The Relation of the Glandular Elements of the Clitellum of the Brandling Worm (Eisenia foetida, Sav.) to the Secretion of the Cocoon.

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With Plate 6 and 1 Text-figure.

INTRODUCTION.

DURING the observations on the coition, cocoon formation, and deposition of E. foetida, an account of which was given in a previous paper (Grove and Cowley, 1926), points were noticed—particularly during the histological investigations—which seemed to throw light upon the problem of the role played by the individual elements in the clitellum during the secretion of the cocoon and its contents. These points have been followed up, and the results arrived at are embodied in the present paper.

Historical.

D'Udekem (1855, 1856) attributed the secretion of the cocoon to certain glands, which he designated the ' glandes capsulogènes ' and which he stated were found in the region of the eighth to eleventh segments in Lumbricus. Hering (1857), however, doubted whether these particular glands did secrete the capsule, but was unable to decide how far the clitellum was responsible, stating (p. 417): ' Inwiefern der Gürtel bei der Eikapselbildung thätig wird, kann ich nicht entscheiden '. Claparède (1869), in his description of the clitellum, says (p. 578) : ' Dass das Clitellum ein drüsiges Organ sei, wird zwar heutzutage von Jedermann angenommen, da ihm die Absonderung der Eierkapsel wahrscheinlich zukommt . . .' Cerfontaine (1890) offers no new observations on this point, but merely suggests (p. 383) certain experiments to determine exactly the part played by the clitellum in cocoon formation. Vejdovsky's (1892) account of cocoon formation in Rhynchelmis appears to be the first record of direct observations upon this phenomenon, and he states that the cocoon is produced in the region of the clitellum. Cole's (1893) criticisms of the view that the cocoon is secreted by the clitellum are so unconvincing that they do not contribute anything to the solution of the problem. Beddard (1895), quoting Vejdovsky's observations, affirms that the cocoon is the product of the clitellum in all the Oligochaeta.

From this point the origin of the cocoon from the clitellum seems to have received general acceptance, but this appears to apply to the cocoon membrane only, for Hering (1857), Vejdovsky (1884), Beddard (1895), and others ascribe to the ' capusologenous glands ' of D'Udekem the function of secreting the albuminous contents of the cocoon. This point will be dealt with in a later section (p. 40).

The literature on the structure of the clitellum is more extensive, and has been summarized by Cerfontaine (1890). A detailed description of the histology of the clitellum of L. terrestris has been given by one of us (Grove, 1925), and the results obtained showed some advances upon the observations of Cerfontaine (1890).

Methods.

In approaching this problem the first essential was to obtain accurate details of the histological structure of the clitellum. As the observations upon cocoon formation and deposition had revealed that the clitellum undergoes changes, often of a profound character, during and after these processes, care was necessary to select material in which the clitellum was in its normal condition, that is to say, in a state of maximum development and ready for cocoon production.

To investigate the activities of the glandular elements it was necessary to obtain material in which the precise stage in the process of cocoon formation was known. In obtaining this, the apparatus used in the observations upon cocoon formation and deposition again proved extremely useful. The tanks were divided into compartments by means of glass strips, and one worm was placed in each compartment. By the use of the technique described in the previous paper, worms exhibiting cocoon formation were kept under observation and killed at the appropriate moment. This was done by removing a section of the side of the tank and allowing the worm to fall into hot water. It was then immediately transferred to a fixative. The fixatives used were Bouin's, and Helly's modification of Zenker's fluid.

For the differentiation of the glandular elements in the clitellum the following stains were used : muci-haematein combined with picro-indigo-carmine, thionin, and Mallory's triple-connective-tissue-stain.

Structure of the Clitellum.

