J. Embryol. exp. Morph. Vol. 18, 1, pp. 67–77, August 1967
Printed in Great Britain

Regeneration and growth control in *Nereis*

I. Growth and regeneration

By D. W. GOLDING[1]
From the Department of Zoology, University of Bristol

The annelid body, as typified by that of *Nereis*, consists of a presegmental prostomium, a number of segments, and a postsegmental pygidium. The larval trochosphere is unsegmented. Once proliferation of segments has started, their number increases steadily throughout most of the life of the adrenal, though the rate of proliferation declines (Clark & Clark, 1962). New segments are added posteriorly by proliferation and differentiation of tissue comprising the growth zone, which forms the anterior border of the pygidium. After loss of the pygidium and posterior segments, wound healing is followed by the formation of a new growth zone. Amoebocyte migration (Stephan-Dubois, 1955, 1956, 1958) and dedifferentiation of cells adjacent to the level of transection (Herlant-Meewis & Nokin, 1962) are important in the early stages of regeneration. Proliferation of segments recommences and they are produced more rapidly than they are in the intact animal, as will be shown later.

All animals are liable to be damaged by predators and physical agents, and annelids, with their soft cylindrical segmented bodies, are more vulnerable than most. Loss of part of the body may involve loss of specialized sensory or reproductive structures, or may impair locomotion and reduce the potentiality for gamete production. The ability to regenerate lost parts of the body is clearly of value to such animals. Secondly, this ability is of value as an essential prerequisite for the process of asexual reproduction by fragmentation exhibited by many annelids—for example, the oligochaete *Nais paraguayensis* (Hyman, 1938) and the polychaete *Procerastea* (Allen, 1921). After fragmentation, sections of the body regenerate lost anterior and posterior parts. Thirdly, the capacity for regeneration has been exploited by annelids in association with the production of sexual individuals. Thus in contrast, to nereids which die soon after metamorphosis and breeding, the reproductive potential of syllids is increased by the survival of the anterior, unmodified part of the body. The posterior sexual region, the stolon, breaks off before releasing the ripe gametes, leaving the anterior region to regenerate posteriorly. Regeneration commences after loss

[1] *Author's address*: Department of Zoology, University of California, Berkeley, California, U.S.A.

of the stolon in *Syllis hyalina*, but before this separation in *S. vitata* (Durchon, 1960).

Regenerative growth and other forms of growth may be considered the result of the interaction between the potentiality for growth possessed by the cells involved and a number of factors which influence the growth activities. The influence may be of an inhibitory or stimulatory nature. If it is inhibitory, it may be described as systemic or local, depending upon the distribution of the agent concerned. Stimulatory influences may also be classified in this way: hormones are systemic agents, whilst the trophic activities of nerves and wound hormones (in some cases) are local influences.

No claims have been made with reference to systemic inhibitors in invertebrates. However, Okada & Kawakami (1942) described the induction of poorly differentiated supernumary 'heads' in *Eisenia* by deflexion or implantation of the ventral nerve cord. They observed that size and differentiation can be increased by amputation of the original 'head' just anterior to the induced bud. Extracts of such auto-inhibitors have been prepared from the maldanid *Clymenella torquata* by Smith (1963). Wound hormones probably play an important role in regeneration in annelids, particularly with respect to the activation and migration of neoblasts (Stephan–Dubois, 1955, 1956, 1958). That the nervous system exerts a trophic influence on regeneration in annelids was first indicated by Morgan (1902) and subsequent work has confirmed this (see Herlant–Meewis, 1964, for review). Work by Avel (1932), Okada & Kawakami (1942), Kawakami (1961) and early work by Sayles (1939, 1940*a*) suggested that the nervous influence is unspecific, that is, the nervous system acts in a generally stimulatory way, but does not determine the character of the regenerate. However, other work has indicated that the trophic influence includes a specific, determining component (Sayles, 1940*a*, *b*). The nervous system also influences growth and regeneration in another way. This is the systemic influence exerted through hormones produced by neurosecretory cells. The action of the supraoesophageal ganglion in influencing regeneration in polychaetes was first noted by Casanova in 1955. Since then others have confirmed his findings. The presence of the ganglion, either *in situ* or implanted in the coelom, is indispensable for segment regeneration (Durchon, 1956; Hauenschild, 1960; Clark & Bonney, 1960; Clark & Evans, 1961; Clark & Ruston, 1963). The control of segment proliferation in the intact animal (the control of growth) has attracted less attention. However, as a result of their investigations, Clark & Scully (1964) suggested that growth in *Nereis diversicolor* is under the hormonal control of the supraoesophageal ganglion. Furthermore, Scully (1964) has suggested that the secretory activity of this organ is directly responsible for growth control.

