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# Caudal Regeneration as a Function of Neuroendocrine Manipulations, in the Earthworm, *Metaphire houlleti* (Perrier)

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Effect of neuroendocrine manipulations like brain ablation and reinjection therapy, on the caudal regeneration of *metaphire houlleti* has been studied. Removal of the brain immediately and within 24 hr after the amputation of the posterior segments totally prevents the caudal regeneration. Removal of the brain 48hr after the amputation retards but does not inhibit the regeneration. Administration of the brain homogenate from 3 day caudal regenerant (and not from normal worms) accelerated the caudal regeneration. This indicates that the brain is the source of a regeneration promoting hormone.

**Key Words : Caudal regeneration, Brain, Regeneration promoting hormone**

## Introduction

According to Hubl (1956) and Chapron and Chapron (1973) a brain neurohormore released soon after the segment loss initiates the process of regeneration in *Eisenia foetida*, *Lumbricus terrestris*, *Allolobophora terrestris* and *Lumbricus rubellus*. However, a number of workers do not agree with this view. This is becuase it has been demonstrated that brain removal and at the same time loss of segments does not prevent the posterior regeneration in *Eisenia foetida* (Herlant-Meewis 1964, Juberthie 1965), *Eophila dollfusi* (Gallissian 1973) and *Allolobophora icterica* (Saussey 1966). Stephan-Dubois (1980, 1983) while studying influence of cephalic region on posterior regeneration of *Tubifex tubifex* and neurosecretory activity during regeneration suggested an inhibitory effect of the brain and a stimulatory effect of the anterior nerve ganglia on the posterior regeneration. Alonso-Bedate and Sequeros (1985) found in young *Allolobophora molleri* that the brain is required for caudal regeneration. However, it is noteworthy that a reduction of regenerative activity (but not its elimination), may, in some cases, follow decerebration (Golding 1974). Nevertheless, in *Eisenia purenaica* (Juberthei & Mostrov 1965) and in *Allolobophora icterica* (Saussey 1963) it has been suggested that segmental ganglia although not indispensable for posterior regeneration but may augment it. A good amount of literature on various earthworm species has also been contributed regarding leukocyte interaction, changes in conduction properties of gaint fibres and tail flattening reflex response during caudal regeneration (Balter et al. 1980, Vining & Drewes 1982, Cooper & Roch 1984, Gras 1984).

Keeping in view the foregoing literature on regeneration in different oligochaetes, the present work is designed to determine the involvement of brain neurohormones, if any, in the process of caudal regeneration in the megascolecid worm, *Metaphire houlleti*.

## Material and Methods

A large number of earthworms, *Metaphire houlleti* were collected from fields around the Aurangabad city. They were kept in glass troughs filled with moist soil from the habitat for acclimation to the prevailing laboratory conditions for 3 days under normal day/night illumination at 26±0.5°C. The worms used were of the same size (6-7 cm) and weight (1.5±0.5g).

The brains were excised by taking an incision dorsally upto front 4 segments using well sterilized scissors and forceps. The homogenates were prepared in earthworm saline (Oka et al. 1984) and injections were given using hypodermic syringe fitted with 27 gauze needle.

### Amputation of caudal segments

To study the hormonal control, if any, of caudal regeneration a specific number of caudal segments were amputated using fine, sterilized scissors. After the transection worms were returned to the moist soil. For amputation the worms used were not anaesthetized. The counting of regenerated segments is easy due to their vascularized state, transparency and dimensions. Such counting, however, is possible upto a period of two months, after which new segments assume normal dimensions and colouration. For calculating the

number of segments regenerated the worms were lightly anaesthetized (because of their extreme agility) in 0.5% ethanol (V/V) in tap water and were held on paraffin tray.

After observing the number of segments regenerated in 30 days following treatments, the regenerating efficiency of worms after each treatment was calculated using the following formula:

$$\% \text{ regenerating efficiency} = \frac{\text{Number of animals regenerating more than 50\% cadual segments}}{\text{No. of regenerants}} \times 100$$

The histomorphological features of brain such as size, shape, location and neurosecretory material staining intensity, of neurosecretory cell types I and II were already described (Bedre 1986) by staining 7μm sections with Mallory's triple (Mallory 1944) and paraldehyde fuchsin (Ewan 1962) stains.