The number of segments included in the clitellum in the Brandling worm shows some variation in different specimens. It may commence on segments 24, 25, or 26, and usually ends on 32. In its normal condition its appearance is pronounced, with sharply defined limits, and much lighter in colour than the other segments of the body. Dorsally and dorso-laterally the segmentation is not distinct, but can be usually demonstrated

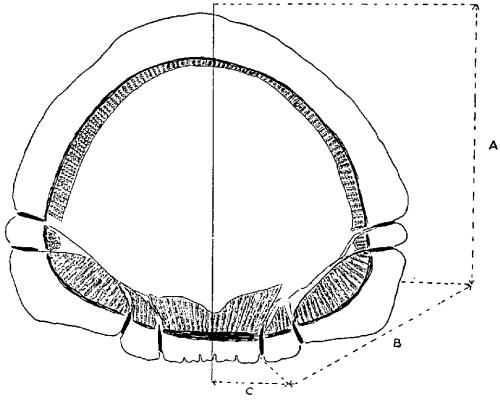
on the ventro-lateral surface. On the ventral surface longitudinal striae are plainly visible, giving this region a wrinkled appearance. In transverse section (Text-fig. 1) the clitellum is roughly circular in outline but shows some flattening ventrally. On the ventro-lateral surfaces the clitellum is thrown into pronounced ridges in segments 28–30 or 31, and papillae are visible just ventral to these ridges in the position occupied by the outer ventral setal pores, but their distribution shows some variation.

In histological structure the clitellum of E. foetida exhibits the same differentiation into regions which has been previously described for L. terrestris (Grove, 1925). Thus in a transverse section through a segment in the middle of the clitellum, it can be divided into three regions on each side of the body (Text-fig. 1). Region A comprises the dorso-lateral portion extending from the mid-dorsal line to a point just below the lateral setae; Region B the portion extending from just below the lateral setae to a point just inside the ventral setae; and Region C the ventral surface. This differentiation is based upon the types of glandular elements present in the respective regions.

Region A.—In this region (fig. 1, Pl. 6) the same three types of glandular elements distinguished in L. terrestris can be demonstrated, namely (*a*) mucin-secreting gland-cells. (*b*) gland-cells with large granular contents, and (*c*) gland-cells with fine granular contents. The mucin-secreting gland-cells are always superficial, and are to be found over the whole surface. Typically each consists of a cell showing the characteristic reticulate contents of a goblet-cell of the epidermis. In some instances, however, they may be more elongated and extend deeper into the thickness of the clitellum. The large granule cells are more deeply seated, often penetrating to a third of the distance between the cuticle and the circular muscles. These cells are elongated, usually expanding towards their lower extremity. The contents consist of large granules which, in the expanded portion, are closely packed together in rows of three or four abreast, but in the narrow extension which opens on the

surface they are commonly found arranged in a single column one above the other. The fine granule gland-cells extend through the whole thickness of the clitellum, and are arranged in groups separated from one another by partitions of connective tissue. Each group consists of a number of glandular cells arranged one above the other in columns. Each cell consists of an expanded basal portion in which lies the nucleus,

TEXT-FIG. 1.



Semi-diagrammatic section through the middle of the clitellum to indicate the positions of the regions mentioned in the text.

and a slender elongated portion which functions as a duct and extends upwards through the thickness of the clitellum until it opens on to the surface.

Region B. (fig. 2, Pl. 6).—The following types of glandular elements are present in this region: (*a*) mucin gland-cells, (*b*) large granule gland-cells, (*c*) fine granule gland-cells, and, in addition, (*d*) special gland-cells occupying the position of the pronounced ventro-lateral ridge, and (*e*) gland-cells associated with the diverticula of the setal pores. The mucin gland-cells are distributed in the superficial tissue in the same manner as

in Region A. The large granule gland-cells are found throughout the region but their distribution may show some variation. The fine granule gland-cells have precisely the same arrangement as in Region A, but in that portion which is marked by the ridge which is so obvious in spirit specimens, the fine granule gland-cells are replaced by groups of cells having the same arrangement and extending throughout the thickness of the clitellum, but which give a different staining reaction from the fine granule cells. These cells also penetrate beyond the circular muscles into the coelomic cavity in the form of a mass of cells lying between the blocks of longitudinal muscles. The glands in association with the diverticula of the setal pores show the same laminate appearance which has been observed in L. terrestris.