The work reported here was carried out to investigate the relationship between growth and regeneration, to obtain data with respect to the rate of growth during regeneration and to explore the possible implication of the supraoesophageal ganglion in growth control.

MATERIALS AND METHODS

Nereis diversicolor were collected from the intertidal mud of the River Avon, Bristol. They were anaesthetized in a solution of 0·5 % M.S. 222 (Sandoz products) in 50 % sea water. Decerebration was carried out by the removal of the intact supraoesophageal ganglion, together with the overlying epidermis. Ganglia were usually implanted by inserting them through the aperture left by extirpation. Animals were maintained in filtered 50 % sea water to which was added 100 units of benzyl penicillin per millilitre.

Further details have been given elsewhere (Golding, 1965, 1967).

RESULTS

(i) *Growth and regeneration*

The proliferation of new segments is the essential feature of both these processes. The following experiment was performed to discover the relative rates of proliferation in the two cases.

Table 1. *Segment proliferation in intact and regenerating animals*

Stage of experiment (weeks)...	1–3	4	5	6	7
No. surviving...	5	5	5	5	5
No. proliferating no segments	0	5	0	0	0
No. proliferating 1 segment	1	0	0	2	4
2 segments	3	0	0	1	1
3 segments	0	0	0	1	0
4 segments	1	0	2	0	0
5 segments	0	0	2	1	0
6 segments	0	0	1	0	0
Mean no.	2·2	0	4·8	2·4	1·2
S.E.	0·5	.	0·4	0·7	0·2

Fifteen segments removed from each animal after 3 weeks.

Five intact animals, each 55–65 segments long, were kept individually for 3 weeks. Each had the right parapodium of the 6th segment from the posterior end removed at the beginning of the experiment and, by virtue of this, the number of segments proliferated by each animal was determined. Fifteen segments were then amputated from each, and they were kept for a further 4 weeks during which the number of segments regenerated during each week was noted. The results are expressed in Table 1 and Fig. 1.

This experiment is typical of many in that it shows that when posterior segments are removed from immature worms, regeneration occurs (i.e. new segments are proliferated), in all cases.

The results also show that after loss of segments and wound healing, segment proliferation is very rapid compared with that occurring during normal growth.

As regeneration proceeds, the rate soon declines towards the normal growth rate. Segment regeneration occurs after loss of part of the posterior of the body and reformation of the pygidium, and can therefore be distinguished from previous growth which is terminated by segment loss. It cannot be distinguished from subsequent growth, however, since it is impossible to say when regeneration ceases and growth takes over. They appear to be essentially the same process, the only difference, apart from the regeneration of the pygidium, being the contrasting rates of proliferation.