## Results

For establishing the relationship between brain secretion and caudal regeneration (if any), the clitellum regressed adults were used. The worms were divided into eight groups and every worm of each group was subjected to an amputation of fifteen posterior segments. Following amputation, worms from each group were debrained at the specific time interval like immediately, and 1 to 6 days after the amputation of caudal segments. In the sham-operated control worms, the brain was left untouched after they were subjected to anaesthetization and mid-dorsal incision upto first 4 segments. The data from table 1 denote the fact that the worms whose brains were removed immediately, and within 24 hr after the amputation of caudal segments, developed only non-vascular pygidium. On the contrary, worms debrained 24 hr after amputation of caudal segments exhibited greater capacity to replace the lost segments which is evident from regeneration of varying number of segments by worms in these categories. Further, as the interval of debraining prolonged after amputation of posterior segments, the regenerative capacity was found to be more. These observations clearly indicate that minimum latent period of 24 hr is essential for the activation of brain neurosecretory apparatus, which in turn synthesize and release the regeneration promoting hormone (RPH). Further partial regeneration observed in worms debrained at different intervals may perhaps be because of drop in the blood titers of RPH due to the brain removal resulting in the retradation in the process of regeneration. The results in table 2 indicate the rate of caudal regeneration in 24 hr debrained worms injected with brain homogenate (25 μl= 2 brains)/worm (from normal) and 3 day caudal regenerants, worms injected with saline served as controls. The data reveal two interesting facts: (i) There is no significant difference between the rate of frequencies of caudal regeneration in 24 hr debrained worms injected with saline and brain homogenate from non-regenerants. No doubt the latter had relatively a slight edge over the former, (ii) When the rate of regeneration is compared between worms

**Table 1** *Caudal regeneration of* ***Metaphire houlleti*** *following debraining at various time intervals*

| Experiment | Groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Debraining (days after amputation) | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| No. of worms used | 20 | 20 | 20 | 25 | 25 | 30 | 30 |
| No. of worms surviving after 30 days | 15 | 12 | 11 | 15 | 16 | 18 | 20 |
| No. of worms displaying no regeneration | 11 | 8 | 1 | 1 | 1 | – | 1 |
| No. of worms displaying non vascular pygidium | 4 | 4 | – | – | – | – | 1 |
| No. of worms displaying vascular pygidium | – | – | – | 1 | – | – | – |
| No. of worms regenerating | | | | | | | |
| 1 segment | – | – | 7 | – | – | – | – |
| 2 segments | – | – | 3 | 1 | – | – | 8 |
| 3 segments | – | – | – | 3 | 3 | 2 | 5 |
| 4 segments | – | – | – | 3 | 3 | 2 | 5 |
| 5 segments | – | – | – | 5 | 2 | 4 | 1 |
| 6 segments | – | – | – | 1 | 3 | 3 | 1 |
| 7 segments | – | – | – | – | 4 | 7 | 1 |

**Table 2** *Rate of caudal regeneration in debrained* ***Metaphire houlleti*** *after different treatments*

| Days after caudal segments amputated | Number of segments regenerated in | | |
|---|---|---|---|
| | 24hr debrained earthworms injected with saline | 24hr debrained earthworms injected with the extract of brain of normal worm | 24hr debrained earthworms injected with the extract of brain from 3 day caudal regenerants |
| 0 | – | – | – |
| 2 | – | – | Pygidium |
| 6 | – | Pygidium | 2 |
| 10 | Pygidium | 2 | 5 |
| 14 | Pygidium | 3 | 6 |
| 18 | 1 | 4 | 8 |
| 22 | 2 | 5 | 9 |
| 26 | 2 | 5 | 11 |
| 30 | 3 | 6 | 12 |

