The Tegumental Glands in the Land Isopoda

A. The Rosette Glands

By

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With 11 Text-figures

CONTENTS

I. INTRODUCTION.

THE tegumental glands of the Decapod Crustacea have been described by many workers, but in the case of the Isopoda the corresponding glands have been much less fully investigated. The literature consists very largely of a rather confused mass of unrelated facts, and none of the published accounts gives a complete survey of all that is known. In many cases the author is concerned only with one particular type of gland, or with the glands of one particular region of the body. Often he fails to distinguish between different types of gland, and frequently he has not been fully acquainted with previous work.

In the Decapoda the tegumental glands consist of 'small, rosette shaped organs which are distributed everywhere beneath the surface of the integument, in the foregut and hindgut and in the gill chamber as well as over the entire surface of the body' (Yonge, 1932). In the terrestrial Isopoda or woodlice they are remarkable for their abundance, ubiquity, and variety. They are found in almost every part of the body, sometimes almost completely filling the structure in which they lie, and, unlike those of the Decapoda, they are of several different kinds.

In the terrestrial Isopoda tegumental glands may be present in the head, thorax, and abdomen; lateral plates and telson; antennae, mouth-parts, legs, gills, and uropods. Structurally they fall into at least five groups, and if, as some investigators have suggested, 'Zenker's Organ' in Asellus is to be regarded as a group of modified tegumental glands, then the Isopoda possess no less than six distinct varieties of tegumental gland. Only one of these is known to occur outside the group Isopoda, and only two, apart from 'Zenker's Organ', have been described before. The glands of three of the six types are unicellular (or rather in one case acellular), and of the other three compound.

They may be classified as follows:

A. Rosette Glands.

These are confined to the head and mouth-parts, and in essentials resemble those of the Decapoda.

B. Lobed Glands.

These glands are often greatly developed in the uropods and lateral plates and may be present in or near the gills. They have been variously named, usually in accordance with their position in the body, or, as in the case of 'Weber's Glands', after the investigator who first described them (Weber, 1881). They appear, however, to have precisely the same structure in all parts of the body, and since in their lobed structure they possess a striking and unique characteristic, it is proposed to call them 'Lobed Glands'. They are totally unlike any glands described in other groups of Crustacea.

C. Compound Glands in Antennae.

A group of three elongated, compound glands is often present in the long (fourth) segment of the antenna. They are somewhat similar in appearance to glands which are found in the legs of certain Amphipoda and which secrete cement for building nests (Nebeski, 1880). They cannot be called 'Antennal Glands' as that name has already been used for certain excretory organs sometimes present in the antennae of the Isopoda and other groups. They are quite distinct from these.

D. Small Compound Glands.

These consist of small groups of cells scattered throughout the body, and particularly numerous in the legs and antennae.

E. Large Unicellular Glands.

Groups of large gland cells are found near the anterior edge of the lateral plates in the thorax.

F. 'Zenker's Organ'.

This structure is confined to the genus Asellus, and consists of a number of extraordinarily large cells on each side of the intestine. These cells may be homologous with the lobed glands.

Of these structures, only the lobed glands and, to a less extent, the rosette glands and 'Zenker's Organ', have been previously described. Owing to their unusual appearance and their relatively enormous dimensions, and to the fact that their secretion is clearly visible to the naked eye, the lobed glands have attracted the attention of a considerable number of workers, and various functions have been assigned to them. A few investigators have studied the rosette glands in the Isopoda, but there is no complete account of their distribution and structure. Also, since the appearance of the most recent account, it has been shown (Yonge, 1932) that in the Decapoda the rosette glands are concerned with the formation of cuticle, and it appeared desirable to investigate the possibility of their having the same function in the Isopoda. The compound glands in the antennae have not, as far as is known, been described before.

This is believed to be true also of the small compound and large unicellular glands. They certainly do not appear to have been described in detail. The present paper deals exclusively with the rosette glands. Accounts of the other types of gland will appear in due course.

This work was carried out in the Department of Zoology at the University of Bristol, at the suggestion and under the supervision of Professor C. M. Yonge, whose unfailing help and encouragement it is a pleasure to acknowledge.

II. Material and Methods.

The glands were studied from the point of view both of structure and of function. Their structure was determined by examination of transverse and longitudinal sections of *Porcellio scaber* Latr., since in this species all types of gland (except 'Zenker's Organ') are well developed. Collinge (1921) states that 'it has frequently been pointed out by investigators upon the minute anatomy of the Terrestrial Isopoda, how numerous are the difficulties that are presented in connection with the preservation and preparation of the different organs for histological investigation'. In the present work, a variety of fixatives (including Bouin's, Flemming's and Zenker's fluids, Dubosq-Brazil and Zenker-Formol) was used, but in every case the results were poor. It was eventually realized that this was at least partly due to a reaction between the acid present in the fixative and the calcium carbonate impregnating the integument. This reaction results in the formation of a considerable volume of carbon dioxide, and it is apparently this evolution of gas (bubbles of which accumulate in the alimentary canal) that causes distortion of the tissues. This difficulty was overcome by fixing specimens in neutral or alkaline solutions, and slowly decalcifying afterwards. Corrosive sublimate and corrosive formol gave better fixation. Excellent results, however, were obtained by fixing in 95 per cent. alcohol, both cold (room temperature) and hot (60° C.), and this reagent was subsequently used in the preparation of all sections, except where particular staining methods required special fixation. Specimens were then embedded in paraffin wax, and

microtome sections were prepared in the usual way. More recently some excellent series of sections have been obtained by means of the new ester wax technique (Steedman, 1945). For detailed study, Heidenhain's haematoxylin and eosin was found to be the best combination of stains. Mallory's triple, and Masson's trichrome, stains both gave excellent results, and various specific stains were also used.