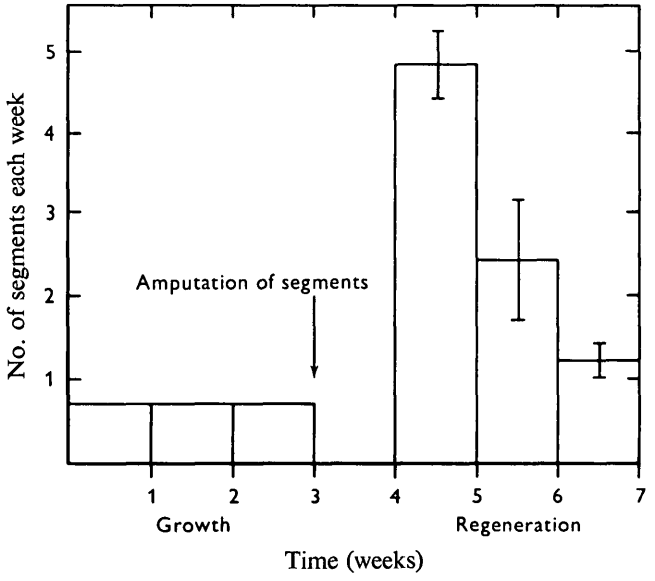

Fig. 1. The effect of segment loss on the rate of proliferation of new segments in immature *Nereis*.

(ii) *Segment proliferation during regeneration*

In this series of experiments, each animal was numbered by the removal of one of its parapodia. Consequently, the progress of regeneration in each animal could be followed. Animals losing segments as a result of entanglement in mucus were eliminated and results compiled only from those surviving undamaged until the end of the experiment.

The first experiment involved the use of 30 animals, each 65–75 segments long. Each specimen had approximately one-third of its posterior segments removed. The ganglia were left *in situ* and the worms were kept individually, without food, for nearly 2 months. The number of segments proliferated by each individual was determined at frequent intervals (Table 2).

Apprehension that starvation was exerting a significant influence on regeneration prompted the next experiment, which differed only from the last one in that the animals were fed twice weekly. The results are given in Table 2.

Thirty decerebrate hosts with implanted ganglia were used in the last of this series of experiments. The procedure was exactly as above (except that decerebrate animals cannot feed). The results are given in Table 2. The rate of segment proliferation at different stages of regeneration was determined from these data (Fig. 2).

Table 2. *Segment proliferation during regeneration in starved animals with ganglia in situ*

Days after amputation...	9	11	13	15	20	24	34	41	48	53
Mean no. of segments regenerated...	0·3	2·2	3·9	4·3	5·3	5·8	7·3	8·4	8·7	8·7
S.E.	—	0·3	0·3	0·3	0·3	0·3	0·4	0·5	0·5	0·5

Results obtained from 19 animals.

Segment proliferation during regeneration in fed animals with ganglia in situ

Days after amputation...	7	14	20	27	33	38	43
Mean no. of segments regenerated...	0	6·7	10·1	11·7	12·7	14·2	15·1
S.E.	—	0·4	0·7	0·9	0·9	1·1	1·1

Results obtained from 15 animals.

Segment proliferation during regeneration in animals with implanted ganglia

Days after amputation...	9	11	13	15	20	24	34	41	48	53
No. showing no regeneration	23	3	0	0	0	0	0	0	0	0
No. regenerating 1 segment	3	14	3	3	1	0	0	0	0	0
2 segments	0	9	8	4	2	3	1	0	0	0
3 segments	0	0	11	11	8	6	6	6	4	4
4 segments	0	0	4	7	10	10	7	6	6	6
5 segments	0	0	0	1	5	7	7	7	7	7
6 segments	0	0	0	0	0	0	4	5	7	7
7 segments	0	0	0	0	0	0	1	2	2	1
8 segments	0	0	0	0	0	0	0	0	0	1
Mean no.	0·1	1·2	2·6	3·0	3·6	3·8	4·4	4·7	4·9	4·9
S.E.	—	0·1	0·2	0·2	0·2	0·2	0·2	0·2	0·2	0·3

Results obtained from 26 animals.

The results obtained in these experiments show that little or no regeneration of segments occurs in the first week after amputation. Segment proliferation is most rapid during the second week, but its rate declines thereafter. The rate of proliferation during the second week is about twice that in the third, and four times that in the fourth. Subsequently, the rate declines more slowly. These characteristic features of the process, revealed by the experiments involving

both fed and starved animals with ganglia *in situ*, can also be seen in those involving decerebrate worms with implanted ganglia, though such animals regenerate fewer segments at all stages.