* 25 μl = 2 brains/worm

**Table 3** *Total number of caudal segments regenerated by non-clitellate* ***Metaphire houlleti*** *subjected to different experimental conditions (Number of segments amputated from each worm = 15.)*

| Experimental condition | No. of worms used | No. of days at the end of which segments counted | No. of worms surviving | No. of worms showing no regeneration | No. of worms regenerating segments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Control | 20 | 30 | 20 | – | – | – | – | – | – | 4 | 5 | 9 | – | – |
| Debrained simultaneously with amputation | 20 | 30 | 15 | 15 | – | – | – | – | – | – | – | – | – | – |
| Debrained 48 hr after amputation | 20 | 30 | 14 | – | – | 6 | 3 | 4 | 1 | – | – | – | – | – |
| 4 anterior and 15 caudal segments amputated simultaneously | 20 | 30 | 15 | 15 | – | – | – | – | – | – | – | – | – | – |

receiving injections of brain homogenate from non-regenerants and regenerants (amputated for 3 days) a clear distinction is observed. The latter group exhibited a high rate of caudal regeneration when compared with the former. These worms regenerated 12 segments as against 6 by the control worms 30 days after the amputation of caudal segments. To ascertain further the status of brain is the source of RPH and its involvement in the regulation of regeneration if any, clitellum regressed adults were subjected to different experimental conditions as described in table 3. After a period of 30 days from the transection of 15 caudal segments total number of segments regenerated were counted. In the first experimental condition worms were debrained simultaneously with the amputation of caudal segments while in the second category worms were debrained 48hr after the amputation of the caudal segements. Each worm of the third group was subjected to simultaneous amputation of first 4 and last 15 segments. The worms deprived of 15 caudal segments with mid-dorsal incision upto 4th segment served as the sham-operated controls. The results tabulated in table 3 clearly indicate that debraining as well as transection of anterior 4 segments results in total failure of regeneration ultimately due to the elimination of brain, the source of RPH as was histologically confirmed by following the cytomorphological changes in the brain neurosecretory cells during caudal regeneration. For this purpose the course of regeneration was divided into five stages based on ocular observations:

Stage-I *Wound healing*-With the contraction of muscle around the wound, the oozing out blood stops. It lasts for few hours.

Stage-II *Appearance of vascular pygidium*-At the end of 5 to 7 days a vascular pygidium appears extending from the transected area.

Stage-III *Appearance of striation on vascular pygidium*-Between 2nd and 3rd week from amputation, thin striations start appearing on the vascular pugidium.

Stage-IV *Segmenation*-In the fourth week the external segmentation becomes more prominent. The new segments at this stage can be identified by depigmented skin on them.

Stage-IV *Growth and pigmentation*-After 5 to 6 weeks from the day of amputation the segments grow to the size of normal and subsequent pigmentation makes them difficult to identify from rest of the segments.

For this study clitellum regressed adults were selected. After the amputation of 15 caudal segments worms were fixed at the intervals of 1,2,3 days and 1,2,3,4, and 7 weeks. The interval of fixation of the brain corresponded with the stages of caudal regeneration described above. The observations of brain neurosecretory picture are presented in table 4 and figure 1. At an interval of 24hr clear vacuolization in the cell bodies of both type-I and type-II cells were clearly evident, thereby suggesting an efferent release of neurosecretory material in response to the afferent stimulus by the loss of caudal segments. In two days amputated worms the picture was entirely different. All the regions i.e. cell bodies, axons and neuropile were fully studied with neurosecretory material, possibly RPH. An increased size of nucleus was another feature

