The Effect of Delayed Brain Extirpation and Replacement on Caudal Regeneration in *Nereis diversicolor*

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INTRODUCTION

THE normal regeneration of amputated posterior segments in the polychaete *Nereis diversicolor* is possible only if the supra-oesophageal ganglion is intact (Clark & Bonney, 1960). Worms fail to regenerate if the ganglion is extirpated before the segments are amputated, but if the ganglion is removed 3 days after amputation of the segments, regeneration proceeds, though at a slower rate than in cerebrate animals. Histological examination of the supra-oesophageal ganglion of regenerating worms at various times after the loss of the posterior segments reveals an increase in cerebral neurosecretory activity within a few hours of the loss of segments. This increased production of neurosecretory material is consistent with the view that cerebral hormones initiate or otherwise promote regeneration, a point we have attempted to confirm in the present paper, and in this respect the Nereidae are comparable with the lumbricid oligochaetes (Hubl, 1956) and nephtyid polychaetes (Clark & Clark, 1959).

In both lumbricids and nephtyids the changes in the appearance of the neurosecretory cells in the supra-oesophageal ganglion are sudden and unmistakable. In *N. diversicolor* they are much less obvious, suggesting, among other possibilities, that the ganglionic influence upon regeneration is exerted more gradually than in the other worms. In order to investigate this we have studied the effect of delayed brain extirpation, and also of reimplanting brains or injecting minced brain into decerebrate worms at various times after the loss of the posterior segments.

The rate at which *N. diversicolor* regenerates is extremely variable. Apart from temperature, sex, the number of segments lost, &c., all of which are known to influence the regeneration rate and which can be controlled, there remains a considerable individual variation in the number of new segments proliferated within a given period after caudal amputation. In the design of the experiments and the assessment of the results we have therefore attached greater importance

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to the type of regeneration that occurs than to the actual number of new segments formed.

Following Stéphan-Dubois (1956) we recognize the following stages in the regeneration of this worm:

1. Wound-healing, when only a cap of epidermal tissue forms over the wound.
2. Pygidium formation, in which a small knob-like pygidium, generally with a pair of anal cirri, is formed.
3. Vascularized pygidium formation, in which the dorsal blood-vessel, generally with lateral side-branches, extends into the new pygidium.
4. Segment proliferation, indicated initially by the appearance of ventro-lateral parapodial rudiments on the new pygidium.

Under the conditions of our experiments, regeneration may be arrested at any of these stages.

MATERIALS AND METHODS

The *N. diversicolor* used in these experiments were collected at Newquay (Cornwall), Portishead (Somerset), and Cullercoats (Northumberland) during the autumn and winter. Stéphan-Dubois (1956) claims that worms approaching sexual maturity regenerate more slowly than immature specimens, but we have not found this to be so, and worms in various stages of sexual maturity have been used in our experiments. The general procedure has been similar to that described previously (Clark & Bonney, 1960), except that MS/222 (Sandoz Products) has been used in place of isotonic magnesium chloride solution as an anaesthetic. Using a 0·05 per cent. solution of MS/222 in sea-water, medium-sized worms are completely narcotized within 5–7 minutes and recover equally rapidly on being returned to fresh sea-water. Loss of blood following removal of the brain was prevented by cauterizing the wound with a small electric cautery. Owing to faults in the sea-water system during the course of the experiments, mortality among the worms, including our stock of intact specimens, was higher than usual, and infections of worms that had suffered cerebral extirpation or amputation of posterior segments was reduced by adding benzylpenicillin (about 50,000 i.u./litre) to the aquaria from time to time.

