Gut and nerve-cord interaction in sabellid regeneration

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Studies of annelid regeneration have considered interactions which may exist between the gut and nerve-cord. Although the influence of the gut has often been assigned a secondary role (Kroeber, 1900; Morgan, 1902; Hunt, 1919; Faulkner, 1932; Sayles, 1932), the necessity for its presence has been demonstrated in certain instances (Okada, 1938). Attention therefore has been primarily directed to the nerve-cord, particularly the trophic influences of this structure at the wound site (Goldfarb, 1914; Bailey, 1930 & 1939; Avel, 1932; Crowell, 1937), and its influence posterior to the wound area, presumably hormonal in nature (Kropp, 1933; Clark, R. B. & Clark, M. E., 1959; Clark, R. B. & Bonney, 1960; Clark, M. E. & Clark, R. B., 1962; Scully, 1964; Golding, 1967a–c). Regardless of the organism or the approach used, previous investigators have pointed to one persistent problem: the independence or interdependence of these two organ systems in the structuring of the regenerate bud. Specifically, what does the gut contribute to the regenerate bud and in what manner, if any, does the nerve-cord direct the formation of the bud? Because of the lack of histological data, plus conflicting views on the exact ordering of these regenerating organ systems, this study will attempt to resolve the problem of tissue interaction involved in normal regeneration.

A unique developing system provided by the sabellid polychaetes can be employed to clarify this situation. Sabellids are marine annelids characterized by the presence of three distinct body regions: (1) a bilobed branchial crown of tentacles used as a respiratory and feeding organ; (2) a thoracic region generally 5–11 segments in length, with dorsal filiform chetae and ventral uncinigerous hooks; and (3) an abdominal region composed of 0–300 segments with dorsal hooks and ventral chetae (Fig. 1). When the animal is cut through the thoracic or abdominal region, anterior regeneration will be epimorphic and limited (hypomeric), i.e. only the prostomium, peristomium and first thoracic segment will be replaced, regardless of the level of amputation. If less than the original number of thoracic segments remain, new thoracic segments will be replaced by a process of post-cephalic reorganization, i.e. the orientation of the

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abdominal chaetae will be reversed and the overall characteristics will become thoracic in nature. These observations are easily corroborated using a high-power dissection microscope. Posterior regeneration involves an initial 2-day wound-healing period. Subsequently the extension of the digestive tract, with commensurate growth in related regions, forms a pygidium which directs normal growth (Nicol, 1930; Berrill, 1931, 1952, 1961; Huxley & Gross, 1934; Gross & Huxley, 1935; Berrill & Mees, 1936; Herlant-Meewis, 1964).

Fig. 1. Dorsal view of the whole normal worm exhibiting the three characteristic body regions. The branchial crown (bc) is a respiratory and feeding organ; the thoraco-abdominal junction (th–ab) marks the division of the two regions of the trunk while the pygidium (py) denotes the last segment.

Transection of the animal at any level therefore reveals the presence of three consistent structural elements—gut, ventral nerve-cord and body wall—any of which could contribute to the regenerative process. Because of the precise positioning of these structures at the wound region, their interactions will of necessity be quite limited. In this paper the mutual dependence of both gut and nerve-cord in the initiation and maintenance of regeneration will be demonstrated. Evidence in support of this will be drawn from surgical manipulation and treatment with colchicine.
**Materials and Methods**

The polychaetes *Sabella melanostigma* and *Branchiomma nigromaculata* are obtained commercially from Tropical Atlantic Marine Specimens, Big Pine Key, Florida. Animals are maintained in 78 and 195 l aquaria at room temperature (22.5 ± 0.5 °C) in constantly aerated artificial sea water ('Instant Ocean', Aquarium Systems Inc., Wickliffe, Ohio). Stock cultures are fed daily with tropical fish food. Fresh tap water is added periodically to the tanks to maintain a specific gravity of 1.025.

Experimental animals are kept in 11 cm finger bowls if 1–5 are used, or in 19 cm finger bowls if more than five are cultured simultaneously. ‘Instant Ocean’ (IO) is changed daily and the finger bowls continuously aerated. Animals are starved during the entire experimental period. All animals are anaesthetized 10–15 min in 0.1 % chloretone for all operations and/or photographic recordings.