Region C.—In transverse section (fig. 3, Pl. 6) this region has a crenulate appearance and consists of a series of blocks of cells separated from one another by deep and narrow invaginations to which are due the striae visible on the surface. Each block of cells is made up of a number of superficially lying mucin-secreting gland-cells relatively more numerous than in the other regions of the clitellum, large granule gland-cells which extend practically through the whole thickness of the clitellum to the circular muscles in this region, and, lying between the bases of these, cells of an interstitial nature with fine granular contents.

Differentiation of the Glandular Elements.

It will be useful at this point to give the distinctive reactions which the respective elements in the clitellum give with the particular stains used.

Muci-haematein and Picro-indigo-carmine.

(*a*) Mucin cells	Deep purple.
(*b*) Large granule cells	Dark green.
(*c*) Fine granule cells	Faint pink.
(*d*) Cells occupying ridge in Region B .	Middle green.
(*e*) Glands associated with diverticula of setal pores	Light green.

In the case of the last two the differentiation was not good.

Thionin.

(*a*) Mucin cells	Deep red.
(*b*) Large granule cells	Greenish blue.
(*c*) Fine granule cells	Deep blue.
(*d*) Cells occupying ridge in Region B .	Faintly tinged blue.
(*e*) Glands associated with diverticula of setal pores	Unstained.

Mallory's Triple Connective-tissue Stain.

(*a*) Mucin cells	Unstained.
(*b*) Large granule cells	Deep red.
(*c*) Fine granule cells	Deep blue.
(*d*) Cells occupying ridge in Region B .	Opalescent or deep blue.
(*e*) Glands associated with diverticula of setal pores	Neutral.

From the above record it will be seen that each of these particular stains gives a distinctive reaction for each of the characteristic gland-cells found in Region A, and this differentiation enables these particular gland-cells (*a*), (*b*), and (*c*) to be distinguished in the other regions of the clitellum. The consideration of the other glandular elements (*d*) and (*e*) may be left for the present, for, as will be shown later, the important elements concerned with the production of the cocoon are (*a*), (*b*), and (*c*) only.

Determination of the Role of the Glandular Elements.

In a previous paper (Grove and Cowley, 1926) it was shown as the result of direct observations that during the process of cocoon formation there is secreted first an enveloping slime-tube extending from two to three segments posterior to the clitellum forwards to about the sixth to eighth segment; later the cocoon membrane is secreted around the clitellum giving this region its characteristic appearance; and, finally, the albuminous contents are deposited between the cocoon membrane and the clitellum prior to cocoon deposition. These observations showed that the portion of the slime-tube immediately surrounding the cocoon, the cocoon membrane, and the albuminous contents of the cocoon were the products of the clitellum.

It seemed reasonable to expect, therefore, that the application of these special staining reactions to sections through the clitellar region of worms killed at various stages of cocoon formation should afford evidence as to the precise role played by each of the glandular elements present in the clitellum in the production of these constituent structures of the cocoon.

It will be well to consider first a transverse section (fig. 4, Pl. 6) through the clitellum of a worm killed just prior to cocoon deposition, this condition being substantiated by the fact that eggs were present in the cocoon. Such sections stained with the stains already mentioned gave the following reactions:

Muci-haematein and Picro-indigo-carmine.

Mucin cells . .	Purple.	Slime-tube . .	Purple.
Large granule cells	Dark green.	Cocoon membrane .	Green.
Fine granule cells .	Some pink and some light green.	Albumen . .	Pink.

Thionin.

Mucin cells . .	Reddish purple.	Slime-tube . .	Same.
Large granule cells	Greenish blue.	Cocoon membrane .	Same.
Fine granule cells .	Light purple.	Albumen . .	Same.

Mallory's Triple Connective-tissue Stain.