RESULTS

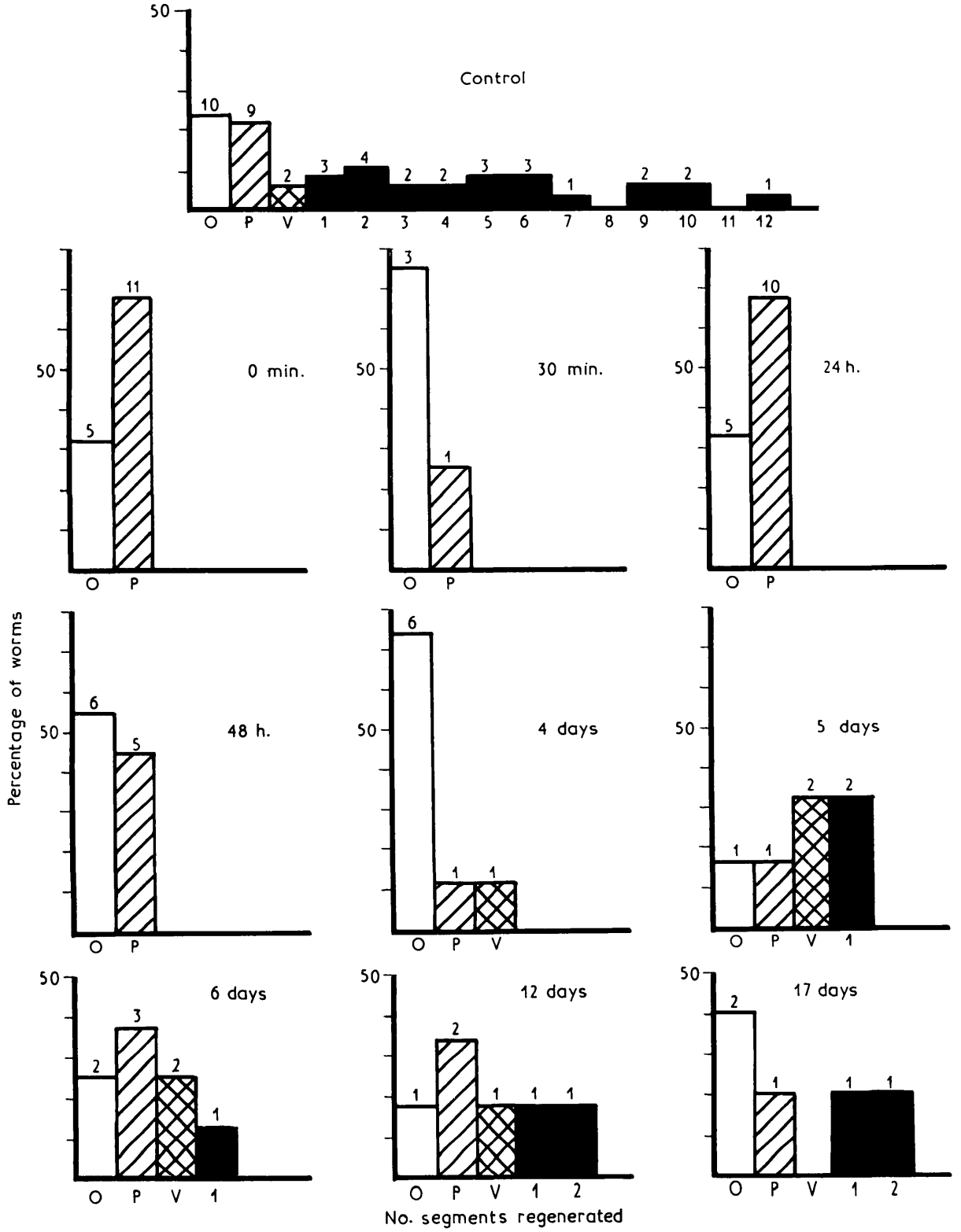
Effect of delayed decerebration upon caudal regeneration

The worms were divided into nine lots (*a–i*) and treated as follows (the numbers in brackets represent the survivors in each group after 30 days).

(*a*) Supra-oesophageal ganglion extirpated before amputation of the posterior segments (16 survivors).

In the remaining groups the supra-oesophageal ganglion was removed after amputation of the posterior segments:

(*b*) 30 minutes after amputation (4);
(*c*) 24 hours after amputation (15);
(*d*) 48 hours after amputation (11);
(*e*) 4 days after amputation (8);



TEXT-FIG. 1. Effect upon regeneration of extirpation of the supra-oesophageal ganglion at various times after amputation of the posterior segments. *Controls*: ganglion intact throughout. O, no regeneration; P, pygidium regenerated; V, vascularized pygidium regenerated; the succeeding numbers indicate the number of segments proliferated. The number of worms represented by each block is indicated above the block.

(*f*) 5 days after amputation (6); (*h*) 12 days after amputation (6);
(*g*) 6 days after amputation (8); (*i*) 17 days after amputation (5).

In addition, two groups of control worms were set up, corresponding to the two periods when these experiments were carried out. The supra-oesophageal ganglion of the controls remained intact throughout the period of regeneration after amputation of the posterior segments. Of these, 44 survived to the end of the experiment.

All the worms were fixed and examined 30 days from the start of the experiments. The extent of regeneration is shown in Text-fig. 1.