**Surgical procedures**

Experimental animals were derived from whole worms severed at the thoraco-abdominal junction. These abdominal portions were further subdivided into ten segment pieces and therefore were regenerating simultaneously anterior and posterior portions. Experimental animals regenerated individuals in the same manner and at the same rate as animals severed only anteriorly or posteriorly. Abdominal segments also showed post-cephalic reorganization (parapodial inversion) clearly and rapidly. This, plus the repetitive histological organization of the abdominal metamere, reduced experimental variation. Both *Branchiomma* and *Sabella* regenerated similarly under all conditions tested, and therefore results of any one section apply to both genera (see Table 1).

Excision of the gut at the anterior end was done by means of a lateral incision

<table>
<thead>
<tr>
<th>Time after operation (days)</th>
<th>Morphological features</th>
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<tbody>
<tr>
<td>1</td>
<td>Eversion of gut; wound healing</td>
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<tr>
<td>2</td>
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</tr>
<tr>
<td>3</td>
<td>Gut resorbed; wound covered by epithelium</td>
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<tr>
<td>4</td>
<td>Regenerate buds</td>
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<td>5</td>
<td>Prominent buds</td>
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<td>6</td>
<td>Finger-like bud stage</td>
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<td>7</td>
<td></td>
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<tr>
<td>8</td>
<td>Growth with elaboration of tentacles</td>
</tr>
<tr>
<td>9</td>
<td>Initiation of post-cephalic reorganization</td>
</tr>
<tr>
<td>10</td>
<td>Collar segment</td>
</tr>
<tr>
<td>11</td>
<td>Palps; first thoracic segment</td>
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into the body wall 3–4 segments long, with a subsequent dorsal incision to produce an epidermal flap. Bending this flap over exposed the gut, which could be removed by grasping with forceps (Fig. 2).

The ventral nerve-cord was removed similarly from the anterior end by an incision on either side of the cord. This piece of body wall plus the closely adhering nerve-cord was removed entirely (Fig. 3). Animals with identical sham incisions, plus normal regenerates, served as controls. Three segment portions were preferably removed in all experimental cases since more extensive removal (six segments—total length) of gut and/or nerve-cord resulted in the death of the animals.

For histological investigations, animals were anaesthetized in 0·1 % chloretone, fixed in Baker's dilute Bouin's, dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin wax. Sections were cut at 7 μ, mounted on glass slides, and stained in 0·1 % toluidine blue.

**Colchicine studies**

Stock 2·5 × 10⁻³ M solutions of colchicine (Nutritional Biochemicals) were freshly prepared and stored in light-proof containers no longer than 5 days. Culture solutions were changed daily with experimental animals being removed at specific intervals to 10 and cultured normally. The final concentration of 1·25 × 10⁻³ M was selected from the following data:

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Gross morphological effects</th>
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<tr>
<td>2·5 × 10⁻³</td>
<td>Death</td>
</tr>
<tr>
<td>1·25 × 10⁻³</td>
<td>Delayed regeneration; polarity affected</td>
</tr>
<tr>
<td>2·5 × 10⁻⁵</td>
<td>Normal regeneration</td>
</tr>
<tr>
<td>2·5 × 10⁻⁶</td>
<td></td>
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<tr>
<td>2·5 × 10⁻⁷</td>
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RESULTS AND DISCUSSION

Excision of the gut

To determine the significance of the gut in anterior regeneration, twenty worms were anaesthetized and the gut was removed for three segments behind the wound area. Wound healing occurred in approximately 18 h with a notable constriction around the incision.

The flat surface of the wound area was drawn 1–2 segments into the body, expanding the lateral region of the worm while concurrently positioning itself in the direction of the severed end of the gut. Sometimes the ventral half of the body curled up to the third segment to meet the dorsal epithelium, thus effecting wound closure where the gut had been removed. In both cases, however, the result was the same—the positioning of the anterior wound surface close to the anterior portion of the intact gut. These experimental animals regenerated normally (see Table 1 for staging), with the buds invariably produced dorsally at the original amputation site, never at the point of excision of the gut (Fig. 4).

