Cellular aspects of regeneration hormone influence in *Nereis diversicolor*

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**SUMMARY**

The histology of caudal regeneration in *Nereis diversicolor* is described with special reference to the role of the cerebral 'regeneration' hormone. Wound healing is complete after 8 days; the regenerated stump of tissue then consists of the pygidium, associated anal cirri and a prepygidial region between the pygidium and the posterior margin of the last intact segment. The term segment blastema is used to refer to the cells from which all segmental structures are derived. The segment blastema is situated in the ventral posterior margin of the prepygidial region, and consists of transverse bands of cells with large nuclei (12 μm diameter) and prominent nucleoli. Segment anlagen are formed immediately in front of the segment blastema; the development of the anlagen is described, and six stages of development are defined.

The extent of caudal regeneration in different animals is best compared with reference to the total number and stage of development reached by the segment anlagen. During normal regeneration at 18 °C, segment anlagen are first formed after 8 days, and they continue to be formed at the same rate until at least the 21st day after caudal ablation. The oldest segments reach stage 6 after about 15 days of regeneration. Wound healing, pygidium formation, and cirrus development and establishment of the segment blastema all occur in decerebrate animals following the loss of caudal segments, but segment anlagen formation is almost completely inhibited. Implantation of ganglia taken from intact donor animals into the coelom of decerebrate animals which have lost caudal segments initiates segment anlagen formation. The segment blastema of decerebrate animals remains competent to respond in this way for at least 15 days.

The regeneration hormone produced by the cerebral ganglion is essential for continued segment anlagen production throughout the 2nd and 3rd weeks of regeneration at 18 °C. Delayed decerebration leads to an arrest of anlagen production, and anlagen younger than stage 3 fail to develop further. Older anlagen (stages 4 and 5) are independent of the regeneration hormone and can continue to differentiate in its absence.

**INTRODUCTION**

*Nereis diversicolor*, in common with most polychaete annelids, can regenerate lost caudal segments; however, they are only able to do so in the presence of a hormone released by the cerebral ganglion (Durchon, 1956; Clark & Bonney, 1960; Durchon & Marcel, 1962) and the secretion of this hormone ceases in

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sexually mature animals, which are therefore not able to regenerate (Golding, 1967e; Porchet & Durchon, 1968).

The role of the cerebral hormone in the process of regeneration, however, is unknown, although much experimental work has been directed towards discovering whether the hormone has a triggering effect on regeneration in response to segment loss (Clark & Bonney, 1960; Clark & Evans, 1961; Durchon & Marcel, 1962; Clark & Ruston, 1963; Clark & Scully, 1964; Scully, 1964) or if the hormone has a prolonged effect on regeneration, rather in the manner of a growth hormone (Golding, 1967a–d). Recent reviews have considered the latter hypothesis to be the more likely (Clark, 1965; Golding, 1967e; Clark & Olive, 1973).

At the cellular level, Herlant-Meewis & Nokin (1963) and Boilly (1965, 1969) have made detailed descriptions of wound healing and tissue activation, but most of their observations relate to events occurring during the first few days of regeneration. Herlant-Meewis & Nokin (1963) thought that either the accumulation of coelomocytes to form the wound plug, or tissue activation, both of which occur during the first 3–4 days of regeneration, might be processes which only occurred in the presence of the regeneration hormone. However, since that time, experimental evidence has emphasized the long-term influence of the regeneration hormone, and a re-examination of the possible role of the hormone at the tissue level is essential. The present paper, which describes the histology of regeneration in relation to experimental investigation of the effect of the cerebral hormone, is the first part of such a study. A report of an investigation into the influence of the regeneration hormone on the kinetics of cell division in the regenerating tissue will follow.

**MATERIALS AND METHODS**

**Routine maintenance**

Animals were collected from the river Wansbeck estuary near Blyth, Northumberland. They were maintained in the laboratory in filtered 100% sea water at 16–18 °C. Experimental conditions and treatment were based on those described by Golding (1967c, d) in similar work. The animals were kept in groups of up to 30 in plastic containers with aeration and an abundant supply of glass tubes. Where animals were kept together without decerebration the jaws were removed after narcotization with MS 222. Ganglion extirpation was performed using fine scissors, the posterior two-thirds of the ganglion being removed, together with overlying epidermis and associated glandular structures. The palps, tentacles and cirri were left intact. Caudal segments were removed by autotomy (provoked by tightly grasping the animals with forceps at the 25th segment) at the 25th–28th segment from the pygidium. Experimental animals had between 65–80 segments and were not sexually mature. (Females with oocytes greater in diameter than 120 μm were excluded.)
In some experiments, ganglia were implanted dorso-laterally through the body wall into the coelom of a previously decerebrated host animal. Donor animals for such implantations were similar to experimental animals and did not have caudal segments removed prior to decerebration. Apart from the use of filtered sea water, which was changed every 2nd or 3rd day, no further attempt to produce sterile conditions was made.