In attempting to determine the functions of the glands, two methods were adopted: comparison of sections, and experimental work. Sections of male, female, and young Porcellio scaber, and of specimens fixed at different stages of the moulting cycle, were examined in order to determine the correlation, if any, between gland development and reproduction, age and moulting respectively. Correlation with terrestrial life was investigated by comparing sections of a number of Isopoda with different habits. The following species were used:

Limnoria lignorum Rathke (marine).
Idotea granulosa Rathke (found at low-water mark).
Ligia oceanica Lin. (found at high-water mark).
Asellus aquaticus Lin. (fresh-water).
Oniscus murarius Cuv. (terrestrial, without tracheal organs).
Porcellio scaber Latr. (terrestrial, with tracheal organs).
Armadillidium vulgare Latr. (adapted to withstand still drier conditions).
Hemilepistus klugii Brdt. (adapted to withstand the extreme conditions of the sandy desert. Specimens of this species were kindly supplied by Professor H. G. Jackson).

Experimental work was carried out mainly on Porcellio scaber. Land Isopoda will live for long periods if kept in a damp atmosphere. Food is less important, and although they eat a great variety of substances, they are capable of living for several weeks without food. A diet consisting exclusively of fresh carrot, first suggested by Gunn (1937), and used more recently by Heeley (1941), is the most convenient for experimental work, and appears to provide the animals with all the

constituents necessary for normal health and activity. Numbers of Porcellio, Oniscus, and Armadillidium were kept in large museum jars containing soil, bark, moss, &c., and covered with a glass plate. A humid atmosphere was maintained by attaching a piece of moist blotting-paper to the under side of the glass plate. Single specimens were kept in Petri dishes, or in round tobacco tins, each containing a piece of moss, and having moist blotting-paper attached to the lid.

Later a special vivarium was made. It measures $16'' \times 16'' \times 1\frac{1}{2}''$ and is divided into sections 3″ square by strips of wood $1\frac{1}{2}'' \times \frac{3}{8}''$. Each section is covered with a glass plate used in making lantern slides, and the base of the whole is covered with perforated zinc. Each section is numbered and contains a $\frac{1}{4}''$ layer of bulb fibre. The vivarium is kept in a shallow zinc tray to which water can be added from time to time, and the fibre is kept damp by the absorption of water from the tray through the perforated zinc.

III. Historical Review.

Many workers have described the 'salivary glands' in various groups of Crustacea as ordinary racemose glands, with central ducts that unite to form a common trunk. Huet (1882) extended this theory to the point of suggesting that the small connective tissue cells attached to the surface of the glands are replacement cells, as in vertebrate salivary glands. He believed the glands to be present without exception throughout the Isopoda.

The first detailed account of these glands in the Isopoda was given by Ide (1891). He showed that their structure is quite different from that of the racemose type, each gland consisting of a regular mass of cells opening by chitinous canals into a central duct. They are always found, he states, in the neighbourhood of the mouth, either round the oesophagus or inside the mandibles. Ide made a special study of the glands in Asellus, two of which are larger than those of any other genus, but he also examined Anilocra, Idotea, Oniscus, and the parasitic Gyge and Ione. He observed considerable variation in the size and number of glands in related species (Asellus, for example, has only four rosette glands), and in

different parts of the same individual, and classified the genera accordingly.

In his monograph on Ligia, Hewitt (1907) describes two pairs of 'salivary glands' on each side of, and opening into, the oesophagus, each being made up of a large number of rosette glands. Ter-Poghossian's description (1909) agrees in general with that of Ide, except where he points out that in the terrestrial forms the rosette glands have a less restricted distribution than either Huet or Ide supposed. They are found, he states, throughout the whole of the head and its appendages. He found histological study very difficult in the land Isopoda, and was unsuccessful in his attempts to determine the course of the ducts.

The most detailed study of rosette glands has been made on those of Decapods. Farkas (1927), using Astacus fluviatilis, divided the activities of the glands into four periods (of construction, rest, reconstruction, and destruction), and believed that, unlike most glandular structures, they never return to a former condition, but eventually degenerate.

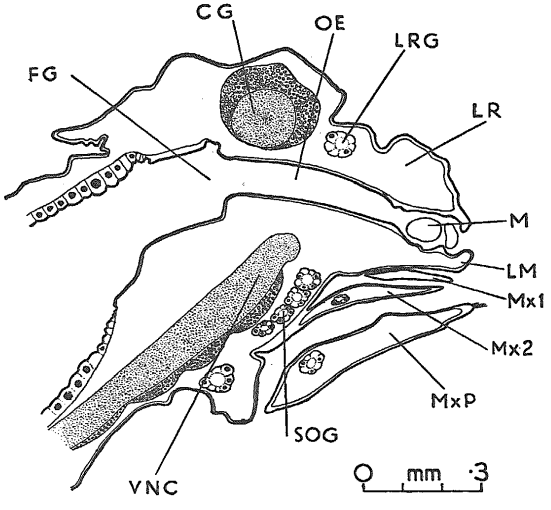
IV. Distribution and Structure.

