The Swimming Setae of *Daphnia carinata*

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**Summary**

1. The structure, renewal, embryonic development, and regeneration of the swimming setae of *Daphnia carinata* are described.
2. Each seta is formed by four giant cells, two (the core cells) forming the distal segment, and two (the sheath cells) the proximal segment. The inner ends of these cells extend back, as the seta strand, through the whole length of the rami of the antenna to be inserted into the hypodermis half-way down the protopodite.
3. The proximal segment of the seta is formed as an inverted sac, enclosing the distal segment. At ecdysis, the sac is everted.
4. The mechanics of the extrusion of the new setae at ecdysis are described.
5. In embryonic development the seta-forming cells are greatly enlarged hypodermal cells, which grow back from the tip of the antenna to the protopodite.
6. The primary embryonic setae consist of the distal segment only of the adult seta.
7. If a ramus of the antenna is amputated, the missing segments are never regenerated. Formation of new setae takes place, however, from the hypodermal membrane which grows across to close the wound. These setae are formed in the same way as in embryonic development.
8. The muscles in the segment through which the amputation was performed degenerate and are not replaced.
9. The potentiality of enlarging and becoming a seta-forming cell is possessed throughout life by all the hypodermal cells of the two rami of the antenna, but not, apparently, by the cells of protopodite hypodermis.

**General Anatomy of the Antenna**

This work is concerned with the setae of the second antenna of *Daphnia carinata*. There are similar setae on the thoracic appendages, and a pair of them at the posterior bend of the abdomen.

The antenna consists of a protopodite carrying a dorsal and a ventral ramus. Each ramus has three segments, which will be referred to as T (terminal), M (middle), and B (basal). In addition the dorsal ramus has a very small wedge-shaped segment between B and the protopodite. Each ramus carries three *terminal setae*. In the dorsal ramus, segment M, and in the ventral ramus segments M and B, carry a single seta on the ventral border of their distal ends. These will be referred to as the *lateral setae*. Thus there are four swimming setae on the dorsal and five on the ventral ramus. Individual setae will be referred to as S1–5 as in Text-fig. 1a. The length of a seta is about equal to the combined length of the three segments of the ramus.

Each seta is divided by a joint which allows ventral flexion of the distal on the proximal segment. The joint is situated below the middle of the seta, the lengths of the distal and proximal segments being in the ratio of about
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1:25 : 1:00. The greater length of the distal segment is an essential part of the mechanism of extrusion of the new seta at ecdysis.

At its base, the seta is very slightly enlarged to form the *insertion bulb*, which constricts to a very narrow opening at its insertion into the antenna. This bulb, and especially its constricted basal opening, also plays an essential part in the extrusion of the new seta at ecdysis.

Each seta is provided with two lines of fine hairs, one on its dorsal and the other on its ventral surface, the dorso-ventral plane being reckoned with the antenna extended in the antero-posterior axis of the body, and the setae parallel with the antennar axis. A longitudinal line of similar hairs runs almost the whole length of the ventral ramus, but on its dorsal border only. These hairs are absent from the dorsal ramus. In addition, the whole surface of the antenna is closely set with fine pointed spines.

Immediately after ecdysis, the centre of the seta is occupied by a protoplasmic axial fibre, which can be traced almost to the tip of the seta. Later in the instar, this fibre is restricted to the lowest third of the proximal segment.

The complicated muscular system of the protopodite has been described by Klotzsche and by Binder. We, however, are only concerned with the muscles of the two rami. These can be flexed, in the ventral direction only.

As is common in Arthropods, the antennar muscles consist of a strand of sarcoplasm with a bundle of striated contractile fibres running along one side of it.

In segment B of the ventral ramus there are two muscles, a larger flexor and a smaller extensor (Text-fig. 1). The sarcoplasmic portions of these are closely applied together. At its distal end the extensor is inserted by a long tendon near the dorsal margin of the joint between B and M. Near the distal end of B, the flexor divides into two, one branch being inserted between the origins of S5 and segment M; the other branch runs on into M, to be inserted in the corresponding position at the end of that segment. The extensor does not enter M, nor does the flexor enter T. The latter segment is therefore devoid of muscles.