Controls

In earlier experiments (Clark & Bonney, 1960) about 25 per cent of the control worms failed to regenerate even a pygidium, but as the posterior part of the worms became necrotic they were omitted from further consideration. On repeating these experiments we again found that nearly a quarter of the control worms failed to regenerate a pygidium or new segments, but since they remained healthy we are unable to discount them and conclude that some specimens of *N. diversicolor* regenerate extremely slowly or not at all. We are unable to correlate this failure to regenerate with size, sex, or sexual maturity.

Of the remaining worms, 21 regenerated new segments (average 5·0 segments) and 11 a new pygidium, of which 2 were enlarged and vascularized. An average number of 2·6 segments were regenerated by the controls as a whole.

Group (*a*). The conclusion of Clark & Bonney (1960) that extirpation of the ganglion before amputation of the posterior segments prevents segment proliferation, though not the formation of a new pygidium, is confirmed.

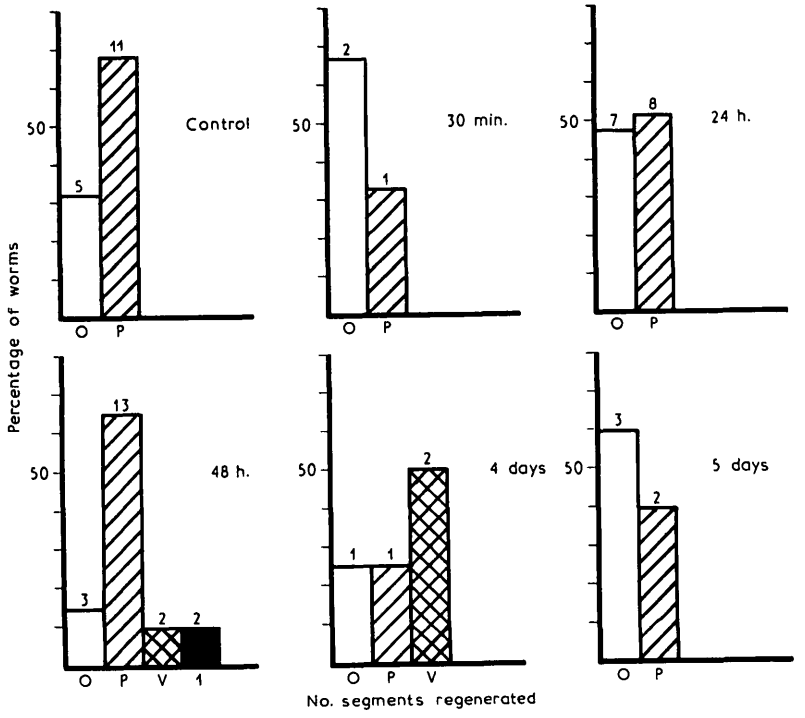
Groups (*b*)–(*d*). Extirpation of the ganglion within 48 hours of amputation of the posterior segments prevents caudal regeneration as completely as decerebration before the amputation of segments. Of the 30 worms in these groups, 16 regenerated a pygidium, but in none was it vascularized.

Groups (*e*)–(*i*). Removal of the ganglion 4 or more days after amputation of the posterior segments does not prevent regeneration, though the number of new segments proliferated is much smaller than that in worms in which the ganglion remains intact. These results may be compared with those obtained previously (Clark & Bonney, 1960). Ganglion extirpation 3 days after amputating posterior segments was then found to result in segment proliferation (a single segment) in 3 of 19 worms; the remainder regenerated at most a pygidium.

Effect of ganglion implantation and injection of ganglion extract during the initial stages of regeneration

The inhibition of regeneration by decerebration either before or shortly after the amputation of a number of posterior segments demonstrated in the previous experiments, and the changes in the neurosecretory activity of the brain of regenerating *N. diversicolor* observed by Clark & Bonney (1960), suggest that

the ganglion is the source of regeneration hormones. We have attempted to confirm this by implanting a whole supra-oesophageal ganglion into the anterior coelom of decerebrate, regenerating worms or by injecting minced ganglion in sterile sea-water into the coelom of the recipient. All the recipients were decerebrated before amputation of the posterior segments and all the donors had been regenerating, with their supra-oesophageal ganglion intact, for the