(iii) *The quantitative effect of segment loss*

In these experiments, the influence of the number of segments amputated on the extent of regeneration was investigated.

In the first experiment, 60 animals, each having 65–75 segments and each having been collected at the same time, were randomly distributed into six groups of 10. No segments were removed from one group; 5, 10, 20, 30 and 40 segments from each member of the other groups respectively. The animals were kept individually, without food, and the number of segments grown or regener-

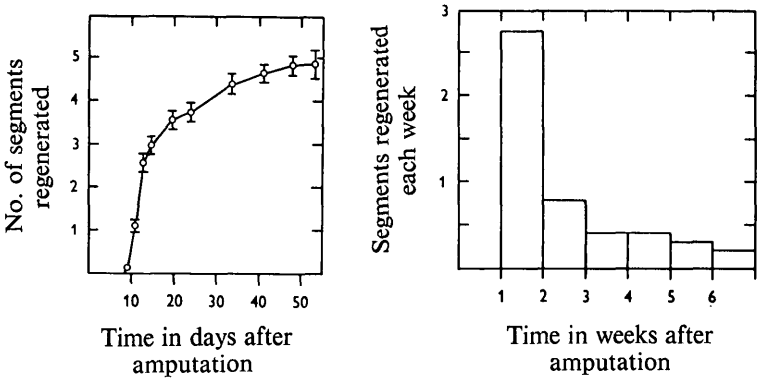

Fig. 2. The rate of segment proliferation during regeneration in animals with implanted ganglia.

ated was determined after 28 days. The results are expressed in Table 3. It can be seen that the rate of growth under these conditions is very slow. However, the rate of regenerative segment proliferation is faster, the speed being dependent on the number of segments amputated. In the second experiment the effect of the removal of different numbers of segments from decerebrate host animals having implanted ganglia from comparable donors was studied. Forty animals, each 67–75 segments long, each having been collected at the same time, were randomly distributed into four groups of 10. The ganglion of each animal was extirpated and implanted into the coelom of another member of the group *before* amputation of segments. Ten, 20, 30 and 40 segments were removed from each group respectively. The different groups were kept together in one bowl and after 21 days, the number of segments regenerated was determined. The results are given in Table 3 and Fig. 3.

The results of this experiment support the conclusions drawn from the previous one. It shows that there is a direct correlation between the number of segments amputated and the number regenerated, whereas Scully (1964)

Table 3. *The effect of segment loss in animals with ganglia in situ*

No. of segments amputated...	0	5	10	20	30	40
No. of animals	10	10	10	10	10	10
No. surviving 28 days	9	8	9	9	8	7
Mean no. of segments regenerated	1·1*	4·3	5·2	7·8	10·6	14·7
S.E.	0·3	0·2	0·2	0·5	0·9	1·3

* Number of segments grown.

The effect of segment loss in animals with implanted ganglia

No. of segments amputated...	10	20	30	40
No. of animals	10	10	10	10
No. surviving 21 days	8	10	9	9
No. showing no regeneration	1	0	0	0
No. regenerating 1 segment	1	1	0	1
2 segments	4	0	0	0
3 segments	1	3	3	0
4 segments	1	5	3	0
5 segments	0	0	1	0
6 segments	0	1	2	2
7 segments	0	0	0	3
8 segments	0	0	0	2
9 segments	0	0	0	1
Mean no.	2·0	3·6	4·2	6·6
S.E.	0·4	0·4	0·4	0·8