The musculature of the dorsal ramus differs from that of the ventral in that there is no extensor muscle.

The details of the movements of the antenna in swimming are too complicated to make out without photographic aid. We need only concern ourselves, however, with the movements of the setae. These are of two kinds: (1) a flexion of the distal on the proximal segment at the joint between them; this takes place in the ventral direction only; (2) a movement of the seta as a whole at its insertion into the antennar cuticle.

During the swimming stroke the segments of the rami, and of the individual setae, are in extension; the lateral setae stand out at right angles to the antennar axis and the terminal setae are in abduction like a hand with the fingers straightened and spread apart. The whole seta apparatus forms a fan-like pattern presenting the maximum resistance to the water. During the forward stroke, which is in preparation for the next swimming stroke, the segments of the rami are flexed, and the seta fan-work is collapsed; the lateral setae rotate
to a position nearly parallel with the antennal axis (their tips pointing forwards), the terminal setae are no longer spread apart but form a loose bunch, and each individual seta is flexed at the joint between the two segments. These

![Text-Fig. 1. A. The left ramus of the antenna showing seta strands, muscles, and the bases of the setae. The setae and the segments of the antennae are designated as in the text. B. A seta at half the magnification of A, showing the joint in the axis and the two opposite lines of hairs. These are actually much finer and more numerous than shown in the figure.](image)

movements of the setae, which can be induced by manipulation of an isolated antenna with a needle, are brought about partly by the resistance of the water and partly, in the case of the lateral setae, by their mode of insertion into the antenna. As can be seen in Text-fig. 1A, it follows from the mode of insertion of the flexor muscles that their contraction to flex the segments of a ramus on
each other (as in the forward stroke) will pull down the cuticle between the setae and the points of attachment of the next antennar segments, thus causing the setae to take up a position pointing forwards, and nearly parallel to the antennar axis. There is no direct insertion of muscles into the setae as was erroneously stated in my paper of 1930.

A nerve runs up each ramus of the antenna (Text-fig. 2). Near the tip of segment T it divides into three branches which run into the bases of the terminal setae. A little lower down, about the middle of T, ganglion cells are inserted on its course, usually arranged in an upper group of three, and a lower one of two, cells.

In the dorsal ramus only, a twig from the main nerve, with two ganglion cells on it, runs to the hypodermis at the base of the short spine on the anterodorsal tip of segment B. This must be interpreted as a sense organ.

I have been unable to find any nerves to S4 or S5 even in preparations where the nerve trunk and its branches to S1–3 and to the sense organ on segment B of the dorsal ramus are all very clear.

FORMATION AND RENEWAL OF THE SETAE

The seta is produced by four giant cells, each more than a millimetre long in a large Daphnia, and with correspondingly large nuclei. Two of these cells are concerned with the formation of the proximal segment of the seta. These we shall call the sheath cells. The other two produce the distal segment, and will be referred to as core cells. It is probable, however, that these latter, though originating as two hypodermal cells, coalesce during embryonic development into a single binucleate cell.

Shortly after ecdysis, the arrangement of these cells is as follows (Text-fig. 4c). The two core cells (or single binucleate cell) form a protoplasmic fibre extending forwards to the tip of the antenna and backwards into a sac, the seta sac, situated just below the base of the seta. The seta sac, which is formed by the two sheath cells, is at this stage a shallow pocket, which will deepen during the course of the instar. At its base, the sheath and core cells taper, and fuse into a thread which we shall call the seta strand. This runs back through all the segments of the ramus into the protopodite.

The three seta strands of the terminal setae are often distinguishable from one another to about the base of segment T, but below that they are fused into a single strand showing no evidence of its tripartite formation. The seta strands from S4 and S5 also unite with the strand from the terminal setae (Text-fig. 1) so that a single seta strand from each ramus enters the protopodite. Sections show that the seta strands end by attachment to the hypodermis about half-way down the protopodite.