**Histological techniques**

All experimental animals were narcotized at the termination of the experiment and the last few segments plus regenerated tissue were fixed in sea-water Bouin. The material was paraffin embedded, cut at 6 µm in frontal or sagittal planes and stained with azan trichrome stain.

**RESULTS**

*Morphological and histological observations*

Segmentation during caudal regeneration in *Nereis diversicolor* begins after the completion of wound healing (Herlant-Meewis & Nokin, 1963), which occurs at 18 °C about 8 days after caudal ablation. By this time the components of the regeneration system are fully established, and two main regions can be distinguished: the pygidium and associated structures, and the prepygidial region, in the floor of which lies the segment blastema.

**The structure of the pygidium after wound healing**

The pygidium is separated from the more anterior parts of the regenerate by a muscular septum carrying a large blood vessel, which connects the dorsal and ventral blood vessels running into the regenerating stump from the previous segment. Two extensions of the damaged nerve cord extend into the newly formed pygidium; they enter mid-ventrally, pass diagonally across the pygidal floor and enter the anal cirri, which by 10 days are well developed. The cirri are solid, with an axial neuropile; mitoses are frequently seen in them, and at their base in the floor of the pygidium. This is thickened latero-ventrally to form the pygidal cushions, which contain two types of secretory material. In the surface epithelium there are goblet cells which stain deep red with azan, while the cells of the pygidal cushions are interspersed with coiled capsules containing a finely granular substance staining pale blue with azan.

The space within the pygidium surrounding the opening of the gut via the anus becomes lined with muscle cells. Occasionally, large numbers of coelomocytes remain within the pygidium, but they have usually dispersed prior to segmentation.
The structure of the prepygidial region and the location of the segment blastema

The prepygidial region consists of a truncated cone of tissue between the pygidial septum and the muscular septum at the posterior edge of the last complete segment. The gut projects through this cone without interruption. Surrounding the gut is a ring-shaped coelomic cavity which is at first asegmental. Prior to the growth of the segment anlagen, the greater part of this region is derived from the pre-existing tissues of the damaged segment and consists of modified epidermis, muscle cells, fibroblasts and coelomocytes. The ventral floor, however, is dramatically different and it is here that the segment anlagen are formed. Mid-ventrally, in the epidermis, there are two neuropile tracts which lead into the pygidium and on to the anal cirri. Within the prepygidial region the neuropile is surrounded by small, densely staining cells which are mitotically active. These do not accompany the neuropile tracts into the pygidium. At the boundary between the prepygidial zone and the pygidium, two small nerves pass outwards. Immediately in front of these lies the segment blastema (Figs. 1, 2). The blastema is characterized by large cells with spherical nuclei about 10–12 μm in diameter with prominent, deeply staining nucleoli about 5 μm in diameter. These large cells can be recognized both in the ectoderm and in the mesoderm; in the latter, being interrupted by the ventral nerve cord extensions and the ventral blood vessels, they form two bands of prominent cells described as the ‘bandelettes mesodermique’ by Boilly (1969).
ectoderm of the segment blastema lies below the mesoderm, and is a somewhatroader zone which merges posteriorly with similar cells in the ventral floor of
the pygidium. The latter, however, are smaller (diameter 3–8 μm, Fig. 1).

Hofmann (1966), describing regeneration in Platynereis, also recognized the
importance of these cells, which he termed the ‘embryonalzellen der Prolifera-
tionszone’.

It must be emphasized that the blastema cells are not the only cells which
are dividing by mitosis, but they are thought to play a stem cell role, maintaining
a source of undifferentiated cells throughout regeneration, and indeed, through-
out normal growth.

The development and differentiation of segment anlagen

During caudal regeneration, several segment anlagen form, which will
differentiate into fully developed segments. In any regenerating individual the
oldest segments are most advanced and are situated farthest from the segment
blastema, while the youngest are in contact with it.

