed and attributed to the additional function in the labial glands of assisting in rejection. The suboesophageal glands were similar to the labial glands of Lepas hilli (where suboesophageal glands are absent), and an examination was therefore made to see if the finely granular secretion and nozzle-shaped pores of the labial glands occurred in any of the available pedunculate cirripedes. A negative result after the examination of sections of Lepas anatifera, Scalpellum scalpellum, and Lythotrya valentiana indicates that rejection may not occur in pedunculates, and this habit in the operculates may be associated with their abundance between tide marks where the water is frequently muddy. That the glands are incompletely adapted to this accessory function is indicated by their rapid exhaustion and their inability to continue to supply material for rejection over a period corresponding to the normal time of immersion of a barnacle at or below half-tide level.

Yonge (1932) has shown that the cuticle is much harder than the chitin, and the thick cuticle near the mouth of the Decapoda has been attributed to the need for a strong surface layer where the passage of food materials causes wear. A similar thickening of the cuticle near the mouth is present in the cirripedes and can be attributed to the oral position of the main tegumental glands.

7. Summary.

1. In the Cirripedia the exo-skeletal integument consists of a thick layer of chitin with a thin superficial layer of cuticle.
2. The cuticle agrees in properties with that of the Decapod Crustacea, and is also secreted by tegumental glands.
3. Unicellular or compound glands, here first described, secrete the cuticle of the outer surface of the peduncle and capitulum; the labial and suboesophageal ('salivary') glands secrete that of the surface of the mantle cavity.
4. In the Operculata the labial glands also secrete between moults, the cuticular material serving to entangle excess material which enters the mantle cavity and so assist in its rejection. The restriction of this accessory function to the Operculata may be correlated with their abundance in frequently turbid waters between tide-marks.
5. The cement is identical in properties with the cuticle and the cement glands are regarded as modified tegumental glands.
6. In the Pedunculata all the gland-cells regenerate after secretion. The labial and suboesophageal glands secrete only at the moult, in the other glands some of the cells are always active.
7. In the Operculata secretion is confined to the moult apart from the secretion produced by the labial glands in connexion with rejection of particles.
8. In the Operculata the cells of the cement glands degenerate after secretion, new cells developing from the walls of the duct.
9. The specializations of the tegumental glands are correlated with the sessile habits of the Cirripedia.

8. References.

Broch, H., 1919.—"Anatomical studies on Anelasma and Scalpellum", 'K. Norske Vidensk. Selsk. Skrift'.

Campbell, F. L., 1929.—"Detection and Estimation of Insect Chitin, etc.", 'Ann. Entom. Soc. Amer.', **22**.

Darwin, C., 1851–1853.—'Monograph on the Cirripedia.'

Gruvel, A., 1905.—'Monographie des Cirrhipèdes.'

Koehler, R., 1889. "Structure du Pedoncule des Cirrhipèdes", 'Revue Biol. du Nord'.

Krüger, P., 1923.—"Studien an Cirripedien. III. Zementdrüsen von Scalpellum", 'Arch. Mikr. Anat.', **97**.

Lang, D., and Yonge, C. M., 1935.—"Function of Tegumental Glands in the Statocyst of Homarus vulgaris", 'Journ. Mar. Biol. Ass.', **20**.

Mawson, M. L., and Yonge, C. M., 1938.—"Origin and Nature of Egg Membranes in Chirocephalus diaphanus", 'Quart. Journ. Micr. Sci.', **80**.

Yonge, C. M., 1932.—"Nature and Permeability of Chitin. 1. Function of Tegumental Glands", 'Proc. Roy. Soc. London', B, **111**.

—— 1935.—"Origin and Nature of Egg Case in Crustacea", 'Nature', **136**.

—— 1936.—"Nature and Permeability of Chitin. II. Permeability of the Integument", 'Proc. Roy. Soc. London', B, **120**.

—— 1938.—"Nature and Significance of Membranes Surrounding Developing Egg Case of Homarus vulgaris and other Decapoda", 'Proc. Zool. Soc. London', A, **107**.