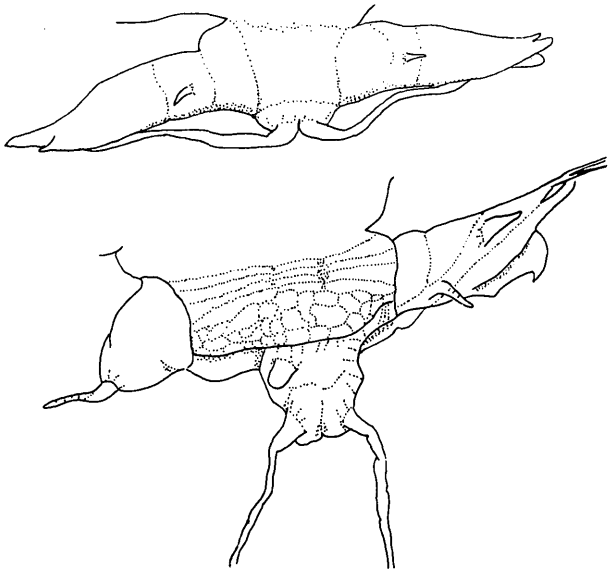
TEXT-FIG. 2. Effect of delayed replacement therapy upon regeneration of posterior segments in decerebrate worms. The time stated is the interval between amputation of the segments and the transplantation or injection of a ganglion from a cerebrate worm that had been regenerating for the same period. Controls: decerebrate worms. The number of worms represented by each block is indicated above the block.

same length of time as the recipient, when the ganglion was removed for implantation. The majority of worms into which foreign ganglia were implanted died, probably because they had to undergo a double operation, once when decerebrated and again when the wound was reopened and the ganglion implanted. We have therefore relied chiefly upon injection of minced ganglion which, although it involves a second narcotization of the recipient, is not a lethal operation.

Implantations were made 30 minutes (3 survivors) and 48 hours (6 survivors) after amputation of posterior segments. Injections were made after intervals of 24 hours (15 survivors), 48 hours (14 survivors), 4 days (4 survivors), and

5 days (5 survivors) following amputation. All the recipients were examined 30 days after the loss of the posterior segments and a number of worms were maintained for an additional 20 days. The amount of regeneration at the end of 30 days in each of these groups of worms is shown in Text-fig. 2. There was no detectable difference between the effects of the two methods of replacing ganglionic material and the results of both series of experiments have been amalgamated.

Implantation or injection of ganglionic material during the first 24 hours of regeneration does not induce the formation of new segments. Nothing more than a pygidium was formed in these worms, and not even that in a third of them. This is comparable to the extent of regeneration in worms decerebrated before or up to 48 hours after amputation of the posterior segments (cf. Text-fig. 1).



TEXT-FIG. 3. *Upper*: dorsal view of a regenerated pygidium and anal cirri of a decerebrate worm. *Lower*: ventral view of a regenerated, vascularized pygidium with anal cirri and a unilateral parapodial rudiment in a decerebrate worm, injected with ganglion extract 48 hours after amputation of the posterior segments.

Replacement of ganglionic material 2–4 days after amputation of the posterior segments was followed within 30 days by the formation of a new pygidium in the great majority of worms, by the formation of a large vascularized pygidium in 4 specimens, and by the appearance of a pair of parapodial rudiments in 2 others (Text-fig. 3). These worms were kept for another 20 days before fixation, but no further regeneration took place in that time.

Replacement therapy on the 5th day appears to be unsuccessful, but since only a small number of worms in this group survived to the 30th day it is probably safer to ignore this result.

DISCUSSION

It is clear from the first series of experiments that the supra-oesophageal ganglion exerts a decisive role in initiating regeneration, and that it must be intact for at least 48 hours after injury for any new segments to be regenerated. However, although new segments may be formed if the ganglion remains intact for 3 days or more, removal of the ganglion at any time during the first 2 weeks after the loss of posterior segments retards regeneration, indicating that the ganglion continues to exert some influence upon regeneration during this period.