In order that comparisons could be made rapidly and accurately between
experimental animals, it was found necessary to develop a criterion of regenera-
tion other than merely the number of segment anlagen, as used by previous
authors. The following sequence of stages was developed to describe the
development of an individual segment anlagen. As will be seen below, in a
normally regenerating specimen there will be a number of segment anlagen
developing simultaneously, with perhaps several in each of these stages.

Stage 1. The earliest indication of the onset of segmentation is the appearance
of lateral segmental nerves from the ventral nerve cord extensions. This is very
soon followed by the onset of segmentation in the mesoderm. Some of the
mesoblasts derived from the segment blastema begin to differentiate as fibro-
blasts which form the septal anlagen. As they do so, they extend dorsally to
contact the ventral blood vessel and eventually the gut, so that the asegmented
cavity of the prepygidial zone becomes segmented by the dorsal growth of the
septal anlagen. The fibroblast-like differentiation does not affect all the meso-
blasts, and between successive septal anlagen are masses of mitotically active
cells which will form the mesodermal component of the chaetal sac complex
(Figs. 2, 3).

At the end of wound healing, the prepygidial region is already between 50
and 150 μm in length, and as many as three segment anlagen may be established
almost simultaneously (Fig. 2). Subsequent anlagen arise by the differentiation
of transverse bands of mesoblasts as septal anlagen, with chaetal sac anlagen
between.

A space begins to develop which separates the chaetal sac anlagen from the
septal anlagen and which will become the parapodial coelom (Fig. 3). Mesoderm
segmentation proceeds as if the underlying ectoderm were growing more rapidly,
causing successive rows of mesodermal cells to be separated from each other.
Stage 2. Stage 2 can be defined by the appearance of the ectodermal component of the parapodial/chaetal sac anlagen. This is formed by the invagination of ectodermal cells into the mesoderm cell group lying between the septal anlagen. Only small numbers of cells are invaginated, through finger-like intuckings of the ectoderm (Fig. 2), but the invagination invariably includes some large cells which resemble those of the blastema and which ultimately become the aciculoblasts and chaetoblasts (see also Figs. 4–6).

In frontal sections, the ectodermal and mesodermal components of the chaetal sac can be recognized by the basement membrane (Fig. 3). Mitosis is common in both the ectoderm and mesoderm cells of the chaetal sac.

During stage 2 the septal anlagen continue to develop, and may eventually contact the gut and even the inner dorsal surface of the animal. Simultaneously, growth of the ectoderm carries the chaetal sac anlagen laterally and a prominent segmental ridge may appear.

Stage 3. Stage 3 can be defined by the appearance of the notopodial aciculum. One of the large invaginated ectoderm cells in the chaetal sac becomes flattened on one surface and begins to secrete the aciculum (Fig. 3). This aciculoblast remains at the base of the developing aciculum (Figs. 3–5). During stage 3 segmentation of the prepygidial zone is completed, and the septal anlagen meet at the dorsal surface. The blood system may also become segmented at this
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Anlagen

Blastema

Stage 3

Pygidial septum

Neuropile

Secretory material in pygidium

Mesoderm and ectoderm in the segment blastema

Developing coelom

Invaginating ectoderm of the chaetal sac

50 μm

Fig. 3. Camera lucida drawing of part of a frontal section through the regenerating tail of *N. diversicolor* 21 days after caudal ablation. The drawing shows the segment blastema and 3 segment anlagen at stages 1, 2 and 3.

Segmental blood vessel

Muscle cells

Developing dorsal cirrus

Fig. 4. Camera lucida drawing of a frontal section through the parapodium of a stage 4 segment anlagen in *N. diversicolor*, 21 days after caudal ablation.
time, with the appearance of a segmental blood vessel in the septum. The essential components of the parapodial complex have been established at this stage, and subsequent stages of the development are based on arbitrarily chosen criteria, which have nevertheless proved useful for the purpose of comparisons between individual animals.

Stage 4. Stage 4 is defined by the appearance of the dorsal cirrus of the parapodium and the further growth of the aciculum (Fig. 4). The acicular sac elongates and projects into the now spacious coelom.

Stage 5. Stage 5 is defined by the appearance of the neuropodial aciculum. Differentiation of the notopodium is well advanced. Muscle cells begin to differentiate from the mesodermal component of the chaetal sac and the secretion of the chaetae is initiated, though these do not yet project from the chaetal sac (Fig. 5).