The terminal segment of the antenna immediately after ecdysis as seen in whole mounts is shown in Text-fig. 2. The nuclei of the core-cells may be situated within the seta itself, or below it in the seta sac. The six nuclei of the three sheaths are below the level of the core nuclei. Transverse sections show the three closely applied sheaths, often very irregular in outline, each with
two nuclei and enclosing a protoplasmic core, which is the core-cells, or binucleate single core cell. The enormous nuclei, both of core and sheath, make them easily distinguishable from any other nuclei in the antenna.

Immediately after ecdysis there begins a withdrawal of the central protoplasmic fibre of the seta, doubtless due to the deepening of the seta sac which progresses throughout the instar. This withdrawal can easily be observed in those cases where one or both of the core nuclei have been drawn into the seta at ecdysis. In 174 setae fixed within 30 minutes after ecdysis, 76 had one or both nuclei within the seta. In 90 setae fixed within the limits 3–5 hours after ecdysis, only one still had a nucleus in it, the other 179 nuclei being now below the tip of the antenna in the seta sac.

Observations of the backward movement of the core nuclei were also made on living animals. One Daphnia was anaesthetized in 5 per cent. ether and kept under continuous observation, beginning 20 minutes after ecdysis. In the first 6 minutes two nuclei close together in one seta moved back (towards the base of the seta) through a distance of 16 μ. In the next 5 minutes they moved back another 8 μ. In another experiment seven Daphnia, all large adults, were anaesthetized 10–30 minutes after ecdysis. Those setae which had nuclei in them were selected for observation and the distances of the nuclei from the base of the setae were measured. The animals were then liberated, and the process repeated at intervals of half an hour. In thirty-nine setae so observed, the mean backward movement of the nuclei was 26 μ in the first hour and 13 μ in the second hour.

About 24 hours after ecdysis the axial protoplasmic fibre only extends about one-third of the way up the proximal segment of the seta, the parts above this being devoid of a protoplasmic axis. The disappearance of the fibre is partly due to its withdrawal as described, and partly, apparently, to its disintegration in the upper part of the seta. The part of the fibre that remains in the lower part of the seta becomes much finer, and surrounded by a chitinous cuticle, with two longitudinal lines of hairs, distinct from the cuticle of the seta itself. This is, in fact, the tip of the new seta, enclosed within the base of the old one. No further withdrawal takes place. The mechanics of the extrusion of the new seta at ecdysis, to be described later, furnish an explanation of
this fact. The long hairs now projecting from the surface of the new seta present sufficient frictional resistance to prevent further withdrawal through the narrow opening at the base of the insertion bulb.

The further deepening of the seta sacs and backward elongation of the core is most easily followed by observation of the movements of the very conspicuous nuclei of the sheath and core cells. As the seta sac deepens and

the core therefore lengthens, there is a slight backward movement of the sheath nuclei, but a much greater movement of the core nuclei. These are at first in front of the sheath nuclei (Text-figs. 2, 4c) but travel back along the seta strand so as to lie far behind them (Text-figs. 3c and 4A). In the case of the terminal setae, these nuclei move back into segment B; in a large Daphnia this involves a backward movement of a millimetre from their original position. The core nuclei of the lateral setae move back along their seta strands into the protopodite.

During the instar, the sheath cells secrete a layer of chitin on the inner surface of the sheath, forming a cylinder enclosing the seta core. This sheath is
the inverted proximal segment of the new seta. With the deepening of the seta sac this chitinous sheath grows back, finally extending to below the middle of segment M in the case of the terminal setae. This corresponds to the length of the proximal segment of the new seta; its lower end marks the joint between the two seta segments (Text-figs. 3c and 4A). The final condition, just before the next ecdysis, is shown in Text-fig. 4A. The seta core is now completely invested with chitin down to the base of the seta sac, where the cuticles of core and sheath are continuous. Below this, the tapering ends of the sheath and core cells are continued back as the seta strand.