A detailed study was made of the rosette glands in Porcellio scaber Latr., and the glands of other genera were then compared with these. Their ducts are extremely fine, and Ter-Poghossian was able to trace their course only in the case of the large glands of Asellus.

1. Porcellio scaber Latr.

As mentioned above, the rosette glands in the land Isopoda are found only in the head and mouth-parts. In Porcellio a number of separate groups of glands may be distinguished. One group lies in front of the oesophagus at the base of the labrum, and comprises a single pair of glands (Text-fig. 1, LRG). These occupy the same position as the labral glands of Asellus, described by Ter-Poghossian, and are rather conspicuous in transverse section. Their ducts appear to run forwards towards the edge of the labrum. The majority of rosette glands are to be

found in two large groups, one at each side of the head (Text-figs. 2 and 3, LMG). Each group is somewhat flattened in a transverse plane. It extends from between the eye and second

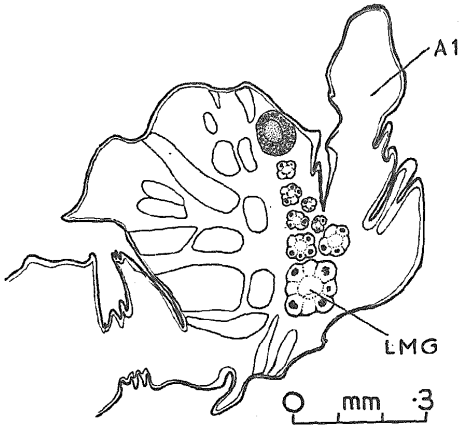


TEXT-FIG. 1.

Longitudinal section near middle line through head of young individual, showing rosette glands (the glands are more numerous in fully developed individuals). This and all the following figures refer to Porcellio scaber Latr. CG, cerebral ganglion; FG, foregut; LM, labium; LR, labrum; LRG, labral rosette gland; M, mandible; MX1, first maxilla; MX2, second maxilla; MXP, maxilliped; OE, oesophagus; SOG, suboesophageal rosette glands; VNC, ventral nerve cord.

antenna to the suboesophageal ganglia, and runs alongside, and lateral to, the peri-oesophageal commissure. The more ventral glands are usually much larger than the others (Text-fig. 2). The ducts from the glands in each group run together in a common trunk, but apparently do not actually unite (Text-fig. 3, DT). They open at the side of the labium (LM), where the

chitin is very thin. It was impossible to determine whether the ducts from all the glands in this region join the common trunk; some appear to open separately at the bases of the mouth parts. A fourth group is composed of a paired series of glands below



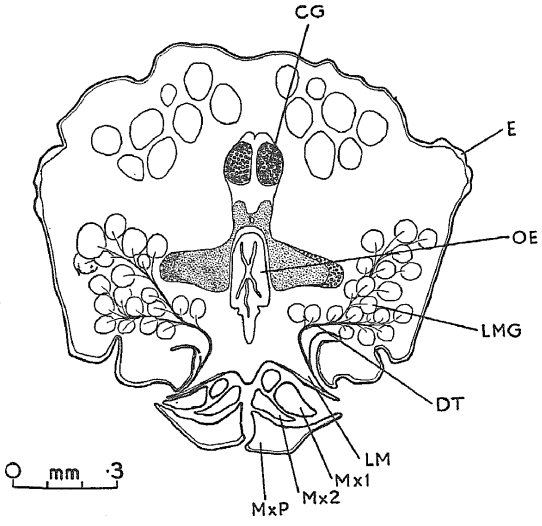
TEXT-FIG. 2.

Longitudinal section through lateral part of head from same individual as Text-fig. 1. A1, first antenna; LMG, rosette glands opening at side of labium.

the suboesophageal ganglia, above the bases of the mouth-parts, and extending into the labium (Text-fig. 1, SOG). Their ducts do not join those of the main group. Finally, rosette glands are found in the maxillipeds and second maxillae, where they occupy the proximal two-thirds of the appendage (Text-fig. 1). They are particularly abundant in the maxillipeds, and their ducts appear to run forwards towards the distal end of each mouth-part.

The rosette glands of Porcellio are very similar in structure to those of the Decapoda. Each consists of a spherical mass of cells surrounded by a delicate capsule of connective tissue (Text-fig. 6, CT). Nuclei of connective tissue cells can

sometimes be seen lying between the gland cells (Text-fig. 6, NCT). The cells composing each gland are relatively larger and fewer than in the Decapoda. Their nuclei, which lie in the



TEXT-FIG. 3.

Composite diagram of transverse section through head, showing arrangement of labial glands and their ducts. CG, cerebral ganglion; DT, trunk formed by ducts of labial rosette glands; E, compound eye; LM, labium; LMG, labial rosette glands; MX1, first maxilla; MX2, second maxilla; MXP, maxilliped; OE, oesophagus.

outer parts of the cells, are very large indeed (Text-fig. 6, NG), and each contains a conspicuous nucleolus. The central fibrillar substance seen in the glands of Decapoda is, in Porcellio, almost entirely obliterated by the gland cells, which extend almost to the centre of the gland. The latter is occupied by the end of the main duct (Text-fig. 8, MD). This is ampulla-shaped and relatively thick-walled. A number of thin-walled collecting

ducts (Text-fig. 8, CD) radiate from it to the gland cells, and in sections their openings can frequently be seen as small circles on the inner surface of the wall of the main duct. The statements of Ide and Ter-Poghossian concerning the number of collecting ducts are inaccurate. Single sections, such as the one figured in Text-fig. 8, suggest that each gland cell is provided with a separate collecting duct, and this is confirmed by examination of serial sections.