Stage 6. Stage 6 is defined by extrusion of the notopodial chaetae through the epidermis (Fig. 6). This represents the terminal event of this staging sequence, though clearly the parapodial anlagen are far from fully developed. At stage 6 the segments have septa with blood vessels, a spacious coelom, and parapodia with dorsal and ventral aciculae, dorsal and ventral cirri and at least
Table 1. Staging systems (designation and definition) for parapodial development in Nereis diversicolor and Platynereis dumerilii

<table>
<thead>
<tr>
<th>Stage</th>
<th>N. diversicolor</th>
<th>Pl. dumerilii</th>
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<tbody>
<tr>
<td></td>
<td>(Olive)</td>
<td>(Hoffmann, 1966)</td>
</tr>
<tr>
<td>Stage 1.</td>
<td>Lateral nerves appear. Septae begin to develop in mesoderm</td>
<td>Stage I. Parapodial anlage, an undivided cup with or without chaetae</td>
</tr>
<tr>
<td>Stage 2.</td>
<td>Ectoderm invagination to produce a chaetal sac anlage</td>
<td>Stage II. Parapodium with notopodium and neuropodium, but no cirri</td>
</tr>
<tr>
<td>Stage 3.</td>
<td>Notopodial aciculum secretion begins</td>
<td>Stage III. Parapodium with notopodium and neuropodium, dorsal cirrus and chaetae</td>
</tr>
<tr>
<td>Stage 4.</td>
<td>Appearance of dorsal cirrus. Extrusion of aciculum</td>
<td>Stage IV. Parapodium with notopodium, neuropodium, chaetae, dorsal and ventral cirri</td>
</tr>
<tr>
<td>Stage 5.</td>
<td>Appearance of neuropodial aciculum. Appearance of chaetae</td>
<td></td>
</tr>
<tr>
<td>Stage 6.</td>
<td>Extrusion of chaetae. Dorsal and ventral cirri</td>
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some functional chaetae. The intrinsic parapodial musculature is also well developed.

In developing this sequence of staging, the appearance of the segmental blood vessels was specifically avoided as a staging criterion, partly because this event is variable with respect to the appearance of the parapodial rudiments, and also because in decerebration experiments to be described below, some segment anlagen became arrested at stage 2 but did nevertheless acquire a segmental blood vessel.
The most detailed comparable staging system for the ageing of regenerating segments in Nereids is that proposed by Hofmann (1966) for *Pl. dumerilii*. The two systems are compared in Table 1. The main differences between the two schemes is in the timing of the appearance of the chaetae, which occurs much earlier in *Platynereis* than in *Nereis*.

Experimental analysis of cerebral hormone function in regeneration

Development of segmental anlagen in normal regeneration

There have often been marked differences in the rate of regeneration recorded for *Nereis diversicolor* by different authors. It is therefore essential to define for any sequence of experiments the ‘normal’ regeneration in the conditions adopted. This is shown in Fig. 7 for results from 123 animals. The number of segment anlagen was determined from histological sections and therefore includes segment anlagen not visible externally. These results agree closely with those of Golding (1967d), which also indicated that the majority of segments appear during the 2nd and 3rd weeks of regeneration. Figure 8 shows the development of the segment anlagen during the first 21 days of regeneration. The first segments reach stage 6 (of the developmental sequence defined above) after about 15 days, and this represents the most numerous class of segment anlagen after 21 days of caudal regeneration. Segment delineation (the appearance of the youngest anlage) starts at about the 8th–10th day following caudal ablation, and continues throughout the 2nd and 3rd weeks, so that stage 1 anlagen are still present at an average of about 1 per individual on day 21.
Regeneration in decerebrate animals

It is now well established that after removal of the supra-oesophageal ganglion, *Nereis diversicolor* loses the ability for caudal regeneration. This has been confirmed in the present investigation. However, sections of the posterior parts of decerebrate animals that have lost posterior segments show that there is not a complete failure of all features of wound healing and regeneration.

In the majority of individuals, wound healing and pygidium formation proceed almost normally, to such an extent that the anal cirri, a pygidium with anal sphincter muscles, prepygidial zone and segment blastema cells can be recognized. In approximately 25% of decerebrate animals one or other of these
features is missing. A similar analysis of all animals with the brain intact reveals these structures to be present in 95% of cases. In many decerebrate animals, segment anlagen are found in the prepygidial zone. Of the 89 decerebrate animals that have been examined, 33 (37%) have at least 1 segmental anlage, and a small number have three recognizable anlagen.