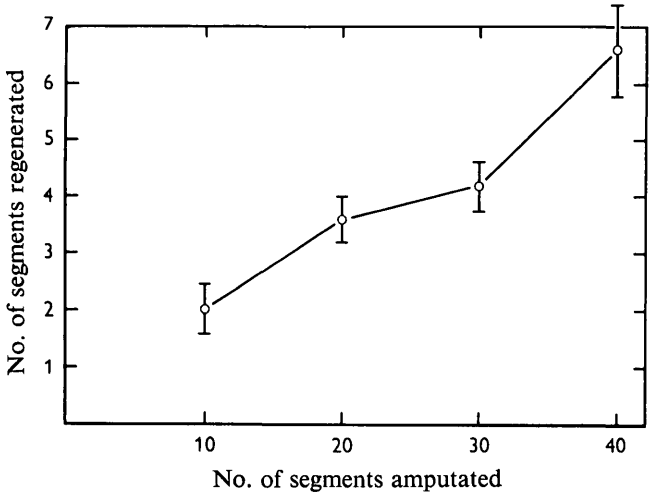

Fig. 3. Effect of the amputation of different numbers of segments on regeneration in hosts containing ganglia extirpated from intact donors.

reported that hosts receiving comparable ganglia as implants regenerate the same amount whether 20–70 segments were removed from them.

As a corollary to this work, the effect of varying the number of segments removed from the donor was investigated. Eighty animals, each 60–70 segments long and each having been collected at the same time, were randomly distributed into four groups of 20. From each animal in groups 1 and 2, the ganglion and 30 segments were removed. They were kept for 3 days (to provide '3-day hosts'). Five segments were removed from each animal of group 3, whereas at least 30 segments were removed from each animal of group 4. Each member of these latter groups was kept for 3 days, after which the ganglion of each member of

Table 4. *The effect of segment loss*

Differential segment loss of donors

No. of segments amputated from donors...	5	30
No. of host animals	20	20
No. surviving 21 days	15	17
No. showing no regeneration	0	0
No. regenerating 1 segment	0	0
2 segments	1	1
3 segments	0	2
4 segments	5	4
5 segments	3	5
6 segments	4	2
7 segments	1	3
8 segments	0	0
9 segments	1	0
Mean no.	5·1	4·8
S.E.	0·4	0·4

group 3 was implanted into the coelom of a member of group 1. Similarly, the ganglion of each member of group 4 was implanted into the coelom of a member of group 2. In this way, the implants were '3-day ganglia' and the hosts '3-day hosts'. After 30 days, the number of segments regenerated by each host was determined. The results are given in Table 4.

Ganglia originating from donors from which many segments were amputated induced no more regeneration in comparable hosts than do those from donors from which only a few segments were removed.

DISCUSSION

The total number of segments possessed by an individual *N. diversicolor* is clearly subject to homoeostatic control. In normal growth, the greater the number of segments already grown the slower the rate of proliferation (Clark & Clark, 1962; Clark & Scully, 1964).

This control is expressed in two ways in regenerative growth. First, during the course of regeneration, the rate of proliferation is initially high but declines rapidly as more segments are produced. This pattern is exhibited in the regeneration induced in a decerebrate animal by an implanted ganglion. Two explanations may be advanced to account for this phenomenon. The first involves both the presence of a fluctuating level of hormone in the body fluids, the rate of proliferation being correlated with this level, and the idea of the exogenous control of normal or implanted ganglia by factors originating from the posterior of the animal. In other words, a feed-back from the regenerating tail to the ganglion is postulated: the more segments proliferated, the more the secretory activity of the ganglion is suppressed. The second explanation is that the concentration of hormone in the body fluids remains constant, but that the inherent growth potentiality of the proliferating region of the pygidium declines progressively as regeneration proceeds.

Growth control is also seen in the correlation between the number of segments lost and the number regenerated. This correlation applies not only to otherwise intact animals (i.e. those with their ganglia *in situ*) but also to animals regenerating under the influence of a foreign ganglion extirpated from the donor *before* loss of the latter's segments. In the first case, the correlation could conceivably be due to the differential nervous stimulation of the neurosecretory cells in the ganglion resulting from section of the ventral nerve cord at different levels. Since the differential 'activation' process postulated by Scully (1964) was thought to result from nervous stimulation, such a mechanism was presumably envisaged. However, this explanation is inapplicable where animals with implanted ganglia are concerned, and since there is no reason to suggest that the phenomenon in one case is different from that in the other, this theory has been discarded.