To avoid confusing the figures, the two lines of hairs on the setae are not shown. They are, however, already present in Text-fig. 4A, on the outside of the seta core and on the inside of the sheath. On both core and sheath the hairs pointed forwards. When the seta sac is everted, the hairs turn to approximately a right angle with the seta axis.

The mode of extrusion of a new seta is shown in Text-fig. 4.

Ecdysis starts with the splitting of the head cuticle on each side along a line running from the rostrum dorsalwards between the eye and the antenna to the mid-dorsal line. The head cuticle in front of this line is detached separately from the rest of the cuticle. The carapace then splits along the mid-dorsal line for about the anterior two-thirds of its length. Now the animal pulls itself out of its old cuticle, withdrawing the antenna through the old antennar cuticle, which remains intact. In the extrusion of the new setae, their tips projecting into the old ones play a vital part. As the antenna is withdrawn from its old cuticle, this tip has to be pulled out of the old seta, and therefore through the very narrow constriction at its base. The two lines of fine hairs with which the seta is provided, and which find room to spread slightly in the small terminal expansion of the seta (the insertion bulb), evidently offer sufficient frictional resistance to withdrawal to cause the new seta to be pulled out of its sac as the antenna is withdrawn from its old cuticle. This causes the eversion of the sheath to form the proximal segment of the new seta (Text-fig. 4B). During this progress there is often a corrugation of the upper end of the sheath.

That this is the mechanics of the extrusion of the seta was shown by amputating setae at their bases, so as to remove the insertion bulb, about an hour before ecdysis. When this is done, the new seta is not extruded at all, or sometimes only partly extruded, though unoperated setae on the same antenna are pulled out of their seta sacs in the usual way. This operation was also valuable as a means of getting partially extruded setae (as in Text-fig. 4B), for attempts to get setae in this state by fixing animals in the act of ecdysis were seldom successful, owing to the quickness of the withdrawal of the antenna from its old cuticle.

Setae may be artificially everted from amputated antennae in the following way. The animal is killed shortly before ecdysis is due (between liberation of the young from the brood-pouch and the ecdysis which usually follows within an hour or so). The terminal setae of the amputated antenna are held against the microscope slide, and by means of a needle inserted into the cut base of
the antenna, it is pulled out of its old cuticle. By this means the new setae are everted from their seta sacs—often completely, sometimes only partially. This operation can be successfully performed on antennae amputated from animals that have been in a 5 per cent. solution of ether for 2 hours after the heart has stopped beating, and in which therefore the tissues must certainly be dead.

At the extrusion of the seta, the protoplasm of the core cells, being continuous with the axial fibre of the distal seta segment, is drawn into the sheath (proximal segment of the seta) as it is everted.
The pulling forward of its anterior end results in a thinning out of the lower part of the seta strand, since its base is fixed in the protopodite. The two core nuclei are also, of course, pulled forward. Their final position depends on how far below the lower end of the seta sac they were situated before ecdysis (Text-fig. 4A). In the fully everted seta they may both be situated in the now shallow seta sac at the tip of the antenna; or if, before the eversion started, they were higher up, one or both may be drawn into the sheath and so come to lie in the proximal segment of the seta itself (Text-figs. 2, 4C). When this occurs they are soon withdrawn below the tip of the antenna as already described.

E A R L I E R A C C O U N T S O F T H E S E T A E

The formation of setae in pockets from which they are extruded in ecdysis has been described for many groups of Crustacea. In Branchiopods, Claus (1876) gives beautiful figures of the setae on the thoracic appendages of Daphnia similis. These setae and the pair of backwardly projecting abdominal setae are, as I have verified in D. carinata also, of similar structure to those on the antenna. Claus does not, however, describe their formation or renewal at ecdysis.