Fig. 4. Seven-day regenerate with the gut removed for three segments posterior to the wound site. Note that tentacle formation (arrow) is at the most anterior part of the animal, not at the point of excision of the gut.

Fig. 5. Seven-day regenerate with the nerve-cord removed for three segments posterior to the wound site. Note the overall distortion of the animal into an L-shape which projects the regenerate tentacles toward the reader (arrow). Inspection will reveal that the buds are on the dorsal side in their proper position at the wound site, not originating from the cut end of the nerve-cord.

In the normally amputated worm such a structural collapse would be limited to the first injured segment because the gut would support the constricting epidermal wall. In the experimental animals constriction of the outer wall with the ventral constriction combined to draw all the remaining structures as far posteriorly as possible; that is, to the point where the gut had not been removed. Subsequent regrowth of the gut was therefore less than the length of one total...
segment in order to communicate with the outside. Otherwise it would have had to regrow the length of three segments to reach the wound surface.

Histological examination of animals with the gut removed supports the gross observations and has revealed several additional points. Even though the gut has been effectively removed for three segments posterior to the wound area, the anterior part of the gut remains at the wound site. The reason for this is that wound closure after such an operation is generally accomplished by a tight constriction of the outer body wall collecting the remaining structures and forcing them toward the unoperated region like a collapsible accordion. The constriction of the muscular elements does not meet with any resistance since the gut and connected septa have been removed. Normally, constriction of these circular muscles would affect wound closure by tightly constricting around the gut and closing 90% of the wound mechanically. Cicatrical material combined with an overgrowth of epithelium later joins the wall to the gut completing the process of closure.

Therefore, in *Sabella* and *Branchiomma* removal of the gut for three segments posteriorly does not in essence remove the gut from the wound region. The influence of this structure on the anterior regenerating end may have been reduced but certainly not eliminated. The actual positioning of the gut in relation to the regenerate bud has not been clearly demonstrated in previous investigations. Morgan (1902) relies upon stitching to effect healing and reports in some cases that a head develops where the anterior cut surface of the nerve-cord is present whether gut tissue is present or not. Hunt (1919) also attempted a description of the relationship between these structures but the lack of histological pictures makes an adequate interpretation difficult.

*Excision of the nerve-cord*

Removal of the nerve-cord for three segments in twenty animals was done simultaneously with the excision of the ventral groove and a small portion of the body wall. Upon healing the dorsal body wall was drawn ventrally, causing the animal to assume an L-shaped appearance. Because of this body distortion the regenerate buds appeared to be produced ventrally (Fig. 5). Microscopic observation reveals that the buds are dorsal to the gut on either side in their normal position (Fig. 6). Growth of a new cephalic region always occurs at the wound site, no supernumerary heads being produced at the anterior surface of the nerve-cord (as reported in Morgan, 1902).

Histological examination demonstrates an odd relationship between the gut and the nerve-cord under these experimental conditions. The gut is drawn over the cut end of the cord in such a way that a longitudinal section of the animal shows a cross-section of the gut (Figs. 6, 7). This relationship is a direct reflexion of the L-shaped configuration visible at the gross morphological level. Removal of the cord for three segments posteriorly is therefore without effect in the overall regeneration of the animals. The important aspect is again the method
of wound closure. After such an operation there is a very tight contraction of
the circular muscles in the body wall with a concomitant contraction of the
longitudinal muscles. Because part of the ventral structural elements is missing,
the sum total of this reaction draws the worm into an L-shaped figure at the

Figs. 6, 7. Sequential frontal sections of an experimental animal with the nerve-cord
removed. (rb, Regenerate buds; nc, nerve-cord.) Photomicrographs of histological
sections stained with toluidine blue were taken using a Normarski differential
interference microscope to increase structural detail.

Fig. 6. A cross-section of the gut showing its relation to the newly formed
regenerate buds.

Fig. 7. A cross-section of the gut with a longitudinal section of the nerve-cord,
illustrating the distortion seen at the gross morphological level in Fig. 5. This
demonstrates that although the nerve-cord was removed for three segments its influ-
ence on the regenerating region does not necessarily act at a distance.
anterior end. The histological results show that the unsupported structures anterior to the severed end of the cord are drawn ventrally and bent almost at right angles. Frontal sections of such worms show a cross-section of the gut at the point where the nerve-cord ends.