Mucin cells . .	Neutral.	Slime-tube . .	Neutral.
Large granule cells	Orange red.	Cocoon membrane .	Orange red.
Fine granule cells .	Blue.	Albumen . .	Blue.

It will be seen from these results that the slime-tube gives the same reaction as the mucin-secreting cells, the cocoon membrane that of the large granule gland-cells, and the albumen that of the fine granule gland-cells.

The examination of sections through the clitellum of a worm which had been killed immediately after cocoon deposition had been permitted to proceed in the normal way, provided further interesting information. In the previous account of cocoon deposition it was recorded that at the termination of the process the clitellum has a distinctive appearance. It shows marked diminution in bulk, so that it no longer projects beyond the general contour of the body and approximates more closely

in colour to that of the other segments. That this diminution in bulk is, as would be naturally expected, due to the discharge by the glandular elements of their various secretions, is shown at once by the sections. The mucin-secreting cells throughout the whole of the circumference of the clitellum have been practically exhausted, very few of them showing the characteristic reticulate appearance so obvious in the normal clitellum. Such cells as still give a mucin reaction with muci-haematein appear to be cells in the process of elaborating fresh mucin contents. The condition of the large granule gland-cells is striking. In practically none of them can be distinguished the characteristic large granules, but in the positions usually occupied by these cells, scattered elements appear giving their distinctive staining reaction. The most striking contrast is to be seen in the fine granule gland-cells. With the exception of scattered isolated instances, the characteristic staining reaction recorded above is no longer manifested. The gland-cells appear shrunken and the fine granular contents can be distinguished no longer. This exhausted condition of the fine granule gland-cells is made all the more obvious by the condition of the cells occupying the ridge in Region B, and of those associated with the diverticula of the setal pores (fig. 5, Pl. 6). In both these instances the cells still give the distinctive staining reactions, and present the same turgid appearance and characteristic contents that are observed in the normal clitellum. From these observations it is evident that the gland-cells in the ridge in Region B, and those associated with the diverticula of the setal pores do not participate in the secretion of the cocoon. It may therefore now be stated that the mucin gland-cells secrete the slime-tube, the large granule gland-cells the cocoon membrane, and the fine granule gland-cells the albumen. In this connexion it will be remembered that the slime-tube extends forwards to the sixth to eighth segment, that portion anterior to the clitellum being secreted by the mucin gland-cells of the ordinary epidermis of the appropriate segments.

The determination of the fact that the gland-cells occupying

the ridge in Region B and the glands associated with the diverticula of the setal pores do not participate in cocoon formation leads to the suggestion that they are probably solely concerned with coition. In support of this, during the examination of sections through pairing worms, secretion has been seen issuing from the diverticula of the setal pores, and also the condition of the cells occupying the ridge suggest that they are active at this time.

It has already been suggested that the secretion of the glands associated with the diverticula of the setal pores assists in the attachment of the worms together, and for the present no further light has been thrown upon this point. The precise function of the cells occupying the ridge is still more obscure, and although many possibilities present themselves, nothing tangible has emerged during the present observations.

Supplementary Notes.

(*a*) 'Capsulogenous Glands'.

D'Udekem (1855 and 1856) gave the name 'glandes capsulogènes' to certain structures which he described in Tubifex rivulorum and L. terrestris, and to which he ascribed as the chief function that 'de sécréter les éléments qui serviront à la confection de la capsule qui entoure les œufs'. Exception to this view was taken by Hering (1857), who states (p. 419): 'Nahe liegt es, das Eiweiss der Eikapsel als das Product wenigstens der vorderen, im neunten bis zwölften Segmente gelegenen Drüsenkörper anzusehen. D'Udekem hat diese gesehen und als "capsulogènes" bezeichnet. Sie sollen zur Bildung der Eikapselschale dienende Filamente absondern. Aber diese Schale ist nicht aus Filamenten zusammengewebt und ich habe auch keine solchen in den drüsigen Körpern gefunden.' Vejdovsky (1884, p. 149) quotes these conclusions of Hering, and suggests the term 'albumen glands' in place of capsulogenous glands, for he describes in Rhynchelmis special glands lying in the ninth segment to which he ascribes the function of albumen secretion. Vejdovsky's lead appears to