The most recent account of the setae of a Branchiopod which I have found is that given by Nowikoff for Limnadia, a form not, however, very closely related to Daphnia. Nevertheless, the structure and formation of the setae are evidently very similar in the two genera. His description of the formation of a new seta, and its extrusion at ecdysis, is in essentials similar to the account I have given for Daphnia. He does not, however, describe the mechanics of the extrusion. Moreover, his figure 50 is similar in essentials to my Text-fig. 4A, but he interprets what I have called the seta strand as a nerve, continued into the seta itself. In the position of my two core nuclei are four cells which he interprets as sensory cells. The sheath cells are, to judge from his figures, ordinary hypodermal cells. In fact, the sheath is an invagination of the ordinary hypodermis.

The seta strands and their connexion with the setae have been interpreted by some workers as muscles, or at least as tendons (e.g. Binder, for D. magna). In my statistical studies of the regeneration of the antenna (1930, 1931), which were not directly concerned with the histology of the process, I made the same mistake.

Early in an instar, when the seta sacs are depleted and crowded close up under the bases of the setae, the long fine seta strand into which they are continued certainly suggests a nerve or tendon. But later, when the seta sacs have extended down it, it has no such resemblance.

The histology of the seta strand close below the seta sac is shown, somewhat diagrammatically, in Text-figs. 2 and 4. It consists of a central darker staining core, continuous with the core cells, surrounded by a paler sheath continuous with the sheath cells. Lower down, the central core is not always recognizable, and sometimes appears broken up into fibrils. Near its lower end, within the protopodite, the seta strand stains much less strongly and is distinctly fibrillar.
The seta strand is clearly a backward prolongation of the core and sheath cells, and indeed the formation of the strand by backward growth of these cells, derived from the hypodermis, can be followed in embryonic development and regeneration. Its function is to act as a support down which the seta sacs can extend. Fixed as it is at its lower end, when the seta sacs are everted and drawn up to the bases of the setae at ecdysis, it remains as a thin strand, which thickens as the seta sacs spread down it again to form the setae for the next instar.

The mode of formation of bristles in insects (Wigglesworth, 1933, and others) indicates the way in which the complex setae of *Daphnia* and other Crustacea may have evolved. The insect bristle is formed from two cells, a lower hair-forming cell and an upper socket-forming cell. The latter forms a chitinous ring through which a process grows out from the hair-forming cell to form the projecting part of the bristle.

It is easy to see how a sinking down of the hair-forming cell could drag down the socket cell to form a sheath which is everted at ecdysis. In that case, the distal segment of the *Daphnia* seta corresponds to the projecting part of the insect’s bristle, and the proximal segment to the elongated and everted socket.

**Embryonic Development of the Setae**

The egg is in a very fluid state when laid. It has the appearance of being poured through the narrow opening of the oviduct into the brood-pouch. Within a few minutes it changes from an irregular sausage shape into a sphere, and at the same time the egg membrane, excessively thin and flexible before this, thickens to form an elastic transparent membrane. About 2 days later (at ‘room temperatures’) this membrane splits and eclosion of the embryo takes place. The embryo, however, is still enclosed in a very fine transparent membrane, which is not a cuticle in the ordinary sense, for it does not follow the contours of the developing appendages. It must form as a complete internal lining to the egg membrane at a very early stage of development. We shall refer to this membrane as the embryonic membrane.

At the time of eclosion, the rudiments of the antennae have their apices directed backward, slightly pushing out the embryonic membrane from the body. They elongate backwards, between the body and the membrane, till their tips have reached nearly to the hind end of the body. The antennae then rupture the membrane and thus become free. This is brought about by movements of the antennae themselves, which are pulled forward between the body and the membrane, at the same time bending outwards at the joint between the protopodite and the rami. After about half an hour of spasmodic movements of this kind, the membrane is ruptured and the antennae pulled out of it.

After a lapse of a few minutes to an hour or so after their release from the brood-pouch, the embryos undergo an ecdysis which brings the antennar setae into their definitive adult condition.
About 1½ hours after eclosion each seta is represented by an elongating hypodermal cell, its distal end level with the outer boundary of the hypodermis, but internally projecting far below it. These cells are conspicuous, not only for their length, but also for their more densely staining cytoplasm. Owing to the compactness of the tissues at this stage it is difficult to discriminate between the nuclei of these seta cells and those of the hypodermal cells between which they lie.