Figure 9 shows the mean number of segment anlagen and the age distribution of anlagen in decerebrate animals. The regenerating stumps remain arrested at a stage more or less equivalent to that in normal animals at 8 days regeneration, when wound healing is complete. The few segment anlagen that are produced fail to differentiate further.

Regeneration after delayed implantation of supra-oesophageal ganglia

Golding (1974) has shown that decerebrate Nereis diversicolor can be made to regenerate new segments after the completion of wound healing in the absence of the brain, by the implantation of ganglia into the coelom.

Similar experiments have been designed for observation of the histology of caudal regeneration following delayed implantation. Animals were allowed to regenerate for 10 or 15 days prior to implantation and were examined 3, 7 and 15 days later. The mean number of segment anlagen was significantly increased 7 days after implantation, i.e. about 2 days earlier than could be detected by external observation (Golding, 1974). None of the anlagen present 7 days after
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Table 2. The mean number of segment anlagen, ± standard deviation, regenerated by Nereis diversicolor after delayed decerebration

(Number of animals in parentheses.)

<table>
<thead>
<tr>
<th>Days of regeneration prior to decerebration</th>
<th>Days after decerebration</th>
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<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>2.2 ± 1.5 (6)</td>
</tr>
<tr>
<td>10</td>
<td>2.0 ± 1.3 (8)</td>
</tr>
<tr>
<td>15</td>
<td>4.8 ± 1.6 (5)</td>
</tr>
<tr>
<td>20</td>
<td>8.3 ± 2.1 (3)</td>
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</table>

decerebration had progressed beyond stage 2, but 15 days after implantation the most advanced were at stage 6 and the distribution of segment anlagen was similar to that occurring after 15 days of normal regeneration (Fig. 8).

At the time of implantation, the processes of wound healing and segment blastema formation were complete. Nevertheless, there was a delay of 7 days prior to the appearance of new anlagen, and the rate at which they differentiated was very similar to that following caudal ablation of animals with the cerebral ganglia in situ.

The effect of delayed decerebration on regeneration

In these experiments the animals were left for various times after caudal ablation before the supra-oesophageal ganglia were removed. In the first experiment of this kind, four groups of animals were allowed to regenerate for 5, 10, 15 and 20 days prior to decerebration. They were left for a further 5, 10, and 20 days before fixation and preparation for histological examination. The mean number of segment anlagen regenerated and the age distribution of these was determined. The results are shown in Table 2 and Fig. 10, but these should also be compared with the results for normal regeneration shown in Figs. 7 and 8. Those animals decerebrated on or before the 10th day of regeneration, when segment anlagen production is just beginning, are arrested at this stage of regeneration. The number of segment anlagen produced remains low, and those anlagen that are present are arrested in early stages of development (prior to stage 3 in almost all cases). Those animals that were decerebrated 10 days after caudal ablation, and allowed to regenerate for a further 10 days, have regenerated for a similar period of time to those in Fig. 8 (21 days) but are obviously very different from them.

The animals decerebrated at the 15th day are particularly interesting. At that time more anlagen are established (mean number = 4.5) and of these a small number may have reached stages 5 and 6 of the developmental sequence. Figure 10
Days regeneration post decerebration | 5 | 10 | 20

| Days regeneration prior to decerebration | 5 | 10 | 20 |

![Graph showing stage distribution of segment anlagen](image)

Fig. 10. Stage distribution of segment anlagen regenerated after delayed decerebration. Details of experimental treatment in text.

shows that the more advanced of the segment anlagen at the time of decerebration are able to continue to develop in the absence of the brain. However, further production of new segments appears to decline, and the younger segment anlagen remain in stages 1–3. The results for animals decerebrated on the 20th day are similar.

A second experiment was designed to investigate in greater detail and with more animals the role of the supra-oesophageal ganglion during the critical period of segment production between the 8th and 12th days of caudal regeneration.

The experimental design consisted of the following groups:

(i) Brain intact during 21 days regeneration (code: 21/—).
(ii) Decerebrate throughout 21 days regeneration (0/21).
(iii) Decerebrated after 8 days regeneration, examined after 21 days of regeneration (8/13).
(iv) Decerebrated after 10 days regeneration, examined after 21 days of regeneration (10/11).
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(v) Decerebrated after 12 days regeneration, examined after 21 days of regeneration (12/9).