This mechanical aspect of wound healing essentially accomplishes two things, wound closure and the drawing into apposition of the gut with the nerve-cord, thus eliminating the necessity of anterior regeneration of the nerve-cord for three segments. Subsequent development shows regenerating nerve fibers innervating the region laterally around the gut in the formation of a new supracesophageal ganglion. Thus, as in the case of the gut, excision of the nerve-cord for three segments does not preclude a role for the nerve-cord at the regenerating end.

This is the main factor overlooked in both Morgan's and Goldfarb's experiments because their major supposition is that the nerve-cord is adequately removed spatially from the regenerating region. Bailey (1930) and Crowell (1937) overcame this situation to some extent when they performed similar operations on Eisenia and Allolobophora respectively, by looping the nerve-cord back into the body. However, following these manipulations, Bailey reported no regeneration while Crowell demonstrated that regeneration proceeded normally. Comparable results were obtained by Okada & Kawakami (1943) and Okada & Tozawa (1944).

The combined removal of gut and nerve-cord served as an operational control. Initial regeneration in this group was considerably slowed, but bud formation and subsequent development proceeded normally. The positioning of the gut and nerve-cord in respect to each other and to the regenerate bud was completely normal.

Interdependence of the intact gut and nerve-cord

Additional evidence for mutual interplay of the gut and nerve-cord during regeneration may be demonstrated by achieving conditions in which there is either, (1) an intact gut with a severed nerve-cord, or (2) an intact nerve-cord with a severed gut. To obtain these situations twenty whole normal worms were subdivided into two groups in the following manner: worms in group 1 were bent in a U-shape so that both ventral surfaces of the worm were in contact and secured with cotton thread; worms in group 2 were similarly bent with their dorsal sides in contact and secured. A one-to-two-segment divit removed from the exposed bend in these animals with micro-dissecting scissors produced an everted gut at two surfaces with an intact nerve-cord in group 1, and an intact gut with two cut ends of the ventral cord in group 2.

The results of this study were as follows. Animals with intact nerve-cords and severed guts (group 1) healed by a tight constriction around the wound area, drawing the two ends of the severed gut toward each other, and forcing them back into their original position. Regrowth of the epidermis completed wound closure with no unusual effects observed.
Wound healing in group 2 was incomplete or absent. No tight constriction of the ventral body wall occurred to aid wound healing as was observed for the dorsal side.

Thus, an interesting aspect concerning possible mechanical differences between the dorsal and ventral body walls is noted. Healing is easily accomplished from the dorsal aspect by a tight constriction of the body wall around the ends of the everted gut, thus restoring the individual to normal. The ventrally operated region does not contract and in several cases the wound is widened by the constant writhing of the animal. Failure to close the wound results in the death of the animals.

The most prominent anatomical feature of the ventral body wall is the ventral shield, a glandular cushion on every segment, which secretes the mucus lining the tube (Nicol, 1930). This ventral shield, as compared to the dorsal body wall, is semi-rigid. This explains why the ventral wall is less pliable and poses a limiting factor in wound closure. These structural differences do not imply qualitative differences in the ability of the tissue to respond to stimulation, but rather show the limitations of the body wall as a whole in controlling nerve-cord and gut interaction.

A simple model of this idea may be constructed by using tubing of different diameter. Similar incisions produce equal surface areas at the wound region. Closer inspection, however, will reveal that the total surface area which communicates with the coelom (which must be closed) will be much less in the case of the severed gut than of the severed nerve-cord.

The fact that no supernumerary heads or tails are observed when the ventral nerve-cord is severed is surprising. It is reported that such growths can be achieved by deflexion of the nerve-cord in the sabellid Spirographis spallanzani (Kiortsis & Moraitou, 1965). Failure to respond adequately to the initial wounding may have prevented the nerve-cord from interacting with the surrounding tissue to elicit this response. This interaction has been demonstrated in lumbricid oligochaetes by looping the nerve-cord back through a small hole in the body wall and obtaining supernumerary structures (Avel, 1947).