Although a large number of sections, prepared by a variety of fixing and staining methods, was examined, it was impossible to determine with certainty how the collecting duct communicates with the cytoplasm of the gland cell. The region of the cell immediately surrounding the collecting duct has a reticulate appearance, and stains more deeply with cytoplasmic stains such as eosin (Text-fig. 8, CA). It seems highly probable that, in the mature condition at least, a cluster of extremely fine, branching canals is formed in this part of the cell, and that these canals open in the collecting duct. A duct cell nucleus can always be seen (Text-fig. 6, ND). It lies nearer the centre of the gland than in the Decapoda, and is oval in shape, although somewhat crescentic in transverse section owing to its apposition to the duct. Other, more elongated, nuclei are found at intervals along the duct, their number apparently varying with its length.

2. Other Genera.

Rosette glands were found in all the Isopoda examined. They are similar to those of Porcellio in distribution and structure in Oniscus, Armadillidium, Hemilepistus, Ligia, and Idotea. In Hemilepistus and Ligia the cells of each gland are larger and fewer than those of Porcellio, while in Idotea they are smaller and more numerous. Ter-Poghossian's description of the glands in Asellus was confirmed. There are two pairs of very large rosette glands, one pair situated above the oesophagus and corresponding to the labral glands of Porcellio, and the other pair occupying the proximal half of the first maxillae. In Limnoria there are two large, many-celled rosette glands ventral to the foregut,

and from ten to twelve smaller ones at the base of the mouth-parts.

V. Function.

1. Secretion of Digestive Fluid.

Various functions have been assigned to the rosette glands of the Decapoda, but in the Isopoda, owing to their restriction to the head region, they have always been assumed to be salivary glands. It was Ide's opinion, for instance, that their ducts pass to the cuticle of the mouth-parts, and that their secretion plays a part in digestion. He adds in support of this that they are better developed in parasitic forms, but this is a fallacious argument, since one of the most characteristic features of parasitic animals is the possession of a digestive system that is not better, but less well developed than that of their free-living allies.

Hewitt does not question the salivary function, while Ter-Poghossian points out that the glands occur most abundantly in the mouth region and mouth-parts, and appear to function as salivary glands since their secretion passes down the ducts and mixes with the food. There is no evidence in support of this assumption. Indeed, no salivary secretion appears to be necessary in the Isopoda (or in any Crustacean). It was shown by Murlin (1902) that the secretion of the 'hepatopancreas' contains enzymes which act on proteins, carbohydrates, and fats; while according to Nicholls (1931) the foregut of *Ligia* is merely an elaborate filter mechanism, digestion taking place entirely in the hepatopancreas. This has been found to be true also of *Porcellio*. It is very improbable, therefore, that the rosette glands function as salivary glands.

2. Formation of Cuticle.

The integument of the Decapod Crustacea consists of two layers, a thin superficial cuticle, and a much thicker underlying chitin. Yonge (1924, 1932) has shown that these two substances are quite distinct, and that while the chitin is formed by the chitinogenous epithelium, the cuticle is secreted by the tegumental glands. The evidence for the glands having this function

is threefold. In the first place the cuticle does not appear until after the formation of the new layer of chitin has begun, and then increases in thickness with it. Secondly the contents of the ducts of the tegumental glands have the same properties as the cuticle. Lastly there is a close correlation between the activity of the glands and the moulting cycle, the glands showing the greatest signs of activity shortly before ecdysis, when the cuticle is being formed most rapidly. After ecdysis the active glands degenerate and new glands appear. The secretion spreads out as a thin, continuous layer over the surface of the chitin, probably as a result of its low surface tension. The layer increases in thickness, and exposure after ecdysis apparently causes it to solidify.

The close similarity in structure between the rosette glands in Isopoda and the corresponding glands in Decapoda suggests that they may have the same function in both groups, and that the rosette glands of Isopoda may also be concerned with the formation of cuticle. Cuticle is undoubtedly present in the Isopoda, and forms a much thicker layer over the mouth-parts than over any other part of the body. Elsewhere it is very thin and in sections tends to break up and separate from the underlying chitin. The cuticle is thus best developed in precisely that part of the body in which the rosette glands are situated, and the possibility of these glands being concerned with cuticle formation was investigated by fixing specimens of Porcellio scaber Latr. at different stages of the moulting cycle, and sectioning in order to compare the conditions of the glands.

The moulting process in Porcellio occurs at irregular intervals, except in the case of young individuals and of breeding females (Heeley, 1941), and the cycle may be affected by external conditions as suggested in the case of Asellus by Unwin (1920). Ecdysis in the Isopoda takes place in two stages. The integument of the posterior half of the body (behind and including the 5th thoracic segment) is moulted first, that of the anterior half being shed two or three days later. By making longitudinal sections of an animal at the stage between posterior and anterior moults, it is possible to examine the conditions of the integument before and after moulting in the same section.