The two explanations advanced above to account for the declining rate of proliferation during regeneration are also applicable to the correlation between the number of segments lost and the extent of the subsequent regeneration. First, loss of a large number of segments may result in a rapid rate of neurosecretion, a higher concentration of hormone in the body fluids, and rapid segment proliferation. The ganglion may be under an inhibitory influence in the intact animal, as far as its secretory activities are concerned. This inhibition may be lifted by the loss of posterior segments. Alternatively, removal of a large number of segments may result in the formation of a proliferating region that is more sensitive to a given level of hormone as compared with a proliferating region formed as the result of the loss of fewer segments. In this case the rate of regeneration would depend on local factors, responsible for the inherent growth potentiality at a given level, instead of on the concentration of hormone in the body fluids generally.

Experiments designed to shed light on this problem are reported in the following communication.

SUMMARY

'Growth' and 'regeneration' in *Nereis* are essentially similar consisting of the proliferation of new segments. However, the rate of segment proliferation is more rapid after loss of part of the posterior of the body. The first regenerated segmental rudiments appear about 1 week after loss of segments. The rate of proliferation is initially high, but declines rapidly as regeneration proceeds. There is a direct correlation between the number of segments lost and the number regenerated by animals with *in situ* ganglia, and by decerebrate hosts receiving ganglia removed from intact donors. Comparable hosts receiving ganglia originating from donors from which different numbers of segments have been amputated regenerate approximately the same number of segments. Contrasting rates of segment proliferation may either be the result of fluctuating hormone concentrations or of differences in the inherent growth potential of the tissues.

RÉSUMÉ

Régénération et contrôle de la croissance chez Nereis.
I. Croissance et régénération

Croissance et régénération chez *Nereis* sont essentiellement analogues. Cependant la vitesse de prolifération des segments est plus rapide après la perte d'une partie de la région postérieure du corps. Les premières ébauches des segments régénéres apparaissent environ 8 jours après la perte des segments. La vitesse de prolifération est grande au début, elle diminue rapidement au cours de la régénération. Il y a une relation directe entre le nombre de segments perdus et le nombre de segments régénérés chez des animaux dont les ganglions cérébraux sont restés en place, et chez des animaux décérébrés qui ont reçu des ganglions prélevés sur des donneurs intacts. Des animaux sur lesquels on greffe des ganglions provenant de donneurs amputés d'un nombre variable de segments, régénèrent à peu près ce nombre de segments. La discussion de ces observations porte sur la concentration de l'hormone et sur le potentiel intrinsèque de croissance des tissus.

This work was carried out during three years of tenure of an award of the Science Research Council. The author is grateful to Professor R. B. Clark for his encouragement and constructive criticism.

REFERENCES

ALLEN, E. J. (1921). Regeneration and reproduction in the syllid *Procerastea*. *Phil. Trans. R. Soc.* B, **211**, 131–77.

AVEL, M. (1932). Analyse expérimentale de la disparition du pouvoir de regeneration d'une tête, dans la region moyenne du corps, chez les lombriciens. *C.r. hebd. Séanc. Acad. Sci., Paris* **195**, 273–5.

CASANOVA, G. (1955). Influence du prostomium sur la régénération caudale chez *Platynereis massiliensis*. *C.r. hebd. Séanc. Acad. Sci., Paris* **240**, 1814–16.

CLARK, M. E. & CLARK, R. B. (1962). Growth and regeneration in *Nephtys*. *Zool. Jb.* (*Physiol.*) **70**, 24–90.

CLARK, R. B. & BONNEY, D. G. (1960). Influence of the supraoesophageal ganglion on posterior regeneration in *Nereis diversicolor*. *J. Embryol. exp. Morph.* **8**, 112–18.

CLARK, R. B. & EVANS, S. M. (1961). The effect of delayed brain extirpation and replacement on caudal regeneration in *Nereis diversicolor*. *J. Embryol. exp. Morph.* **9**, 97–105.