Text-fig. 5 is of a longitudinal section of the terminal segment of an antenna 4 hours after eclosion, the rudiment of S1 being only just grazed by the razor. Each of the rudiments of S2 and S3 consists of two very large cells, one behind the other, possibly already fused in S2. These are evidently the future cores cells. Their nuclei are already larger than those of the cells of the general hypodermis, and their tips project beyond its surface. This is a projection of the cell itself, not of cuticle. Indeed at this stage no cuticle can be detected. It is not yet possible to identify sheath cells.

By the time the antennae free themselves from the embryonic membrane (about 36–48 hours after eclosion) the rudiments of the three terminal setae form together a multicellular mass, which, as evidenced by the number of nuclei, consists of the full complement of core and sheath cells. Cell boundaries cannot, however, be made out satisfactorily. Posteriorly, this cell mass has already grown back to the protopodite. Anteriorly, the short blunt projections of the core cells in Text-fig. 5 have elongated into fine threads covered with cuticle. These primary setae, as they may be called, correspond to the distal segment of the definitive seta. Thus they lack the joint of the adult seta, but are flexible enough to bend into U- or S-shapes.

The primary setae continue to elongate till by the time the young are released from the brood-pouch (about 5 days after the eggs are laid) they have about half the length of the ramus. Each consists of a staining central strand continuous with the protoplasm of the cell and surrounded by soft cuticle. By now the seta sacs have developed, and the whole structure is similar to that in the adult, except that the projecting part of the seta corresponds to the distal segment only of the adult seta. At the ecdysis which follows release from the brood-pouch, eversion of the sheath takes place as described for the adult, and the animal is now provided with jointed setae of the adult type.

The advantage of this type of swimming seta, consisting of two rigid segments bending at the joint between them in one direction only, over the unjointed but flexible setae of the late brood-pouch young, is evident from the sudden change in the nature of the movements of the animal after this ecdysis. Before this, in spite of the violent action of the antennae the movements of the newly released young are very feeble and result in little true locomotion, in striking contrast to the strong, directed movements immediately after the ecdysis.
Regeneration of the Setae

I have dealt with the regeneration of the antenna in Daphnia and Simocephalus in two former papers. That of 1930 was a statistical study of the number and length of setae regenerated, and the factors influencing this. The second paper (1931) demonstrated that amputation and subsequent regeneration for a hundred generations had no detectable influence on either the regeneration or normal growth of the antenna. Neither of these papers dealt with the histology of the regeneration process.

As stated in the earlier papers, and confirmed by the present work, the segments of the antenna removed by the operation are never regenerated; new setae, however, are formed freely from the tip of the antennary stump after amputation through any one of the segments T, M, and B.

One conclusion of my former papers needs to be modified by the results of the new work. In the earlier experiments the dorsal ramus of the right antenna was amputated within a few hours of release from the brood-pouch, through segment B in Daphnia and M in Simocephalus (designated in the earlier papers as II and III respectively). Thus four setae, three terminal and one lateral, were removed in each case. The number and length of setae regenerated were recorded in the first adult instar. In more than a thousand antennae, the number of setae ranged from 0 to 9, and an analysis of the distribution of the numbers showed that it could be interpreted as a normal probability distribution in which the number 4 occurs in excess, with a compensating deficiency of numbers greater than 4. This, together with the fact that 4 was also the number of setae removed, was made the basis of some theoretical discussion.

In the present work, most of the amputations have been performed not on young but on mature and therefore much larger animals, and the number of setae regenerated is substantially greater than in the earlier experiments, numbers above 4 being commoner than those below. (The maximum number, though in a very much smaller total than in the earlier experiments, is also 9.) It appears therefore that the number of setae regenerated is largely influenced by the area of the hypodermis which closes the wound and forms the new tip of the antenna from which the new setae are formed. Therefore the fact that operation in new-born animals tends to be followed by the reproduction of the missing number of setae has not all the significance that I attributed to it.