In the case of Homarus moulting occurs only once or twice a year, but the approach of ecdysis is indicated by the presence of gastroliths, which increase in size as the time of ecdysis draws near. The precise stage in the moulting cycle of an animal preparing to moult can therefore be readily determined. Gastroliths do not occur in the Isopoda, but an even more readily observable sign of approaching ecdysis is the appearance of white patches on the ventral side of the thorax. Herold (1913), who noted them in Armadillidium, believed them to be stores of lime, and thought that this formation of 'weissen Platten' was a means of getting rid of an excess of that substance. He also believed, however, that part of the lime is reabsorbed shortly before the moulting of the posterior half and used in hardening the new integument. This view was confirmed by Numanoi (1934), who worked on Ligia exotica and observed that after the posterior moult, the white plates on the thorax gradually fade away. Meanwhile, a chalky deposit appears on the pleopods, and after the anterior moult, this also fades. He concluded that the calcium for the new posterior integument is obtained from a temporary reservoir in the thoracic segments, and that the calcium for the new anterior integument is stored in a similar way in a temporary reservoir in the abdominal pleopods.

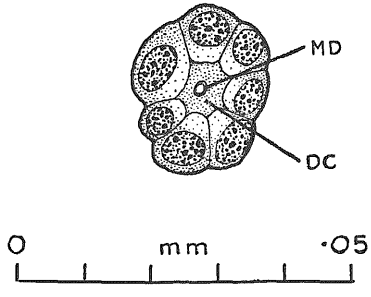
Thus it was a relatively simple matter to obtain sections of Porcellio that were fixed at various stages before and after ecdysis, and during the inter-moulting period. Young animals (approximately two thirds adult size) were used, since these, unlike mature individuals, moult at comparatively regular intervals of three to four weeks. Sections were prepared of specimens in which the white plates were entirely absent, slightly developed, half developed, and fully developed. Other individuals were isolated, allowed to moult, and then fixed at various periods after the moult (immediately after posterior moult, a few hours after posterior moult, immediately after anterior moult, a few hours after anterior moult, five, ten, and fifteen days after posterior moult).

The course of development and degeneration of the glands was traced by comparing the different appearances of the glands,

and arranging them in a progressive series (Text-figs. 4–11). The activity of the glands was then correlated with the stages of the moulting cycle. Furthermore, a comparison was made between the staining reactions of the gland contents and the cuticle.

(*a*) Development of the Rosette Glands.

In the earliest stage examined (Text-fig. 4), the nuclei occupy the outer part of the gland cells and are very large in proportion



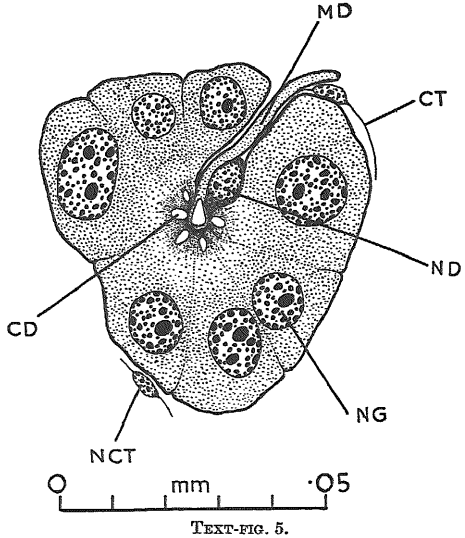
TEXT-FIG. 4.

Section through centre of young rosette gland from animal fixed a few hours after posterior moult. DC, duct cell; MD, main duct.

to the small size of the gland. The gland cells do not extend into the centre of the gland, as in the later stages, but surround what appears to be a very large duct cell (Text-fig. 4, DC). The main duct is clearly visible (MD), but no collecting ducts have apparently yet been formed. The cytoplasm of the gland cells has a granular appearance, and stains readily with eosin. The duct cell is less granular and less deeply stained. The gland increases in size, and the duct cell becomes both relatively and actually smaller (Text-fig. 5). The nuclei move towards the middle of the gland cells and collecting ducts appear. The cells later (Text-fig. 6) become differentiated into two regions: an outer, granular region, staining deeply with eosin as before, and an inner, paler region. The cytoplasm surrounding the collecting duct has now acquired a reticular appearance, staining

red with eosin. It is believed to consist of a system of minute, branching canals as already mentioned (Text-figs. 6 and 8, CA).

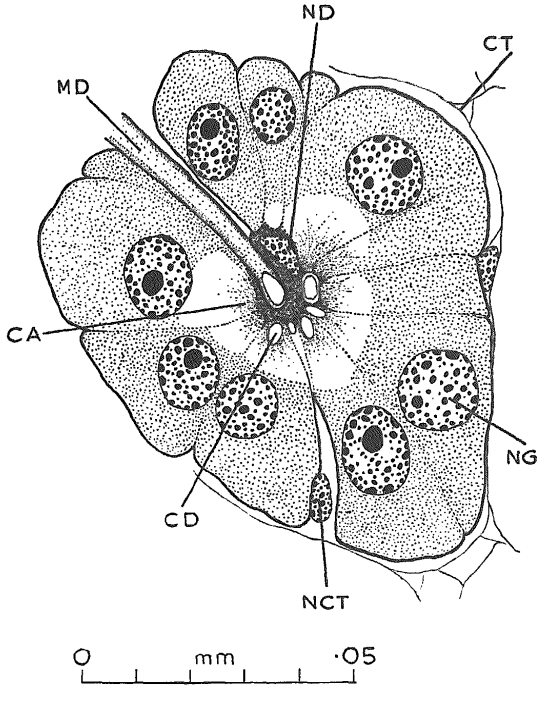
The gland has now reached its maximum size, and the nuclei, which are exceptionally large, even for gland cells, begin to



TEXT-FIG. 5.