(vi, vii, viii) Fixed and examined after 8, 10 and 12 days of caudal regeneration respectively, with the brain intact (8/—, 10/—, 12/—).

The number of segment anlagen and the stage of their development was determined from histological sections. Figure 11 summarizes the mean number of anlagen in each group; an analysis of variance for all the animals fixed on the 21st day showed that animals which regenerated with the brain intact for 21
days had significantly more segment anlagen than all the other groups, including animals which regenerated for 12 days with the brain intact (group 12/9). Decerebration clearly leads to an arrest in the process of segment anlagen production, although a small number of anlagen may be produced after decerebration. There are more segments in all groups of animals that have regenerated for 21 days (8/13, 10/11, 12/9) than there are in the corresponding control groups (8/–, 10/–, 12/–) but the differences are small, and in the case of the animals decerebrate or fixed on the 10th day (groups 10/11 and 10/–), not significant. There is therefore only a limited capacity for further anlagen production after decerebration.

The stage distribution of the segment anlagen for each group is shown in Fig. 12. The eldest segments present at the time of decerebration continue to develop normally and may reach stages 5 and 6. The number of such segments is greater the longer the delay prior to decerebration. Most of the segment anlagen which were at early stages of segment anlagen at the time of decerebration remain arrested at that stage. A few of the stage 2 anlagen may acquire a small centre of aciculum secretion, but they remain small and do not develop further.
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The appearance of the younger segments suggests that their growth is arrested by decerebration, and that before a certain size has been reached the segment anlagen are not able to differentiate and develop in the absence of the brain.

DISCUSSION

Morphological and histological studies

Caudal regeneration in Nereis diversicolor resembles closely the same process in Platynereis dumerilii (Hofmann, 1966) and Nephtys (Clark & Clark, 1962; Clark, 1965, 1968). In all three species, wound healing and pygidium formation are separate processes from, and largely independent of, the formation of new segments. New segments arise from the products of cell division in a narrow band of specialized cells lying ventrally immediately anterior to the pygidium. The cells in this region have a very striking appearance, with a large nucleus which appears empty apart from the prominent, deeply staining nucleolus. In Nereis and Platynereis (Hofmann, 1966) cells of this type are found in both the ectoderm and the mesoderm in the prepygidial region at the junction with the pygidium, but in Nephtys (Clark, 1968) the ectodermal cells lie more posteriorly, in the floor of the pygidium. These cells have been recognized by a number of authors – Herlant-Meewis & Nokin (1963), Hofmann (1966), Clark (1968) and Boilly (1969) – but the terminology used for these cells and other structures in regeneration has not been consistent.

Clark & Clark (1962) and Clark (1968) have used the term ‘blastema’ to include all the cells which accumulate at the wound, although Clark (1968) points out that this may not be ideal, since only a small proportion of these cells actually participate in the regeneration of new tissue. The general term ‘wound plug’ equivalent to the French ‘bouchon cicatricielle’ (Herlant-Meewis & Nokin, 1963; Boilly, 1969) is preferable for this accumulation of cells.

In Nephtys (Clark, 1968), Nereis diversicolor (Boilly, 1969) and Platynereis (Hofmann, 1966) the cells which give rise to the segmental mesoderm have been identified on the anterior ventral face of the newly regenerated pygidium. These cells have been described as the ‘zone of proliferation’ (Clark, 1968) in Nephtys, the ‘bandelettes mesodermique’ in Nereis diversicolor (Boilly, 1969) and ‘die embryonalzellen der Proliferationszone’ in Platynereis (Hofmann, 1966). As these cells are thought to be the ultimate source of all the segmental structures (Hofmann, 1966; Clark, 1968), the term ‘segment blastema’ may be applied to them. This term is equivalent to the term ‘proliferation zone’, but has been preferred in the present report because mitosis and cellular proliferation are equally, if not more, common in the pygidium and anal cirri, in the younger segmental anlagen and amongst the cells of the ventral nerve cord.
Earlier work on the experimental analysis of regeneration and the role of the supra-oesophageal ganglion in *Nereis diversicolor* has been mainly concerned with external features. However, several authors (Clark & Bonney, 1960; Clark & Evans, 1961; Durchon & Marcel, 1962; Golding, 1967c) have noticed that a pygidium will form in the absence of the supra-oesophageal ganglion. The histological part of the present investigation has confirmed that this is the case. Perhaps even more importantly, it has shown that the cells which comprise the segment blastema are formed and appear in the normal position in at least 75% of decerebrate animals. The regeneration hormone is not therefore essential for the establishment of the segment-forming tissues. Instead, it appears that the cerebral hormone is necessary for the proper functioning of the blastema, and also for the growth of the young segment anlagen. This is illustrated most clearly by the delayed decerebration experiment, described in Table 2 and in Figs. 10-12. These experiments have shown that when decerebration takes place at a time when new segment anlagen are being formed, segment production soon stops and the youngest segments are arrested at early stages of differentiation. The older segments, and especially those that have proceeded to stage 4, in which the main components of the parapodium are present, are able to continue to differentiate in the absence of regeneration hormone.