**Colchicine studies**

One controversial point confronting any attempt to define tissue interactions during regeneration is the origin and role of migratory cells (neoblasts, coelomocytes, regeneration cells) in this process. Numerous studies have been conducted (Liebmann, 1942a, b; Stephan-Dubois, 1958; Lender, 1962) but the actual contribution of these cells in forming a regenerate bud has never been explicitly demonstrated. The alkaloid colchicine is known to be an effective mitotic inhibitor (Taylor, 1965). In addition, its ability to inhibit the migration of cells during regeneration has been reported (Lehmann, 1957, 1965). Therefore, this chemical provides an approach whereby one may gain further insight into the mechanisms of reorganization at the cellular level.
To determine the role of cell migration in tissue interactions involved in annelid regeneration, animals were exposed continuously to $1.25 \times 10^{-3}$ M colchicine. Culture solutions were aerated and changed daily as in other experiments. Groups of experimental worms removed daily from colchicine to IO exhibited a delayed pattern of regeneration shown in Table 2.

Table 2. The effect of continuous exposure to colchicine on regeneration

(* = removed to IO; B = buds; T = tentacles; C = controls.)

<table>
<thead>
<tr>
<th>Time in colchicine (days)</th>
<th>Time after operation (days)</th>
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<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16</td>
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<td>6</td>
<td>— — — — — — * — — — — B* B B T T T</td>
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<td>5</td>
<td>— — — — — * — — B B B T T T T T T T</td>
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<tr>
<td>1</td>
<td>* — — — — — — B B B B B T T T T T T T</td>
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<tr>
<td>C</td>
<td>— — — — — — B B B B B B T T T T T T</td>
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</tbody>
</table>

1 Italic indicates expected time of regeneration after treatment (see note 2).
2 Days 1 and 5 of visible regeneration (see Table 1).

The daily lag in the removal of worms from colchicine to IO was reflected in the overall pattern of regeneration. The appearance of regeneration buds was delayed, but this delay was in direct proportion to the time cultured in colchicine. The inhibitory effect was therefore instantaneous and was maintained until the animals were returned to IO. In several instances worms were cultured in colchicine up to 2 weeks with no ill effects (i.e. they regenerated upon return to IO), thereby eliminating sublethal cytolysis as an experimental factor. Normal healing patterns (eversion of the gut with contraction of the outer body wall) were observed in all experimental animals, but there was a definite and substantial delay in the fusion of the two layers. Therefore, colchicine may be acting here to block cell division in the epithelial layer and in the other tissues as well, because an immediate overgrowth of the epithelium was evident when the animals were returned to IO. Further corroboration of these effects is currently being investigated by means of autoradiography.

When these results are compared with those on *Nephtys* and *Nereis*, an interesting hypothesis may be proposed. If regeneration in sabellids is under neurohormonal control, it is possible that colchicine affects not only cellular migration and division but also neurosecretion. If this situation is the case, two alternatives may be proposed. (1) The cells responsible for the formation of the regenerate buds may be incapable of responding while exposed to colchicine, since regeneration does not occur. Returning the experimental animals to IO initiates regeneration but on a delayed time scale. Therefore, it would have to be postulated that a stable hormone (active) is produced with effectiveness lasting
at least up to 2 weeks in certain instances. (2) Alternatively, the rate of manufacture and/or release of the hormone may be critical.

Clark & Clark (1962) on *Nephtys* originally demonstrated in transplantation experiments that decerebrate animals receiving an ‘activated’ ganglion from a post-5-day regenerating donor do not regenerate. The assumption here was that the neurosecretory products of the implanted ganglion had already been released. Golding (1967a–c), using *Nereis*, however, demonstrated that ganglia from intact animals and post-5-day regenerating animals are capable of inducing regeneration in host worms. By doing sequential transplants of the same ganglion into competent hosts, he was also able to demonstrate a prolonged secretory activity in the ganglion. He demonstrated further that half a ganglion was

![Fig. 8. A heteromorphic head produced at the posterior wound surface of an experimental animal exposed to 1.25 x 10^{-3}M colchicine for 6 days and then removed to IO. Both cephalic regions are regenerating simultaneously and are considered 'normal' (negatively phototactic).](image)

incompetent while two halves achieved the same result as a whole normal ganglion; this demonstrates the presence of a threshold for activity of the ganglion. In sabellids, therefore, it may be possible that colchicine is reducing the rate of release of a neurohormone so that the time scale of regeneration is extended proportionately. This allows the essential tissue interactions to produce a normal regenerate when returned to IO before 5 days, and allows a build-up of subthreshold concentrations in tissues at the posterior wound region to produce a heteromorphic head when returned to IO after 5 days.

