Caudal regeneration in the absence of a brain in two species of sedentary polychaetes

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SUMMARY

1. Evidence has been obtained showing that unlike the requirements of errant polychaetes studied by other investigators, at least two species of sedentary polychaetes, Branchiomma nigromaculata and Chaetopterus variopedatus can undergo caudal regeneration in the absence of a brain.

2. Both Branchiomma, when maintained in a brainless condition by repeated removal of anterior segments, and Chaetopterus, when rendered brainless by amputation of cephalic segments at a level at which anterior regeneration does not occur, undergo posterior regeneration following removal of caudal segments.

3. Animals maintained in a brainless condition for 7–10 days prior to posterior segment removal undergo normal posterior regeneration. Consequently, it appears unlikely that a circulating brain hormone present at the time of amputation is responsible.

4. Experiments were performed on the extremely long 13th segment of Chaetopterus. An isolated posterior half of segment 13, which possesses ventral ganglia, regenerates both anteriorly and posteriorly, while the anterior half regenerates only anteriorly. That the tissues of the anterior half of segment 13 are competent to regenerate posteriorly was shown by leaving the anterior half of the segment attached to the first 12 segments. Under these conditions posterior regeneration occurred.

5. These experiments suggest that the ventral ganglia rather than the brain are important in posterior regeneration in these species.

INTRODUCTION

Studies have shown that in many polychaetes the supraoesophageal ganglia exert considerable influence on posterior regeneration (Casanova, 1955; Durchon, 1956; Clark & Clark, 1959; Clark & Bonney, 1960; Hauenschild, 1960; Golding, 1967a, b, c). The hypothesis has been put forward (Clark & Evans, 1961; Clark & Ruston, 1963; Clark & Scully, 1964; Scully, 1964) that following caudal amputation, a temporal sequence of accumulation of neuro-secretory material by the brain and its subsequent release controls replacement of posterior segments in some species of nereids and nephthyids; however, experiments of Durchon & Marcel (1962) and Golding (1967a, b) have shown that transplanted brains from intact animals are equally competent to induce regeneration as brains removed from animals following amputation. Despite lack of agreement concerning the conditions under which a brain can be activated

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and the time of hormonal release, there is a consensus that a brain is necessary in these animals for caudal regeneration to occur, that its role can be filled by an implanted brain which lacks nervous connexions, and that the role of the brain is probably neurosecretory.

This postulated neurosecretory mechanism receives much support from histological studies carried out on a number of species, primarily utilizing the affinity of neurosecretory cells for some of the Gomori stains, particularly paraaldehyde fuchsin. In 1936 Scharrer described neurosecretory processes in the supraoesophageal ganglia of *Nereis virens*. This work has subsequently been extended by several authors to include a variety of oligochaetes and polychaetes (Herlant-Meewis, 1956; Herlant-Meewis & Van Damme, 1962a; Clark, 1955, 1959; Hauenschild & Fischer, 1962; Aros, Vigh & Teichmann, 1965; Teichmann, Aros & Vigh, 1966; Scharrer & Brown, 1962). A discussion of the problems relating to the interpretation of cytological demonstration of neurosecretion is provided by Bern & Hagadorn (1965). Although direct correlation between histological evidence of neurosecretion and regenerative processes remains tenuous, modifications of cells considered to be neurosecretory have been described during the early stages of regeneration by Clark & Clark (1959), Clark, Clark & Ruston (1962), Herlant-Meewis & Van Damme (1962b), Herlant-Meewis & Gallardo (1965), Santhakumari (Nayar, 1966) and others. In addition, specific destruction of neurosecretory cells of the brain of *Platynereis* with a fine ultraviolet beam is reported to inhibit regeneration (Hoffman, 1966). Excellent reviews of annelid neurosecretion have been provided by Clark (1965, 1966, 1969), Gabe (1966) and Durchon (1969).

In the errant polychaetes studied, the brain has proven essential for complete caudal regeneration and it is becoming accepted that polychaetes require a brain for regeneration to occur (Herlant-Meewis, 1964; Tombes, 1970). However, since simultaneous anterior and posterior regeneration occurs in many of the sedentary polychaetes, it seemed appropriate to test the requirement in some of these species.