In *Nereis*, as in *Nephtys* (Clark, 1965, 1968), the early stages of segment differentiation are characterized by a high nucleus/cytoplasm ratio and the developing cells are mitotically very active. The later stages of development, however, involve a gradual change to growth by cell differentiation and in the latter phase the regeneration hormone does not seem to be involved.

In the light of these observations it seems most likely that the cerebral hormone has a mitogenic effect. However, absence of the supra-oesophageal ganglion does not lead to a general failure of mitosis, as is shown by the growth of the anal cirri. Furthermore, preliminary work has suggested that decerebration does not lead to an immediate and complete failure of DNA synthesis in the segmented blastema, nor in the young segment anlagen (P. J. W. Olive, unpublished observations); this aspect of the effect of the regeneration hormone is being further investigated.

The central nervous system of *Nereis diversicolor* has two separate effects on regeneration; in addition to the endocrine influence of the cerebral ganglion, normal regeneration requires the presence of nervous tissue derived from the cut ends of the ventral nerve cord. Without this regeneration can occur, but it is abnormal; parapodia and anal cirri do not form, though other regeneration events occur normally (Boilly & Combaz, 1970; Combaz & Boilly, 1971; Combaz, 1972). The nervous system is necessary for anal cirrus development and
for the inductive events which lead to the establishment of parapodia (Boilly-
Marer, 1971a, b). On the other hand, the main influence of the cerebral
ganglion appears to be on the establishment of the mesodermal components of
the segments and their subsequent growth, without which proper differentiation
of the segments cannot take place.

Evidence in support of the hypothesis that the regeneration hormone is a growth
hormone

The delayed implantation and delayed decerebration experiments establish
that the cerebral hormone is responsible for the appearance of segment anlagen
and their early growth, but that eventually the segment anlagen become
independent and can continue to differentiate in the absence of the hormone.
Segment production does not begin until the 8th day at 18 °C and is then very
active throughout the next 2 weeks. Normal regeneration will only take place
if there is a source of cerebral hormone throughout that period. Several investi-
gators have used the delayed decerebration technique (Clark & Evans, 1961;
Clark & Ruston, 1963; Hofmann, 1966; Golding, 1967d) and all have found that
the number of segments regenerated increased with the length of time that the
brain remained in situ. There have, however, been differences in interpretation
of the results (see Golding 1967a, c, d) which have arisen at least in part from
the failure to adequately define the criteria used to define a segment. The staging
sequence adopted here avoids this difficulty and confirms that the cerebral
ganglion has a prolonged effect on segment formation.

If the later stages of segment anlagen development such as the appearance
of chaetae were used to define 'a segment' then it would appear from external
morphological evidence that segments continued to appear long after decere-
bration – which might be construed as evidence for a 'trigger-like' effect on
regeneration. In reality there is no such effect. In the implantation experiments
it was found that ganglia implanted in decerebrate hosts which had completed
wound healing would initiate segment formation and development. It was not
necessary for the ganglia to be 'activated' by caudal ablation of donor animals
as suggested by Scully (1964), and in all cases the ganglia were taken from
animals which had not lost caudal segments.

Caudal regeneration is not under the influence of the supra-oesophageal
ganglion in all species of polychaete (see Hill, 1972), and in fact, this dependence
has only been demonstrated in members of the families Nereidae and Nephty-
dae. In the Nereidae, but not the Nephtydae (P. J. W. Olive, unpublished
observations), the life-history is divided into a period of somatic growth
followed by a period of sexual development. The change is mediated hormonally
(see Clark & Olive, 1973) and the hormone dependence of regeneration in *Nereis
diversicolor* may be one aspect of this type of endocrine regime.
REFERENCES


Hormone influence on Nereis regeneration


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