For operation, the animal is anaesthetized in a 5 per cent. solution of ether in water, and placed on a microscope slide to which a strip of celloidin has been cemented. The antenna is amputated by a splinter broken off from the edge of a safety- razor blade and mounted on a holder. The amputations were made near the distal ends of segments M or B on either the dorsal or ventral ramus, usually on the corresponding rami of both antennae. The loss of one ramus from both antennae causes no serious disturbance in the life of the animal, as judged by egg-production.

Except when there were reasons for the contrary, the operations were performed within an hour or two after ecdysis, in order to avoid the disturbing
factor of the presence, later in the instar, of the setae to be extruded at the next ecdysis. With operation early in an instar the sheath and core nuclei are removed, or if left in the antenna they disappear. The remains of the seta strand can often be identified for a while contracted to the base of the operated segment. In any case, neither the old seta cells if present, nor the seta strand, take any part in the production of new setae. These are formed entirely from the hypodermis which closes the wound.

The muscles cut through by the operation contract to the base of the operated segment and degenerate; about 4 or 5 days after operation they can still be identified as a nearly homogeneous mass. After two ecdyses after operation no remains of them can be identified in whole months. No formation of new muscles to replace the old ones was ever found. This, however, is not surprising, as the regenerating segment is now the terminal one, and this has no muscles even in the intact antenna.

The nerve disappears from the operated segment, and only once out of a large number of cases was a nerve found after formation of new setae is complete.

The whole process of regeneration concerns therefore only the formation of new setae.

Amputation is followed by an out-gush of blood, which clots to form a plug closing the cylinder of cuticle which is the outer wall of the antenna. After about 24 hours (at winter room temperatures, at which an adult instar lasts about 5 days) this plug has been transformed into a densely pigmented fibrous structure, containing cellular elements consisting doubtless of leucocytes and cells from the hypodermis, which becomes disorganized for a short distance below the wound.

The hypodermis lining the cuticle has now grown across the wound below the plug, forming a thin membrane consisting of a single layer of flattened cells like the rest of the hypodermis.

About 40 hours after the operation the condition is as shown in Text-fig. 6. The reconstituted part of the hypodermis now forming the tip of the regenerating antenna, though still only one-layered, is becoming thicker than the rest of the hypodermis owing to the enlargement of its cells. In the figure, one cell in particular is seen to be extending below the inner boundary of the rest of the hypodermis. Examination of later stages shows that this is a future seta core cell. Its nucleus is already larger than those of typical hypodermal cells.

The number of leucocytes present in the injured segment is much greater than in the normal antenna. They are distributed throughout the segment, but are specially numerous close under the terminal hypodermal membrane from which the new setae are to be formed.

About 50 hours after operation there has been a general enlargement of the cells of the hypodermal membrane closing the wound. Some of them are sending out long processes into the lumen of the antenna; these are the future core cells. They become arranged in pairs, one still in the hypodermis, the other sunk below it. They soon, however, come to constitute a binucleate body in which a separating cell boundary cannot be distinguished.
Text-fig. 7 shows the condition after the lapse of one full instar, and therefore also of one ecdysis, after operation. (The smaller size of Text-fig. 7 compared with Text-fig. 6 is due to the fact that the former is a section through segment M, and the latter through segment B.) The cells of the hypodermis closing the wound have now enlarged enormously. On the left of the section a seta is seen growing out of a binucleate cell mass formed by two core cells. On each side of it is an elongating hypodermal cell, no doubt the future sheath cells. On the right can be seen a multicellular mass, cut obliquely; in neighbouring sections at least two setae can be seen to originate from this mass.

Like the primary embryonic setae, the regenerating seta at this stage is formed from the core cells only, and therefore corresponds to the distal segment of the adult seta.

The ecdysis at the end of the instar in which the operation was performed carries away the plug at the end of the amputated segment, and the primary setae, which had grown out between the hypodermis and the plug, now project to the exterior.