Experiments were performed on the sabellid fanworm *Branchiomma nigromaculata*, which is capable of regenerating a head and tail following transection of the body at almost any level, and on *Chaetopterus variopedatus*, which has more restricted regenerative capacities. Animals of the latter group were very useful for these experiments because removal of the anterior portion of the worm behind segment 14 (Fig. 1) is followed by failure to regenerate anteriorly (Berrill, 1929). In this way a brainless animal can be readily obtained. In addition, the 13th segment, which is capable of regenerating an entire worm, is extremely long and operations can be performed on one isolated segment, thus excluding the influence of adjacent segments. Finally, around Hawaii, a small form of *Chaetopterus variopedatus*, averaging about 2.5 cm in length, is found. Because of their size, these animals can be maintained with ease in large numbers during experimentation, and regeneration proceeds rapidly.
MATERIALS AND METHODS

Animals of the species, *B. nigromaculata* and *C. variopedatus*, were collected from the waters around Coconut Island, Oahu, Hawaii. Frequent collections were made, so that animals were held in the laboratory for only short periods of time prior to amputation. Operations were usually performed without the use of anesthetic; however, when necessary, a saturated solution of chloretone in sea water was added drop-by-drop until narcotization was achieved.

It was found that mortality could be greatly reduced by maintaining numerous worm fragments in filtered sea water in large containers instead of individually in small finger bowls. Consequently, regenerating animals were usually housed in aquaria of 2 or 3 gal (7.6 or 11.4 l) capacity containing a layer of well-washed sand and provided with a continuous air supply. Sea water in finger bowls and aquaria was changed daily. Animals were maintained at room temperature which was 25 ± 2 °C at the time of the experiments.

RESULTS

Normal regeneration

*Branchiomma nigromaculata*

Following amputation of either anterior or posterior segments, the edges of the wound are pulled together apparently by muscular contraction. Under the conditions of the experiment, epidermis usually covers the anterior amputation surface within the first 24 h. By 68 h a bi-lobed anterior regeneration blastema has formed. The first apparent signs of differentiation are usually seen around 72 h when the blastema acquires a slightly uneven outline. Within the next 12 h, finger-like projections corresponding to the future tentacles become apparent. These elongate rapidly and within about 8 days well-differentiated heads are present. Pinnules line the developing tentacles, palps have formed, and rudiments of the new collar and particle-sorting apparatus can be observed. A similar time sequence for this species has been observed by Fitzharris & Lesh (1969).

Within 3 days of posterior amputation, a well-developed blastema is usually present. New posterior segments are progressively added. These are clearly distinguishable for some time after their formation by their smaller size and by their coloration which is considerably lighter than the heavily pigmented original segments.

*Chaetopterus variopedatus*

In these animals, regeneration proceeds very rapidly after amputation. Following removal of the anterior 12 segments, a blastema is visible in 36 h. By 48 h the blastema has elongated and the anterior tip is slightly enlarged. It continues to increase in length, and acquires the appearance of an un-segmented
tube curving dorsally over the old part of the worm. Numerous somites appear to be produced simultaneously by segmentation of the blastema. Within 72 h external segmentation is evident and rudiments of the long notoform alipodia of segment 12 and of the tentacles can be seen.

During posterior regeneration, usually by 48 h, the blastema has acquired an irregular appearance corresponding to the formation of future segments. By
72 h it is quite long and demarcation of segments is more apparent. Subsequent differentiation is dependent upon which segments were initially amputated, since *Chaetopterus variopedatus* is one of the species which replaces precisely what is missing. This ability to 'count' has been described previously by Berrill (1929). During posterior regeneration, proximal segments are differentiated first. If the posterior segments are removed at the level of segment 16, about three tiny genital segments are distinguishable by 72 h. If the amputation occurs at the level of segment 13, the fan segments, 14, 15 and 16, are apparent before the genital segments. By 72 h, an enlargement corresponding to one fan is apparent and the elongating blastema retains its uneven appearance. Three fans are usually apparent by 120 h, and elongation and differentiation of genital segments continues. Because of the very different morphology of segments, most segments can be recognized at an early stage of development.

In the following experiments utilizing *Chaetopterus*, some degree of variability of time of regeneration was observed, especially in cases in which the operation involved bisecting a segment. Delays probably corresponded to difficulty in closing the wound and they did not exceed 24 h.