Section through centre of half-developed rosette gland from animal fixed immediately after posterior moult. CD, collecting duct; CT, connective tissue; MD, main duct; NCT, nucleus of connective tissue cell; ND, nucleus of duct cell; NG, nucleus of gland cell.

show signs of activity. Small globules which stain a bright pink colour with eosin appear between the chromatin granules in the nucleus (Text-fig. 7, GL). This nuclear substance may also have the form of a single large globule, or may be granular, these differences in appearance probably representing successive stages in the formation of the secretion. The latter now passes out of the nucleus and forms a pink, granular mass in the cytoplasm at the outer end of the cell (Text-figs. 7 and 8,

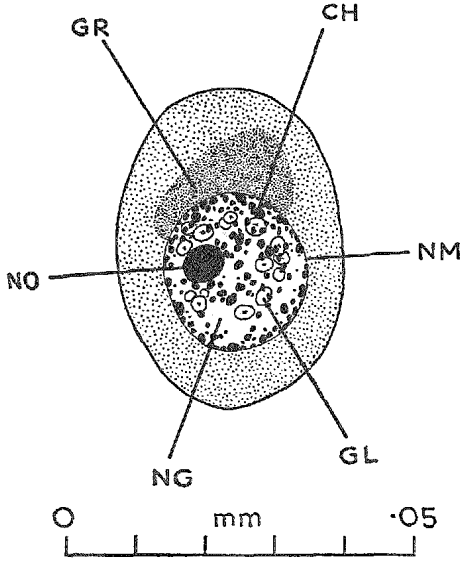


TEXT-FIG. 6.

Section through centre of immature rosette gland from animal fixed immediately after posterior moult. CA, fine canals in cytoplasm; CD, collecting duct; CT, connective tissue; MD, main duct; NCT, nucleus of connective tissue cell; ND, nucleus of duct cell; NG, nucleus of gland cell.

GR). This gradually disappears, and the cytoplasm becomes vacuolated and stains increasingly deeply with haematoxylin (Text-fig. 8, s). In the mature gland (Text-fig. 9) the cytoplasm is full of secretion and stains very darkly.

After this the gland begins to degenerate, the cytoplasm no longer has any affinity for either eosin or haematoxylin and the cells appear to be pouring their secretion into the duct.

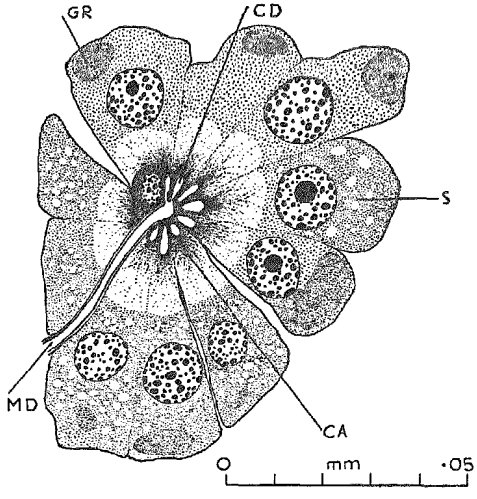


TEXT-FIG. 7.

Section through single cell of rosette gland from animal fixed immediately after posterior moult, showing formation of secretion inside nucleus. CH, chromatin granules; GL, globule of secretion inside nucleus (stained bright pink with eosin); GR, finely granular mass of secretion in cytoplasm (stained red with eosin); NG, nucleus of gland cell; NM, nuclear membrane; NO, nucleolus.

This emptying process begins at the inner end of the cell (Text-fig. 10, CR), and extends outwards. Finally (Text-fig. 11), the whole of the cytoplasm takes on a gray and coarsely granular appearance, collecting ducts are no longer visible, and the nuclei, obviously degenerating, now have a shrunken appear-

ance and pass to the outer edge of the cell. The gland then presumably breaks up and disappears.



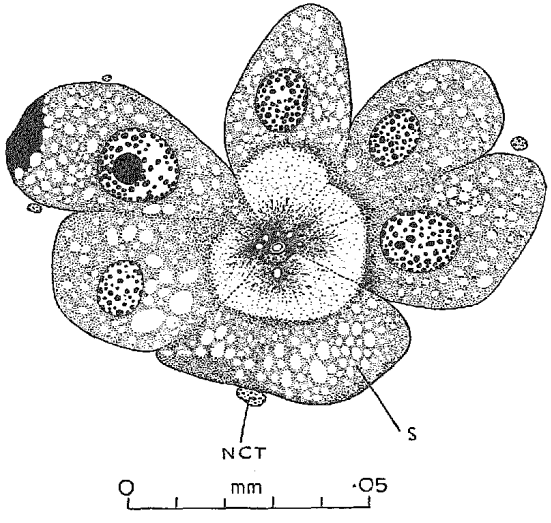
TEXT-FIG. 8.