have been generally followed, for Benham (1890, p. 207), in his section on nomenclature, states : ' The glandular structures met with in Lumbricus, . . . and others, and usually called " capsulogenous glands ", are misnamed. As far as we know they have nothing to do with the formation of the capsule or cocoon, which is formed by the hardening of the secretion of the gland-cells of the clitellum ; but they give rise to the albuminous fluid found in the cocoon, in which the ova and spermatozoa are deposited, and which serves as nourishment for the developing embryos. Vejdovsky suggests the word " albumen-glands " for these structures, a term which I retain.' Beddard (1895), again, in describing certain genital papillae in the Perichaetidae compares the glands associated with these structures with the capsulogenous glands of Lumbricidae, stating (p. 144) : ' The function of these papillae is a matter of doubt ; more generally they have been held to be organs which allowed the worms to maintain a firm hold upon each other during coitus ; I have suggested that their function may be that of producing albumen for filling the cocoon, and have in consequence compared them to the so-called capsulogenous glands of the Lumbricidae—glands which occur in the neighbourhood of the spermathecae, but whose structure is at present not known.'

Reference to the existence of glandular elements in the region where the ' capsulogenous glands ' are considered to be, has been made in previous papers (Grove, 1925, and Grove and Cowley, 1926), and it has been shown that in both L. terrestris and E. foetida the glandular structures are developed in relation with the diverticula of the setal pores (ventral in Lumbricus and lateral in Eisenia) and that their function is to produce a secretion which is abundant during coition and which probably assists in the attachment of the worms together. The present observations have shown conclusively that the albumen is a product of the fine granule gland-cells of the clitellum, so that the term ' capsulogenous glands ', both in its original and later connotations, is incorrect, and it will be best for it to be discarded altogether.

(*b*) Tubercula pubertatis.

Of this term, in spite of the importance which is attached to these structures by systematists in the classification of the Lumbricidae, there appears to be no precise definition. It was apparently originated by Eisen according to most authors (e.g. Vejdovsky, 1884; Benham, 1890; Beddard, 1895), but none of them gives the exact reference. Cerfontaine (1890) attributes the term to Ude (1885), but since Vejdovsky (1884, p. 156) refers to the term, and Ude's paper did not appear until December 31, 1885, it is evident that Cerfontaine was mistaken. Vejdovsky (1884, p. 156) states: 'Die Bildung des Lumbriciden-Gürtels wird eingeleitet durch die bauchständigen paarigen Anschwellungen, die von Eisen als "Tubercula pubertatis" für die Unterscheidung einzelner geschlechtslosen Arten höchst charakteristisch und constant sein sollen.' Ude (1885), in the introduction to the systematic part of his paper, merely says: 'Als Tubercula pubertatis bezeichnet man die auf der ventralen Seite des Clitellums auftretenden Papillen, welche isolirt stehen oder jederseits eine Leiste bilden können.' Cerfontaine (1890), in describing the external features of the clitellum, states (p. 333): '... sur les bords de la face ventrale de la ceinture règnent deux bourrelets très apparents désignés par Ude sous le nom de Tubercula pubertatis. ...' Benham (1890), in the section dealing with nomenclature, writes: 'The lower edge of the incomplete clitellum is sometimes, as in Lumbricus and Rhinodrilus, further modified, presenting the appearance of a linear band, or group of glands over more or fewer somites. To these the name Tubercula pubertatis has been applied by Eisen.' Beddard (1895), in his introduction to the Lumbricidae, states (p. 688): 'Highly characteristic of the Lumbricidae are the structures first called by Eisen the Tubercula pubertatis. These structures in fact, at any rate in the peculiar form which they show in the Lumbricidae, appear to be confined to that family. They are prominent papillae, sometimes paired, and sometimes taking the form of a pair of bands running con-

tinuously over several segments. Their structure is like that of the clitellum, and they appear before that organ on some of the segments which will be occupied by it. Their position corresponds to the interval between the dorsal and ventral setae, and, therefore, to that of the male pores with which they are sometimes united by a groove, which has been noticed by several writers.'