**Experimental**

Anterior and posterior ends were removed from 20 fanworms (*B. nigromaculata*). The posterior-facing surfaces were allowed to regenerate; however, the formation of a new head, which would contain a developing brain, was prevented by amputating the most anterior two old segments each day. Ten animals in which the heads were left intact and caudal portions amputated served as controls. Within 8 days after amputation all animals but one, which will be discussed subsequently, possessed differentiated new posterior segments. Fanworms undergoing repeated anterior segment removal regenerated posterior segments as rapidly and as completely as the controls.

The possibility of a circulating brain hormone present at the time of amputation was investigated by amputating heads prior to tail removal. Heads only were removed from 45 worms. As in the experiment described above, anterior segments were removed in this case, every second day, for the next 10 days, at which time the posterior end was amputated. Caudal regeneration was allowed to proceed, while anterior segment removal continued throughout the course of the experiment. Early removal of the heads in this experiment had no effect on caudal regeneration, and replacement of the tails proceeded at the normal rate. Again, one animal, which will be discussed later, did not replace the tail segments.

As a result of the inability of *Chaetopterus variopedatus* to regenerate anteriorly following transection of the body behind the 14th segment, a brainless animal can be readily obtained without the trauma of repeated injury. Segments 15 and 16 were isolated from 20 worms and their regeneration
Table 1. Regeneration of anterior and posterior halves of segment 13 of Chaetopterus variopedatus. Animals in each category are expressed as percentages of survivors.

<table>
<thead>
<tr>
<th></th>
<th>Posterior halves</th>
<th>Anterior halves</th>
<th>Whole segment controls</th>
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<tbody>
<tr>
<td>No. of experimental animals</td>
<td>50</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>No. of survivors</td>
<td>40</td>
<td>36</td>
<td>17</td>
</tr>
<tr>
<td>Differentiated heads and tails</td>
<td>31 (78%)</td>
<td>0</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>Differentiated heads only</td>
<td>8 (20%)</td>
<td>27 (75%)</td>
<td>0</td>
</tr>
<tr>
<td>Differentiated tails only</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No regeneration</td>
<td>1 (2%)</td>
<td>9 (25%)</td>
<td>0</td>
</tr>
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observed. All 20 fragments underwent a rapid caudal regeneration and differentiating genital segments could be seen within 3 days.

In a second experiment similar to that performed on Branchiomma, 40 worms were sectioned between segments 14 and 15 and the posterior portions maintained for 1 week. At that time the genital segments were removed so that a fragment of worm consisting of segments 15 and 16 remained. Again, the isolated segments replaced the missing posterior portion in the complete absence of a brain or any anterior regeneration.

A third experiment was carried out to investigate the role of the ventral ganglia in Chaetopterus regeneration. In this worm the 13th segment is very long (Fig. 1) and is capable, in isolation, of regenerating an entire worm. A ciliated cup is present on the dorsal surface of the segment and directly opposite, on the ventral side, is a suction disc. Paired ventral ganglia lie above the suction disc, although nerve cell bodies are not restricted to this area. Segment 13 was isolated from 50 worms. Each segment was then divided into an anterior half lacking ganglia and a posterior half containing them by sectioning immediately in front of the ciliated cup and ventral suction disc. Twenty isolated entire 13th segments were established as controls. The results, expressed as percentages of survivors in each category, are given in Table 1.

From the data, it is apparent that the posterior portions of segment 13 possess the capacity to regenerate both anterior and posterior segments (Fig. 2A). Anterior portions, however, produced only new anterior segments. Although healing of the posterior wound occurred in these anterior portions, in no case were caudal segments regenerated (Fig. 2B).

When an amputation is made through the middle of a segment, closure of the wound is not effected as readily as if the amputation is made between the segments. Because of the trauma involved in this experiment, mortality was quite high. Fourteen anterior halves and ten posterior halves died without undergoing any differentiation. A few half-segments, although they did not die
Fig. 2. Regeneration of segment 13 in Chaetopterus variopedatus. (A) Posterior half of segment 13 with regenerated posterior (r) and anterior (h) segments; ciliated cup, (cc). (B) Anterior half of segment 13 with regenerated anterior segments only (h). (C) Anterior half of segment 13 undergoing posterior regeneration (r) when left attached to first 12 segments. (C) × 12.
Fig. 3. Amputation and regeneration of segment 13 of *Chaetopterus variopedatus.* (A) Isolated segment 13 undergoing transection. Anterior portion regenerates only anterior segments (h), while posterior portion regenerates both anterior (h) and posterior (t) segments. (B) Anterior portion of segment 13, when left attached to first 12 segments, regenerates posterior (t) segments.

during the course of the experiment, never produced differentiated segments, probably also as a result of the trauma involved. It is clear, however, that the posterior halves can regenerate segments posteriorly as well as anteriorly, while the ability for posterior regeneration appears lacking in the anterior halves.