A peculiarity occurred if the animals were cultured in colchicine for periods greater than 5 days. In 10–15% of the cases (6 out of 56 worms cultured), heteromorphi c heads were produced at the posterior cut surface as well as normally at the anterior wound region upon return of the animals to IO (Fig. 8).
Thus two regenerating cephalic regions were produced simultaneously. The heads formed at a normal rate and were 'functional', i.e. negatively phototactic. Both cephalic regions induced parapodial inversion, characteristic of that type of regeneration. Reamputation produced two worms which regenerated at a normal rate and according to the original polarity of the animal, i.e. that cut surface which would normally support cephalic regeneration did so and vice versa. Thus two worms were obtained, one 'normal' and another with two heads. It is quite obvious that the colchicine affects the polarity for a brief, but critical, time and that the effects are not permanent. Furthermore, the ability to produce a cephalic region is not limited to the anterior cut surface.

Comparing this observation with the previously described tissue interactions, the development of bipolar worms appears plausible since both wound surfaces possess a severed nerve-cord, gut and body wall, all in their proper spatial relationship. However, during normal regeneration, polarity is expressed in an asymmetrical pattern which produces an anterior head and posterior pygidium. The administering of colchicine may block neurosecretion, and thus permit a different interaction to occur at the posterior wound region. Flickinger & Coward (1962) have obtained similar heteropolar regenerates in the platyhelminthes, but their worms were exposed to Colcemide for only brief periods, generally a day, and the animals were cultured in an agar slant.

It is of interest that these heteromorphic worms no longer possess a growth region. Normally, elongation of the worm is accomplished by cell division and growth in a region just anterior to the pygidium (Dales, 1963). The formation of a heteromorphic head may necessitate the omission of a growth area and therefore commit the worm to a static size. What physiological effects this might have are unknown at the present time.

**SUMMARY**

1. Gut and nerve-cord interaction during regeneration in the marine polychaetes *Sabella* and *Branchiomma* was examined. Surgical manipulation of various organ systems revealed a mutually dependent tissue interaction of gut, nerve-cord, and body wall in order for normal regeneration to occur.

2. Exposure of *Sabella* and *Branchiomma* to $1.25 \times 10^{-3} \text{M}$ colchicine prevents regeneration for a period of time which is directly proportional to the time cultured in colchicine. Removal from colchicine to normal sea water (IO) initiates regeneration without any evidence of sublethal cytolysis.

3. Bipolar heteromorphic heads are observed in 10–15% of the organisms cultured in colchicine for more than 5 days. Reamputation after returning the worms to IO reveals that the effect is transient, the worms regenerating according to their original polarity.

4. The possible interaction of gut, nerve-cord, and body wall with a neurohormone is discussed. It is concluded that if regeneration in sabellids is under
Sabellid regeneration

neurohormonal control, it is possible that colchicine may retard the rate of release of the hormone and therefore permit the regenerating organ systems at the posterior wound region to produce a heteromorphic head.

ZUSAMMENFASSUNG
Die Wechselwirkung zwischen Leibeshöhle und Nervenstrang in der Regeneration der Sabelliden


3. Wurden die Tiere mehr als 5 Tage lang in der beschriebenen Weise mit Colchicin behandelt, so konnte man in 10 bis 15 Prozent der untersuchten Fälle die Entwicklung von bipolaren heteromorphen Köpfen beobachten. Reamputation nach der Überführung in die normale Kulturlösung (IO) zeigte, daß dieser Effekt zu einer ganz bestimmten Zeit eintritt und daß später die Würmer gemäß ihrer ursprünglichen Polarität regenerieren.


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Sabellid regeneration


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