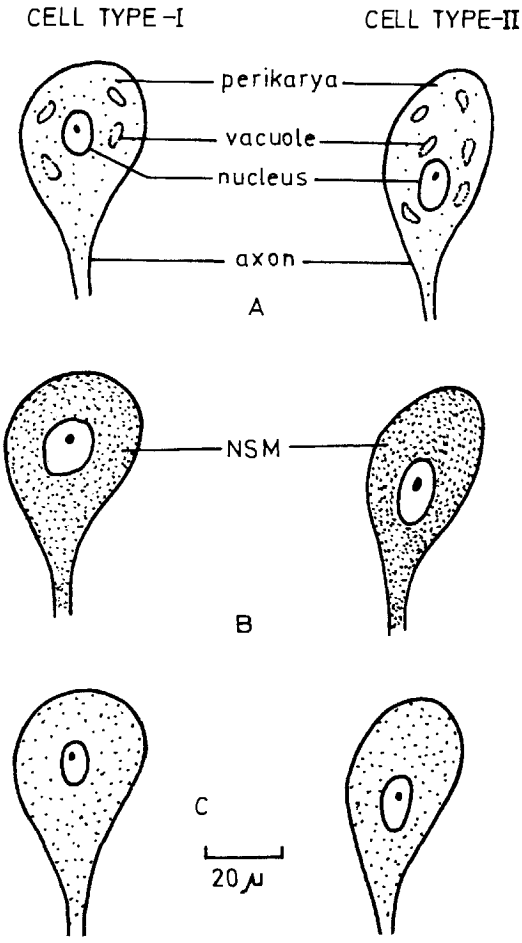


**Figure 1** Histomorphological changes in the brain neurosecretory cells of **Metaphire houlleti** after different periods of caudal segment amputation. A After 1 day (24hr) of amputation. Note prominent vacuoles in the perikarya of both call types I and I and remarkable depletion of neurosecretory material (NSM) B: After 2 Days (48hr) of amputation. Note considerable increase in the neurosecretory material (NSM) alongwith an increase in the nucleus size. No vacuoles were observed. C: Neurosecretory cells with normal appearance. This condition was observed in the brain of both normal worms and amputated for more then 96 hours.

**Table 4** *Number and cytomorphological characteristics of brain neurosecretory cells of* ***metaphire houlleti*** *during regenerations. Number of segments amputated from each worm = 15*

| Interval after amputation | BRAIN | | | | | |
|---|---|---|---|---|---|---|
| | Type-I Cells | | | Type-IInd Cells | | |
| | Number | Area in μm | Histological characteristics | number | Area in μm | Histological characteristics |
| 1st day | 12 | P 10-25<br>N (5-10) | Vascuolizationin perikarya | 20 | P 5-20<br>N (2-5) | Vacuolization in perikarya |
| 2nd day | 11 | P 10-24<br>N (6-11) | Perikarya, axons (contain in NAM) | 18 | P 5-20<br>N (2-6) | Perikarya, axons neuropile studded with NSM |
| 3rd day | 12 | P 10-24<br>N (6-11) | -do- | 20 | P 5-20<br>N (2-6) | -do- |
| 1st week | 15 | P 10-24<br>N (6-11) | Perikarya and neuropile studied with NSM | 22 | P 7-25<br>N (2-5) | Perikarya and neuropile studded with NSM |
| 2nd week to | | P 10-25 | -do- | 20 | P 5-20 | -do- |
| 4th week | 20 | N (6-11) | | | N (2-5) | |
| 5th and 6th week | 18 | P 10-25<br>N (5-10) | NSM confined to perikarya | 18 | P 5-20<br>N (2-5) | -do- |
| 7th week | 13 | P 10-15<br>N (5-10) | Scanty NSM in all the regions of NSM apparatus | 21 | P 5-20 | -do- |

P = Perikarya; N = Nucleus; NSM = Neurosecretory material

suggesting an enhancement in the synthetic activity of neurosecretory cells. More or less same histological picture was evident upto the end of the 4th phase but an increased number of active neurosecretory cells was another significant point. Cells however dropped to normal number with scanty neurosecretory material confined to the cell bodies at the vth stage. These observations clearly indicate a correlation between the process of regeneration and alterations in the neurosecretary picture of brain.