To determine if this regenerative failure of the anterior halves was a result of
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incompetence of the local tissues, ten whole worms were transected through segment 13, again immediately in front of the ventral suction disc. This provided pieces of worm consisting of the first 12 segments and the anterior half of segment 13 which does not regenerate posteriorly in isolation, and which lacks ganglia. Under these conditions, normal regeneration occurs (Fig. 2C). These results are summarized in Fig. 3.

The two specimens of Branchiomma which did not form new tail segments while undergoing repeated anterior segment removal, did form a regenerate at the posterior surface. In both cases, tentacles and a normal head were produced at the posterior end. In each of these animals, following formation of the posterior head, the anterior end was allowed to regenerate, and a second head was produced in both. In one worm the anterior head was amputated again, and one day later the posterior head was amputated. Blastemata formed at both ends and again two new heads were produced.

DISCUSSION

By repeated removal of the anterior segments of Branchiomma, and by amputating the anterior part of the body of Chaetopterus at a level at which anterior regeneration could not occur, it was possible to obtain brainless animals and to eliminate the possible influence which a developing brain might exert during regeneration of a head. Under these conditions, it was found that animals of both species are capable of undergoing caudal regeneration in the complete absence of a brain, and of doing so at the same rate at which they would regenerate if the brain were present. It appears, then, that contrary to the conditions reported for Nereis and Nephthys (Clark and co-workers, 1959–64; Durchon & Marcel, 1962; Golding, 1967a, b, c), the brain exerts little influence upon regeneration in Branchiomma and Chaetopterus. In these animals a hormone produced by the brain cannot be prerequisite to regeneration, unless it is produced by the brain in an intact animal and persists in the body for longer than 10 days.

In the light of the experiments performed on Chaetopterus involving bisection of an individual segment, it seems more likely that the ventral ganglia present in each segment of the worm play an important role in caudal regeneration. The posterior portion of segment 13 which contained the ganglia regenerated caudal segments while in all cases the anterior part of the segment did not. Since the anterior part of the segment did regenerate caudally when the first 12 segments were left attached to it, the failure of this part of the segment to regenerate in isolation is not due to a lack of ability to close and heal the amputation wound successfully, or to an incompetence of local tissues. It appears, rather, that something which is necessary for regeneration can be produced by the anterior segments. It apparently is present in the posterior part of segment 13 but not in the anterior portion. It is possible that the necessary component is an essential type of cell which migrates. In the light of the
investigations cited (Clark and co-workers, 1959–64; Durchon & Marcel, 1962; Golding, 1967a, b, c), however, it is tempting to suggest that neurosecretion is involved. This is in good agreement with the results of experiments performed on some of the oligochaetes in which neurosecretory events have been described throughout the ventral nerve chain (Herlant-Meewis, 1966; Aros et al., 1965) and in which normal caudal regeneration is reported to occur whether or not a brain is present (Herlant-Meewis & Gallardo, 1965; Saussey, 1963).

Although numerous investigations have been carried out on the polychaete brain, less consideration has been given to the nerve cord as a potential neurosecretory organ. Van Damme (1962) has described localized neurosecretory cells occurring within each ganglion of the nerve chain in Nereis diversicolor. She pointed out at that time that the local influence of these cells of the nerve cord upon regeneration should be considered in addition to that of the supraoesophageal ganglia. Moreover, Arvy (1954) investigated four families of sedentary polychaetes, including three species of Sabellidae, and in all cases found cells showing the staining characteristics associated with neurosecretion distributed in the ventral ganglia as well as in the brain. Recently, Dhainaut-Courtois & Warembourg (1967, 1969a, b) described cells with an affinity for paraldehyde fuchsin in the ventral nerve cord of Nereis pelagica and N. diversicolor; however, they point out that neither neurosecretion nor definite homology with similarly staining cells of the brain could be established.