During the instar initiated by this first ecdysis after operation (the instar the beginning of which is represented by Text-fig. 7) the full morphology of the seta apparatus is established. During this instar there is a rapid back-growth of core and sheath cells to form the seta sac and strand, their advancing tips frayed out into pseudopodia-like processes reminiscent of a regenerating vertebrate nerve-fibre. When the operation was through segment M, by the following instar these processes had penetrated into segment B, where some of them came in contact with the muscle strands of that segment.
Whether this is a sufficient fixation basis for them, or whether they continue to grow back to their normal fixation point half-way down the protopodite, has not been ascertained.

At the second ecdysis after operation the sheaths are everted in the usual way, and the typical jointed setae, supplied with the two lines of hairs, appear. The setae are, however, still much shorter than the normal setae corresponding with the shorter seta sacs. They increase in length at subsequent ecdyses, but never reach the normal length in animals operated as adults.

The process of forming new setae after amputation is therefore similar to that in embryonic development; owing to the much larger area of the hypodermis involved, the number of setae regenerated is, however, often much larger than in embryonic development. I have not found any mitoses in the regeneration blastema, if one may so describe the area of hypodermis from which the new setae are formed, nor in the neighbouring parts of the hypodermis. The large mass of new protoplasm involved in the formation of half a dozen setae is provided entirely by the enlargement of individual cells.

As we have seen, the potentiality of seta-formation extends along the whole length of the dorsal and ventral rami, for new setae are formed whatever the level of amputation, even when this is through segment B of the dorsal ramus, which is far below the level of normal seta formation. The potentiality is not even confined to those cells which, as a consequence of the operation, have become constituents of the hypodermis forming the tip of the stump of the amputated antenna. Seta-formation can be induced by pricking an intact antenna anywhere along its length distal to the protopodite. This is followed by an enlargement of the hypodermal cells round the point of injury, which produce setae in the same way as described for the formation of new terminal setae. Seta-formation does not, however, result from a lateral wound so regularly as it does from the tip of an amputated antenna. Out of 37 operations in which a fine needle was passed through the wall of the antenna at various points along its length, 15 produced 1–7 setae each at the point of the wound. In the remaining 22, after throwing off the scar tissue at the next ecdysis, no setae appeared.

The protopodite, however, is either incapable of producing setae, or its capacity to do so is very low compared with that of the rami. Amputation through the protopodite proved too severe an operation, but sixteen protopodites were pricked, and scars formed over the wounds exactly similar in appearance to the scars formed by pricking the rami; not one seta, however, was produced.
Similar small wounds were made in the neighbourhood of the pair of abdominal setae, but no new setae were ever produced. The other parts of the body carrying setae of this type, the thoracic appendages, are not accessible to operation. Small wounds in various parts of the head and carapace heal rapidly, and, as was to be expected, without seta formation.

Thus any hypodermal cell of the antenna distal to the protopodite has the potentiality of becoming a seta-forming cell, given the necessary conditions. It is possible that one of these conditions is that the cell should be relieved from the tension of the thinly stretched hypodermis lining the antennar cuticle, thus giving it opportunity to enlarge. It will be noted that the setae formed in embryonic development are produced at the apices of segments, for even the lateral setae are terminal to the segments from which they spring.

In fact, we are here not concerned with regeneration in the sense of restoring the normal condition, but with the release of a potentiality which is normally inhibited owing to the lack of some further factor necessary for its realization. The problem of why certain cells at constant positions in the embryonic antenna develop setae, is equally the problem of why seta formation is restricted to these positions, since all the hypodermal cells distal to the protopodite are potential seta-producers.

It appears therefore that the determination of a seta cell takes place in two stages. First, there is the acquisition of a state of competence to differentiate into seta-forming cells. This occurs in certain regions of the body, namely, the antennae distal to the protopodite, the thoracic appendages, and a small region at the posterior bend of the abdomen. This competence is retained throughout life in the case of the rami of the antennae. The second stage in the determination is the local morphogenetic stimulus to which the response of a seta-formation is given. The situation is not greatly altered if for the provision of a positive morphogenetic stimulus we substitute the local removal of an inhibiting factor.

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