Section through centre of rosette gland during formation of secretion from animal fixed immediately after posterior moult. CA, fine canals in cytoplasm; CD, collecting duct; GR, finely granular mass of secretion forming in cytoplasm (stained red with eosin); MD, main duct; S, darkly staining secretion forming in cytoplasm (stained dark blue or black with haematoxylin).

(*b*) Correlation with the Moulting Cycle.

Mature glands, filled with secretion, were observed only in material fixed ten days after the posterior moult, or in individuals with half developed white plates. In other words, the glands are fully developed and ready to pour out their secretion at a stage approximately half-way through the moulting cycle, which in the case of the immature animals used lasts three to four weeks. At fifteen days after moulting, most glands had the half-empty appearance of the one shown in Text-fig. 10.

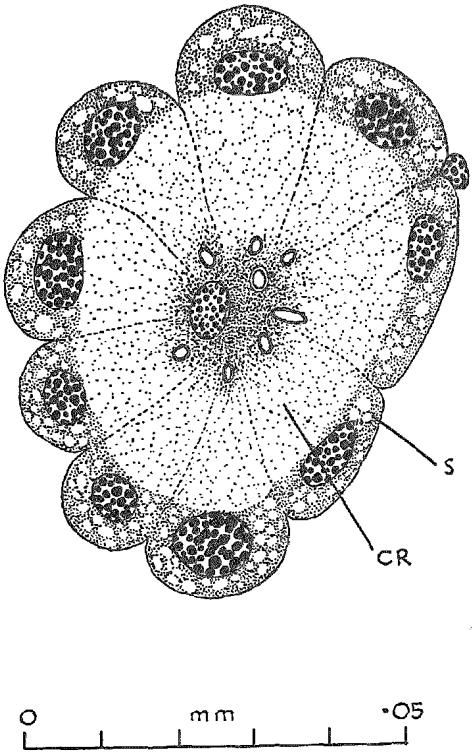
Completely empty glands are present immediately after the posterior moult, and degenerating glands a few hours later. Young glands, similar to that shown in Text-fig. 4, were seen only



TEXT-FIG. 9.

Section through centre of mature rosette gland in state of active secretion from animal fixed ten days after posterior moult. NCT, nucleus of connective tissue cell; s, darkly staining secretion forming in cytoplasm.

immediately after posterior moult, and in the stage fixed a few hours later. Immature glands were also seen at these stages, and at all subsequent stages until ten days after moulting, when all the glands had reached maturity. It may be noted here that formation of the new integument precedes by several days the actual process of ecdysis, and that it is in sections of this latter stage, approximately half-way through the moulting cycle, that the new integument first begins to appear.



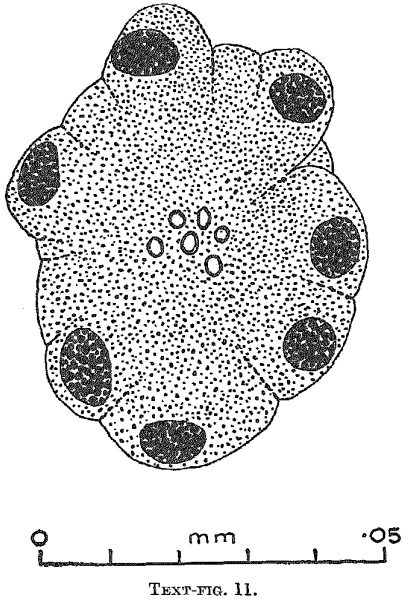
TEXT-FIG. 10.

Section through centre of rosette gland which has partly discharged its secretion from an animal fixed fifteen days after posterior moult. CR, clear region of cell from which secretion appears to have been discharged; S, darkly staining secretion in cytoplasm.

(*c*) Staining Reactions.

Both the cuticle and the secretion in the glands are stained black with iron haematoxylin, while chitin is unstained. With

Mallory's triple stain, the cuticle, the duct contents, and the innermost region of the gland cells stain bright red, chitin blue. Masson's trichrome stain colours the cuticle and glands bright



TEXT-FIG. 11.

Section through centre of rosette gland which is degenerating after discharging its secretion from animal fixed fifteen days after posterior moult.

red as with Mallory, and the chitin green. Thionin and mucicarmine gave negative results, but with Best's carmine the glands are stained red. This indicates the presence of glycogen, but whether this is an essential constituent in the formation of cuticle, or merely a source of energy needed in the elaboration of this, is not certain.

Pryor (1940) has shown that the epicuticle of insects is formed by the interaction of two substances: a water-soluble protein, and a dihydroxyphenol. It is possible that crustacean cuticle may be formed in a similar way. The presence of a polyphenol may be demonstrated by the *argentaffin test* (Lison, 1936). This test was applied to sections of individuals fixed during the period when the glands are actively secreting, but neither cuticle nor glands gave positive results.

(*d*) Discussion.

It is evident from an examination of the rosette glands fixed at different stages of the moulting cycle, that the glands are actively secreting for only a few days before moulting is due to take place. (Their early maturity, half-way through the moulting cycle, is not surprising when it is remembered how short this cycle is in comparison with that of the Decapoda.) At no stage other than between posterior and anterior moults are degenerating and newly formed glands seen. After ecdysis the old glands degenerate and are destroyed. At the same time new ones appear, and these subsequently develop and show greatest signs of active secretion at the time when cuticle is most rapidly being formed. There is therefore a very definite correlation between the activity of the rosette glands in Porcellio and the moulting cycle, and it is clear that they are intimately connected with ecdysis.