That the influence of the ganglion is hormonal is confirmed by the second series of experiments. Implantation of the ganglion, or the injection of ganglion extract, from worms that have themselves been regenerating for 3 or 4 days into decerebrate worms that have lacked the posterior segments for a similar period, sometimes results in the regeneration of a vascularized pygidium or a single pair of parapodial rudiments in the latter. The replacement of the cerebral regeneration hormones is obviously very incomplete, since no more than one segment was regenerated although the worms were kept under observation for a total of 50 days. However, implantation or injection of a single ganglion results in an important advance in the regenerative processes over those seen in decerebrate worms, in which a vascularized pygidium is never formed and segment proliferation has never been observed. The initial stages of regeneration in *N. diversicolor*, as in other annelids, are marked by the migration of free coelomic cells to the wound (Nusbaum, 1908; Dehorne, 1950). In the absence of such a migration a new pygidium with anal cirri may be formed by the reorganization of tissues bordering the wound, but in *Nereis* mesodermal derivatives, including blood-vessels, are not formed, and segment proliferation does not follow upon the formation of a new pygidium (Stéphan-Dubois, 1956). Stéphan-Dubois also demonstrated that the regeneration of an enlarged, vascularized pygidium is a characteristic intermediate stage between normal regeneration with segment proliferation and local reorganization of the epidermal tissues to form only a pygidium. Our results are comparable. Replacement of ganglionic material during the first 2 days after injury is followed by a degree of regeneration characteristic of that when coelomic cells have not migrated to the wound. Replacement on the 3rd or 4th days is followed by regeneration characteristic of a limited migration of the coelomic cells.

We can also draw certain conclusions about the sequence of events following the loss of the posterior segments. Injury is followed by a response on the neurosecretory cells of the supra-oesophageal ganglion within 6–12 hours (Clark & Bonney, 1960). However, regeneration hormones are either not elaborated or not released in sufficient quantity during the first 48 hours to initiate regeneration, because extirpation of the ganglion during this period prevents subsequent regeneration. The fact that replacement of ganglionic material, whether by

implantation of a whole ganglion or by injection of minced ganglion, is incapable of inducing regeneration, suggests that the hormones have not yet been elaborated in quantity, and that the ganglion must remain intact for this to happen. The period around 48 hours after injury appears to be a critical one when the hormones are present in the ganglion (replacement is partially successful in inducing regeneration) but have not yet been released (extirpation of the ganglion at this time still inhibits regeneration). By the 3rd (Clark & Bonney, 1960) or 4th days (present results) the hormones are evidently circulating in the blood or body fluid of the animal, because brain extirpation no longer totally inhibits regeneration. The ganglion is still a source of the hormone and replacement of ganglionic material at this stage induces regeneration in decerebrate worms. Whether by the 5th day the ganglion ceases to secrete the regeneration hormones remains to be investigated. The results of the brain-extirpation experiments suggest that it continues to promote regeneration at least until the 17th day and probably longer, and the contrary evidence of the failure of replacement therapy on the 5th day is probably too slender to be considered at present.

SUMMARY

1. Extirpation of the supra-oesophageal ganglion of *Nereis diversicolor* before or up to 48 hours after amputation of a number of posterior segments inhibits caudal regeneration. Extirpation of the ganglion 3 or more days after amputation of segments retards but does not inhibit regeneration.

2. Injection or implantation of supra-oesophageal ganglia of regenerating worms into decerebrate hosts 48 hours or more after amputating the posterior segments results in segment proliferation in some of the recipients. Replacement of ganglionic material before 48 hours has no effect.

3. The types of regeneration observed following replacement therapy on the 3rd or 4th days is characteristic of that when there has been a limited migration of coelomic cells to the wound.

4. 'Regeneration' hormones appear to be present in the ganglion 48 hours after the loss of posterior segments, but they do not circulate in the body in effective quantity until 24 hours later.

RÉSUMÉ

L'Effet de l'extirpation et du remplacement différés du cerveau sur la régénération caudale de Nereis diversicolor

1. Si l'on extirpe le ganglion supra-œsophagien de *Nereis diversicolor* avant l'amputation d'un certain nombre de segments postérieurs, ou jusqu'à 48 heures après l'amputation de ces mêmes segments, la régénération est inhibée.

2. L'extirpation du ganglion plus de 48 heures après l'amputation des segments retarde mais n'empêche pas la régénération.

3. Le remplacement, par implantation ou injection, du matériel ganglionnaire des vers décérébrés, pendant les 24 premières heures après l'amputation n'est pas suivi de régénération. La même opération, pratiquée 48 heures ou plus après l'amputation, donne lieu à quelque régénération, en particulier à la prolifération de segments.

4. On peut conclure de ces expériences que des hormones de régénération sont élaborées dans le ganglion supra-œsophagien pendant les 2 ou 3 premiers jours après la perte des segments postérieurs. A 48 heures, elles sont présentes dans le ganglion, mais elles ne circulent pas en assez grande quantité dans le sang ou dans le liquide cœlomique pour provoquer la régénération. Vers 72 heures, la régénération est amorcée, mais le ganglion continue à exercer son influence sur la régénération pour une période d'au moins 14 jours.

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