In these statements some confusion is evident as to what extent the Tubercula pubertatis is composed of a row of papillae or is a continuous band. In L. terrestris and E. foetida there is present along the ventro-lateral border of each side of the clitellum a definite recognizable band or ridge occupying in L. terrestris segments 33–6 and E. foetida segments 28–30 or 31. This structure is continuous along its upper and lower borders with groove-like depressions which are so obvious in spirit specimens due to the contraction of the arciform muscles. The margins of the band outline the course of the seminal fluid during its passage from the seminal groove to the ventral side of the clitellum (Grove and Cowley, 1926, Text-fig. 3). In addition to this ridge there is, associated with the outermost ventral setae, a row of prominences, which are the structures to which Beddard refers as appearing before the clitellum on some of the segments which will be occupied by it (Grove, 1925).

Histologically, the ridge and the papillae are distinct. The ridge is occupied by cells arranged in a similar manner to the fine granule gland-cells of the clitellum. Each cell consists of an expanded basal portion from which extends a narrow ductule to the surface. The contents consist of granules of a slightly coarser character than those found in the fine granule gland-cells. The position of the papillae is occupied by the cells with characteristic laminate structure, associated with the diverticula of the setal pores. In this connexion it must be remembered that glands similar to those found in the papillae are present in other regions of the body, so that they are not characteristic of the clitellum alone. Both they and the gland-cells occupying the ridge have been shown by the present observations to be probably concerned solely with coition.

Summary.

The clitellum of E. foetida may be divided into three regions on each side of the body in accordance with the glandular elements present. Region A, which extends from the mid-dorsal line to a point just below the lateral setae, contains (*a*) mucin-secreting gland-cells, (*b*) large granule gland-cells, and (*c*) fine granule gland-cells. Region B, which extends from just below the lateral setae to a point just inside the inner ventral setae, contains gland-cells (*a*) and (*b*) and (*c*) as in Region A, and in addition (*d*) gland-cells occupying the ventro-lateral ridge and (*e*) gland-cells associated with the diverticula of the setal pores. Region C, which comprises the ventral surface, contains gland-cells (*a*) and (*b*), and cells of an interstitial nature.

By the use of differential stains evidence has been obtained, from a similarity of staining reactions, that the cocoon slime-tube is secreted by the mucin gland-cells, the cocoon membrane by the large granule gland-cells, and the albumen by the fine granule gland-cells.

The gland-cells occupying the ridge in Region B and those associated with the diverticula of the setal pores do not participate in the secretion of the cocoon.

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EXPLANATION OF PLATE 6.

Reference Lettering.

alb, albumen; *co memb*, cocoon membrane; *co sl tu*, cocoon slime-tube; *cir mus*, circular muscles; *cu*, cuticle; *div se po*, diverticulum of setal pore; *f g c*, fine granule gland-cells; *gl se po*, glands associated with the diverticulum of the setal pore; *gl r*, gland-cells comprising the ridge of Region B; *l g c*, large granule gland-cells; *long mus*, longitudinal muscles; *mu c*, mucin-secreting gland-cells.

Fig. 1.—A portion of Region A in transverse section, showing three types of gland-cells. × 91·5.

Fig. 2.—Region B in transverse section. × 50.

Fig. 3.—Region C in transverse section. × 59·5.

Fig. 4.—A portion of a transverse section through the cocoon and clitellum of Region A. × 92·5.

Fig. 5.—Regions B and C in transverse section after cocoon deposition. × 39·5.