## Discussion

The failure of regeneration in *Metaphire houlleti*, within 24hr of debraining is a clear indication of the fact that with debraining worms were deprived of a cerebral factor which stimulates regeneration. These results are supported by earlier observations made by Clark and Bonney (1960), Chapron and Chapron (1973), Hanumante (1975), Kodarkar (1979), and Alonso-Bedate and Sequeeros (1985) on other oligochaetes. Hubl (1956) has demonstrated that suprapharyngealectomy (brainectomy) concurrently followed by amputation of number of posterior segments results in the total failure of caudal regeneration in lumbricid oligochaetes. Suprapharyngealectomy if carried out after three days of amputation of posterior segment it result into considerable retardation of regeneration (Clark & Bonney 1960).

The data presented in table 1 give an indirect proof that in *Metaphire houlleti* there is a mechanism which signals the loss of body segments to the brain and probably in response to this stimulus brain secretes neurohormones, involved in the process of caudal regeneration. This stimulatory factor may simply be nervous input or may be the neurosecretory hormone. Further study (table 2) erased the former possibility as the debrained worms injected with brain homogenate of regenerants displayed a higher rate of caudal regeneration than control. This clearly indicates that the brain homogenate contains a factor which provides a signal for the initiation of the caudal regeneration. Juberthie and Mostrov (1965) have followed the modifications in the cycle of neurosecretory cells in *Eophila pyrenaica* in the course of regeneration. Similarly, Marcel (1973a,b) has recorded changes in the number of paraldehyde fuchsin stained neurosecretory cells in nerve ganglion of *Eisenia foetida* during caudal and cephalic regeneration. Chapron and Chapron (1973) have documented the piling up and emptoying of cerebral neurosecretory cells in *Lumbricus rubellus*

during the tail regeneration. Dey and Nanda (1979) found after 24hr of posterior amputation in *Pheretima posthuma* that the brain neurosecretory perikarya showed various degrees of alterations both in their morphology and secretory dynamics. These reports demonstrate that neurosecretory cells from the brain ganglia of earthworms are prone to alterations during regeneration. As summarised in table 4 the neurosecretory cells in the brain of *Metaphire houlleti* exhibit definite changes in secretory characteristics indicating a clear correlation between their secretory cycle and caudal regeneration. The data in table 2 are a physiological evidence for such morphological expression. Further it clearly revealed the fact that there must be a hormonal factor secreted in the brain which enhances the rate of caudal regeneration in *Metaphire*. Moreover, this factor must be in a physiologically ineffective amount in normal (non-regenerating) worms and amputation or loss of segments activates the brain probably after 24hr, the minimum latent period required to initiate synthesis and/or release of brain factor (hormonal), like in *Nereis diversicolor* (Clark & Evans 1961). This is further evident from the failure of caudal regeneration in worms debrained or deprived of first four segments before this period (table 3). Thus, the elaboration and release of this brain neurohormone (s) requires minimum 24hr after severing the posterior segments. An optimum circulating level of RPH is essential not only for the initiation but also for the completion of caudal regeneration. It is further evident from the observation (table 1) that in the regenerants debraining at different time intervals although instantly do not interfere with the process of regeneration, slows it down as is revealed by varying number of segments regenerated in the same period by individuals debrained at different intervals. The number of segments regenerated goes on increasing with longer intervals of debraining and vice-versa. The observed correlation between interval of debraining and number of caudal segments regenerated must be well due to failure in the maintenance of circulatory level of RPH. Once the worm is debrained the process of regeneration is retarded and resulted in the addition of less number of segments as compared to the control worms. In *Metaphire* the normal circulating level of RPH perhaps reaches the physiological levels in 24hr and the worms always fail to regenerate if debrained before this period.

To summarise, the data depicted through tables 1 to 4 clearly indicate that as in other tropical oligochaetes like *Perionyx excavatus* (Hanumante 1975) and *Octochaetoides sudershensis* (Kodarkar 1979), in *Metaphire houlleti* also the brain is a locus for the caudal regeneration promoting hormone unlike inhibitory influence of brain neurohormones, reported in temperate oligochaetes, *Eisenia foetida* (Juberthi 1965), *Allolobophora chlorotica* (Maissiat 1965) and *Allolobophora icterica* (Saussey 1966).

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