CLARK, R. B. & RUSTON, R. (1963). Time of release and action of a hormone influencing regeneration in the polychaete *Nereis diversicolor*. *Gen. comp. Endocrinol.* **3**, 542–53.

CLARK, R. B. & SCULLY, U. (1964). Hormonal control of growth in *Nereis diversicolor*. *Gen. comp. Endocrinol.* **4**, 82–90.

DURCHON, M. (1956). Influence du cerveau sur les processus de régénération caudale chez les néréidiens. *Archs. Zool. exp. gen.* **94**, 1–9.

DURCHON, M. (1960). L'endocrinologie chez les annélides polychètes. *Bull. Soc. zool. Fr.* **85**, 275–301.

GOLDING, D. W. (1965). Ph.D. Thesis, University of Bristol.

GOLDING, D. W. (1967). *Gen. comp. Endocrinol.* **8** (in the Press).

HAUENSCHILD, C. (1960). Abhangigkeit der Regenerationsleistung von der inneren Sekretion im Prostomium bei *Platynereis dumerilii*. *Z. Naturf.* **15***b*, 52–5.

HERLANT-MEEWIS, H. (1964). Regeneration in annelids. *Advanc. Morphogenesis* **4**, 155–216.

HERLANT-MEEWIS, H. & NOKIN, A. (1962). Cicatrisation et premiers stades de régénération pygidiale chez *Nereis diversicolor*. *Annls Soc. r. zool. Belg.* **93**, 137–54.

HYMAN, L. H. (1938). The fragmentation of *Nais paraguayensis*. *Physiol. Zoöl.* **11**, 126–43.

KAWAKAMI, I. K. (1961). Experimental analysis of factors influencing regeneration in the earthworm. *Japan J. Zool.* **13**, 141–64.

MORGAN, T. H. (1902). Experimental studies of internal factors of regeneration of the earthworm. *Wilhelm Roux Arch. EntwMech. Org.* **14**, 563–91.

OKADA, Y. K. & KAWAKAMI, I. K. (1942). Studies on the regeneration of earthworms by transplantation. I. Influence of the nervous system. *Proc. Imp. Acad., Tokyo* **17**, 149–52.

SAYLES, L. P. (1939). Buds induced by implants of nerve cord and neighbouring tissues in the polychaete *Clymenella torquata*. *Biol. Bull. mar. biol. Lab. Woods Hole* **76**, 330–338.

SAYLES, L. P. (1940*a*). Buds induced by implants of anterior nerve cord and neighbouring tissues inserted at various levels in *Clymenella torquata*. *Biol. Bull. mar. biol. Lab. Woods Hole* **78**, 298–311.

SAYLES, L. P. (1940*b*). Buds induced by implants of posterior nerve cord and neighbouring tissues inserted at various levels in *Clymenella torquata*. *Biol. Bull. mar. biol. Lab. Woods Hole* **78**, 375–87.

SCULLY, U. (1964). Factors influencing the secretion of regeneration-promoting hormone in *Nereis diversicolor*. *Gen. comp. Endocrinol.* **4**, 91–8.

SMITH, S. D. (1963). Specific inhibition of regeneration in *Clymenella torquata*. *Biol. Bull. mar. biol. Lab. Woods Hole* **125**, 542–55.

STEPHAN-DUBOIS, F. (1955). Régénération postérieure de *Nereis diversicolor* après irradiation régionale auz rayons-X. *C.r. hebd. Séanc. Acad. Sci., Paris* **241**, 1175–7.

STEPHAN-DUBOIS, F. (1956). Évolution du régénérat caudal de *Nereis diversicolor* après irradiation régionale aux rayons-X. *Bull. Soc. zool. Fr.* **81**, 199–207.

STEPHAN-DUBOIS, F. (1958). Le rôle des leucocytes dans la régénération caudale de *Nereis diversicolor*. *Archs. Anat microsc.* **47**, 605–652.

(*Manuscript received 14 February 1967*)