Since the glands are in a state of active secretion only during that period in the moulting cycle when new cuticle is being laid down, and since the rosette glands in Porcellio bear such a close structural resemblance to those of the Decapoda, it appears highly probable that their function is the secretion of the cuticle. This view is supported by a comparison of the staining reactions of the cuticle and of the contents of the mature glands and their ducts. The affinity for stains is invariably the same, and in no instance does the glandular secretion show a reaction that is not also shown by the cuticle. Furthermore, as in the Decapoda, the cuticle first appears after the formation of the new chitin has begun, and then increases in thickness with it. This process may most easily be explained

on the assumption that, while the chitin is formed entirely by the chitinogenous epithelium, the cuticle is secreted by the rosette glands as a fluid substance, which is carried through the newly formed layer of chitin by the ducts. Finally, the rosette glands in Porcellio are confined to the head region, and here (and particularly in the mouth-parts, on or near which the ducts open) the cuticle is considerably thicker than in any other part of the body.

A similar inequality in the distribution of the cuticle is found in the Decapoda, for although the rosette glands are present under the integument in all parts of the body in this group, they are more abundant in the head region than elsewhere, and the cuticle is especially thick round the mouth, on the labrum, and in the oesophagus. This unequal distribution of the cuticle is apparently correlated with the function performed. Crustacean cuticle appears to have three main functions (Yonge, 1936): (1) protection of the new chitin from chemical action during dissolution of the old chitin in the early stages of ecdysis (Yonge suggests that this may have been its primary function); (2) protection of the chitin (which is a relatively soft and delicate substance) from mechanical and abrasive action; (3) control of permeability (unimpregnated chitin is freely permeable). The cuticle has in addition acquired certain subsidiary functions in the Decapoda, including the formation of the attachment membranes of the eggs (Yonge, 1935, 1937) and of the cement which secures the statoliths to the sensory setae (Lang and Yonge, 1935). It has also been used for a variety of purposes in other groups.

The first of these functions, namely the protection of the new chitin from chemical disintegration during ecdysis, will be performed as effectively by a thin layer of cuticle as by a thicker layer. The relatively thin cuticle covering the greater part of the body in Porcellio, which is presumably formed by the fluid secretion from the rosette glands in the head region spreading backwards as a result of its low surface tension, would thus appear to be as efficient in this respect as the thicker cuticle of the Decapoda, in which the rosette glands are universally distributed below the integument.

In both groups mechanical protection of the integument is provided partly by the cuticle and partly by calcification of the underlying chitin. Mechanical protection is especially important in the region of the mouth-parts and foregut. These have to withstand considerable abrasive action in dealing with a diet which, in the Decapoda, frequently includes hard, sharp, heavily calcified materials, and which, in the Isopoda, is largely made up of the tough, dry food substances associated with life on dry land. It is essential, however, that the means of protection should allow for free movement of the mouth-parts, and stretching of the wall of the oesophagus during the passage of food. This requirement is not fulfilled by calcification, which has the effect of increasing to a considerable extent the hardness and rigidity of the material involved. In both groups, therefore, the integument in the region of the mouth is protected by a greatly thickened cuticle, which is both flexible and elastic, while elsewhere the cuticle is thinner, and protection is mainly dependent on a high degree of calcification.

Control of permeability is a more serious problem for land animals, particularly in its effect on water loss, and the reduced condition of the cuticle over the greater part of the body in Porcellio is a little more difficult to understand. Impregnation of the integument with calcium salts, however, is of considerable importance in this respect, and probably goes a long way towards preventing desiccation. Moreover, there is evidence which suggests that the lobed glands produce a secretion which reduces water loss still further, and which may be largely responsible for the success of the Isopoda in their colonization of dry land. This evidence will be discussed in a later paper.

It is evident, therefore, that the rosette glands in Porcellio are concerned with the formation of cuticle, and the rosette glands of other Isopoda, since they have, in general, a similar distribution and structure, may readily be assumed to have the same function.

VI. Summary.

1. The land Isopoda possess five (or possibly six) distinct varieties of tegumental gland, and these are briefly described.

Of these, only the rosette glands are known to occur outside the group.

2. The rosette glands of the Isopoda are similar to those of the Decapoda, but are confined to the head and mouth-parts. The structure and development of the rosette glands in Porcellio scaber Latr. are described.

3. Activity is intimately connected with the moulting cycle, the glands reaching maturity and liberating their secretion only during the few days before ecdysis.

4. The contents of the glands and their ducts show the same staining reactions as the cuticle.

5. The cuticle first appears after the formation of new chitin has begun and increases in thickness with it.

6. The thickness of the cuticle is greatest in the mouth-parts, where the ducts of the rosette glands open.

7. The function of the rosette glands in the Isopoda is similar to that of the corresponding glands in the Decapoda, namely to secrete the layer of cuticle on the surface of the integument.

8. Seven other species of Isopoda, from a wide range of habitat, were examined. Rosette glands similar in structure and distribution to those of Porcellio, and presumably having the same function, were found in every case.

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