A modifying influence of the nerve cord on posterior regeneration when the brain is left intact has been described in polychaetes (Nusbaum, 1908; Holmes, 1931; Bailey & Bailey, 1938; Sayles, 1939, 1940a, b; Boilly & Combaz, 1970). Most of these authors found that while regeneration occurred in some cases after removal of the nerve cord, it was less than that obtained in the controls. Boilly & Combaz (1970) observed that blastema formation and the early stages of differentiation proceeded normally in denervated segments of Nereis diversicolor but later stages of growth and differentiation were inhibited.

In many tube-dwelling sedentary polychaetes, including the Sabellidae and Chaetopteridae, the brain is quite simple when compared to that of errant polychaetes, probably correlated with the reduction of sense organs in these forms (Bullock & Horridge, 1965). It does not seem unlikely therefore that some of the functions carried out by the large brains of more active species might here be performed by the ventral ganglia. Furthermore, the errant polychaetes which have been studied do not undergo anterior regeneration upon amputation of the head. Since both Branchiomma and Chaetopterus are capable of anterior regeneration, it is probable that they do so in nature and, indeed, animals of both species with regenerated anterior segments are frequently collected. Consequently, it would appear advantageous to these animals to have neurosecretory abilities distributed along the nerve cord rather than localized within the head, which may be lost with some degree of frequency.

The effect of the nerve cord on anterior regeneration is not well understood,
but it seems possible that anterior regeneration may be dependent upon direct contact of tissue or tissues with the nervous system rather than on neurosecretion. Although formation of a head in response to the presence of the cut end of the nerve cord has been described by numerous investigators (Morgan, 1902; Okada, 1932, 1934; Marcel, 1963; Kiortsis & Moraitou, 1965) the necessity of the nerve cord at the wound surface has been disputed by others (Goldfarb, 1909, 1914; Avel, 1932; Bailey, 1939). Some of this conflict may have been resolved by Herlant-Meewis & Deligne (1965), who propose that after removal of the nerve cord, regeneration occurs if the nerve cord is capable of regenerating rapidly enough to reach the cicatrical epidermis prior to differentiation. Herlant-Meewis (1964) also points out that it is possible that nerve stumps left within the wall might be adequate to support regeneration.

Fitzharris & Lesh (1969) have recently proposed a model involving interaction of gut and nerve cord in anterior regeneration in the sabellids Branchiomma nigromaculata and Sabella melanostigma. Numerous results indicate the participation of ectodermal, mesodermal and endodermal derivatives in the formation of the blastema if they are in the proximity of the amputation site (Boilly, 1967, 1968a, b, c; 1969; Thouveny, 1961, 1964, 1967; Buongiorno-Nardelli & Thouveny, 1966; Hill, 1970); however, this need not preclude a specific control exerted over regeneration by the nervous system.

In the experiments utilizing Chaetopterus segment 13, both anterior and posterior halves of the segment supported anterior regeneration. Since the ventral nerve cord, including some nerve cell bodies, was present in the anterior as well as the posterior half, predictions cannot be made concerning controlling factors in anterior regeneration; however, that a difference exists in the requirements for anterior and posterior regrowth in this species is clearly indicated.

The occurrence of bipolar animals in the experiments using sabellids seems to be in some way related to repeated amputation. Although only two animals out of the 60 undergoing operations formed a secondary head, in the course of experiments over a five-year period involving anterior and posterior amputations on sabellids of the species Sabella melanostigma and Branchiomma nigromaculata, I have never previously obtained a two-headed animal. Fitzharris & Lesh (1969) have reported a higher incidence of the same phenomenon in these species following treatment for 5 days or more with colchicine. In both experiments normal head regeneration was prevented for an extended period of time. How this complete lack of a head or developing cephalic structures might affect polarity remains a tantalizing question.

Contribution 392 Hawaii Institute of Marine Biology, University of Hawaii.

I wish to express my sincere thanks to Dr James N. Cather for his encouragement and for providing me with opportunity to carry out this work, to Dr Philip Helfrich for making space available to me, and to Dr Meredith L. Jones and Dr N. J. Berrill for their assistance in species identification.

This work was submitted as partial fulfillment of the requirement for the degree of Doctor of Philosophy, University of Michigan.
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(Manuscript received 8 May 1972)